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Title page

Commentary

Clearance prediction methodology needs fundamental improvement: trends common to rat and human hepatocytes/microsomes and implications for experimental methodology

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Global assessment of in vitro assay performance for prediction of clearance

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Non-standard abbreviations: AFE (average fold error); BDDCS (Biopharmaceutics Drug Disposition Classification System); CL_b (clearance in blood); CL_p (clearance in plasma); CL_{int} (intrinsic clearance); CYP (cytochrome P450); ESF (empirical scaling factor); fu_b (fraction unbound in blood); fu_p (fraction unbound in plasma); HLM (human liver microsomes); PBSF (physiologically based scaling factor); Q_h (blood flow); R_b (blood:plasma concentration ratio); RLM (rat liver microsomes); UGT (uridine 5'-diphospho-glucuronosyltransferase); fm_{UGT} (fraction metabolised by UGT)

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Abstract

Although prediction of clearance using hepatocytes and liver microsomes has long played a decisive role in drug discovery, it is widely acknowledged that reliably accurate prediction is not yet achievable despite the predominance of hepatically cleared drugs. Physiologically mechanistic methodology tends to underpredict clearance by several-fold and empirical correction of this bias is confounded by imprecision across drugs. Understanding of the causes of prediction uncertainty has been slow, possibly reflecting poor resolution of variables associated with donor source and experimental methods, particularly for the human situation. Hallifax et al. (Pharm. Res. 27: 2150-2161, 2010) reported that among published human hepatocyte predictions there was a tendency for underprediction to increase with increasing *in vivo* intrinsic clearance, suggesting an inherent limitation using this particular system. This implied an artefactual rate limitation *in vitro*, although preparative effects on cell stability and performance were not yet resolved from assay design limitations. Here, to resolve these issues further, we present an up-to-date and comprehensive examination of predictions from published rat as well as human studies (n= 128 and 101, hepatocytes and n= 71 and 83, microsomes, respectively) to assess system performance more independently. We report a clear trend of increasing underprediction with increasing *in vivo* intrinsic clearance which is similar both between species and between *in vitro* systems. Hence, prior concerns arising specifically from human *in vitro* systems may be unfounded and the focus of investigation in future should be to minimise the potential *in vitro* assay limitations common to whole cells and subcellular fractions.

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Introduction

For more than a decade numerous studies have reported prediction of clearance of drugs from human and rat hepatic *in vitro* systems (with increasing emphasis on hepatocytes rather than microsomes), all of which have indicated a tendency for underprediction on the basis of physiologically mechanistic scaling (Shibata et al., 2002; Hallifax et al., 2005; Ito and Houston, 2005; Riley et al., 2005; Brown et al., 2007; Stringer et al., 2008; Chiba et al., 2009; Hallifax et al., 2010). These studies used both intra- and inter-laboratory datasets for drugs which were mostly commercially available small molecules (MW 200-600) with a predominant metabolic route of clearance. With an increasing emphasis on human (hepatic) *in vitro* systems, *in vivo* intrinsic clearance (CL_{int}) of these drugs ranged over about five orders of magnitude (1-100,000 ml/min/kg).

To limit the negative impact of prediction inaccuracy on drug discovery and human dosing decisions, empirical correction of prediction bias, as has been suggested, offers a practical and justifiable improvement (Poulin et al., 2012; Sohlenius-Sternbeck et al., 2012; Yamagata et al., 2016). But considerable uncertainty remains, reflecting the poor precision which accompanies the bias seen in published datasets. Reducing both the imprecision and bias requires understanding of their causes and for prediction methodology to progress, a mechanistic approach must be maintained and improved. There have been a number of suggested potential causes of underprediction, but many have not been met with incisive investigation.

The impact of liver model choice is still questioned despite demonstration of marginal effect between the well-stirred and parallel tube models, for a large dataset (Hallifax et al., 2010). Only very high clearance compounds are significantly impacted and those are inevitably a minor proportion of a typical dataset. While the modelling of hepatic clearance has been extended to include transmembrane processes (Chiba et al., 2009), this is more applicable to more recently developed, less permeable, drugs and in any case, cannot be as widely assessed due to the general lack of distinction of uptake processes in available *in vitro* data. Related to liver models, the extent of protein binding is often cited as a factor in prediction accuracy, due possibly to perceived inherent binding measurement inaccuracy, a lack of equilibrium *in vivo* or facilitated uptake of drug (Soars et al., 2007b; Ring et al., 2011; Poulin et al., 2012; Bowman and Benet, 2016). For highly lipophilic drugs, Poulin et al. proposed a methodology relating plasma and hepatic albumin binding based on

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postulated involvement of bound drug with the uptake process (Poulin et al., 2012; Poulin and Haddad, 2013). However, this semi-mechanistic methodology has been shown to offer no improvement on the conventional physiological approach (Yamagata et al., 2016).

Human hepatocytes are inherently variable in drug clearance capability *in vitro*, reflecting a number of unavoidable factors: donor phenotypic variability, potentially detrimental processing (preparation and storage) and lability of metabolising enzymes and uptake transporters – all sources of variation and possibly bias (Hallifax and Houston, 2009). Prediction assay methodology itself, lacking in standardisation, is probably another source of variation (and bias); given the extremely wide range of *in vivo* CL_{int} involved, system artefacts might be expected. Addressing such issues, Hallifax et al. (2010) highlighted a tendency for prediction accuracy from human hepatocytes (but not microsomes) to decrease with increasing *in vivo* CL_{int}, among an extensive dataset from published studies, suggesting *in vitro* artefacts (eg. cofactor exhaustion) or *in vitro* permeability rate limitation to explain underprediction. A lack of relationship between prediction accuracy and permeability was later demonstrated (Hallifax et al., 2012) focussing attention on assay methodology. Bowman and Benet (2016), in a recent comparison of published studies using human hepatocytes and microsomes, saw only slight evidence of difference in prediction accuracy between drugs classified (BDDCS) as transporter substrates or not, or between drugs segregated as highly bound in blood or not. Having confirmed the lack of resolution between sources of uncertainty, they highlighted a need for improved experimental methodology.

Use of rat *in vitro* systems for measurement of CL_{int} has been superseded by the now widely available human equivalent. Although some studies have indicated a tendency towards underprediction of clearance for rat as well as that more widely acknowledged for human (Huang et al., 2010; Sohlenius-Sternbeck et al., 2012), minimal inter-individual and processing variability in rat implies much less prediction uncertainty (Iwatsubo et al., 1996; Iwatsubo et al., 1997; Wilson et al., 2003; Ito and Houston, 2005; Riley et al., 2005; Hallifax and Houston, 2009). It is therefore of considerable potential value to appraise the limitations of clearance prediction in rat, in parallel to human, to enable more incisive understanding of experimental uncertainty. Going beyond previous assessments of prediction of clearance and to attempt to resolve source- and experimentally-based variation and bias, we have compiled the most extensive datasets to date for inter-species (rat and human) comparison of *in vitro*

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(hepatocyte and microsome) predictions of CL_{int} from the literature. This commentary critically examines the accuracy, precision and trends in prediction of clearance between the foundational *in vitro* species assessing the implications for future progress in prediction methodology.

Data collation

Datasets for human (n= 101, hepatocytes; n= 83, microsomes) and rat (n= 128 hepatocytes; n= 71, microsomes) *in vitro* CL_{int} and *in vivo* CL were compiled based on examination of existing published datasets and a further search of the literature. Both approved pharmaceuticals and investigatory proprietary compounds were considered provided that complementary *in vitro* and *in vivo* data were available. Criteria for inclusion of *in vivo* data were that reported blood clearance (CL_b) or plasma clearance (CL_p) was determined from intravenous dosing and that CL_b did not exceed hepatic blood flow (Q_H). *In vitro* CL_{int} determined from either metabolite formation or substrate depletion over a range of substrate concentrations, or from single concentration substrate depletion time profiles was considered. *In vitro* data included both suspended hepatocytes and liver microsomes, reflecting the prevalent use of these systems in the pharmaceutical industry. Microsomal data represents that from incubations with exogenous NADPH (Phase I metabolism) only. Hepatocyte data represents incubations conducted in the absence of serum; in each instance the use of fresh or cryopreserved cells was recorded.

For human hepatocyte data, two key review articles, Hallifax et al. (2010) and Paixao et al. (2010) were identified; from these the original sources were examined and data not previously included were added (for e.g. Hallifax et al. (2010) included only predictions from cryopreserved hepatocytes). Most additional data came from subsequently published studies (Sohlenius-Sternbeck et al., 2010; Akabane et al., 2012a; Akabane et al., 2012b; Sohlenius-Sternbeck et al., 2012); some data came from earlier studies not previously included (Hallifax et al., 2005; Hallifax et al., 2008). For human liver microsomes (HLM), original sources from Ito and Houston (2004) and Hallifax et al. (2010) were examined, with supplementation from Obach (1999), Cubitt et al. (2009), Gertz et al. (2010) and Sohlenius-Sternbeck et al. (2010). Additional data for both human hepatocyte and HLM were provided by R Stringer (Stringer 2006, thesis, University of Manchester) as data supplemental to Stringer et al. (2008). The rat hepatocyte datasets encompassed a previous compilation (Ito and Houston 2005) and several subsequent original research articles; the majority of data were from Huang et al. (2010) and

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Sohlenius-Sternbeck et al. (2010). For rat liver microsomes (RLM), original source data cited by Houston (1994), Houston and Carlile (1997) and Ito and Houston (2004) were collated together with data from Jones and Houston (2004), De Buck et al. (2007) and Huang et al. (2010). The complete datasets (with sources) are given in Supplements 1 (human) and 2 (rat). No data was duplicated by source; where more than one original source provided data for a particular drug, mean CL_{int} was calculated.

The datasets were considered to predominantly comprise highly permeable drugs of which the vast majority would be expected to be cleared by metabolism without rate limitation by transport, although dependency on hepatic uptake transport for clearance would not necessarily preclude inclusion of predictions for the purposes of this analysis. In 2015, Varma et al. proposed a drug classification system for predicting the major clearance route, based on charge, permeability and molecular weight – the Extended Clearance Classification System (ECCS). Using the assignments they presented, the human datasets in the present study (as far as could be assigned – 73 and 72 % hepatocytes and microsomes, respectively) comprised about 86 % deemed to have metabolism as the primary route of clearance – which supports our initial assumption. Of the remainder, half (7 %) could be assigned as rate limited by hepatic uptake transport and half (7 %) dependent on renal clearance. For rat, about 79 and 100 % (hepatocytes and microsomes, respectively) of the commercial drugs which were ECCS pre-categorised (60 and 65 % of commercial drugs, hepatocytes and microsomes, respectively) were predominantly cleared by metabolism. A large proportion of the rat datasets were proprietary compounds but on the basis of their physico-chemical properties at least 85 % for hepatocytes and 100 % for microsomes (assuming all ‘AZ’ compounds were highly permeable as denoted by Varma et al. [$>5 \times 10^6$ cm/sec]) were inferred as predominantly cleared by metabolism. As the above categorisation is inherently imprecise, no particular individual drug prediction was excluded following the initial collation criteria.

***In vitro* fraction unbound**

In studies where the fraction unbound in either microsomes ($f_{u_{mic}}$) or hepatocytes ($f_{u_{heps}}$) was experimentally determined and reported alongside CL_{int} , this value was applied in prediction of *in vivo* unbound CL_{int} ($CL_{int,u}$). Where $f_{u_{mic}}$ or $f_{u_{heps}}$ were unreported, these values were estimated using

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lipophilicity relationship algorithms (Equations 1 (Hallifax and Houston (2006) and 2 (Kilford et al. (2008) respectively).

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

$$f_{u_{heps}} = \frac{1}{1 + 125 \cdot V_R \cdot 10^{0.072 \cdot \log P / D^2 + 0.067 \cdot \log P / D - 1.126}} \quad (2)$$

where P is the microsomal protein concentration, log P/D is either the log P value for basic and neutral drugs or the log D value for acidic drugs and V_R is the volume ratio of hepatocytes to medium (0.005 for 1 x 10⁶ cells/ml (Brown et al., 2007)).

Scaling *in vitro* intrinsic clearance to whole liver

In vitro CL_{int} values were scaled to the *in vivo* whole liver equivalent using Equation 3 (Hallifax et al., 2010), where the physiologically based scaling factor (PBSF) is the microsomal average recovery factor for microsomal predictions and hepatocellularity for hepatocyte predictions, and LW is the liver weight/kg bodyweight.

$$\text{Predicted } in \text{ vivo } CL_{int,u} = \frac{in \text{ vitro } CL_{int} \cdot PBSF \cdot LW}{f_{u_{mic}} \text{ or } f_{u_{heps}}} \quad (3)$$

Microsomal recovery factors of 40 mg microsomal protein/g liver (Hakooz et al., 2006) and 60 mg microsomal protein/g liver (Houston and Carlile, 1997) were used for human and rat respectively; hepatocellularity of 120 x 10⁶ hepatocytes/g liver was used for both human (Hakooz et al., 2006) and rat (Bayliss et al., 1999) and LW was 21.4 g/kg bodyweight for human and 40 g/kg bodyweight for rat (Davies and Morris, 1993).

Determination of *in vivo* intrinsic clearance

In vivo CL_{int,u} was derived from hepatic clearance (CL_H) (blood), fraction unbound in blood (f_{u_b}) and Q_H with a given value of 20.7 ml/min/kg for human (Davies and Morris, 1993) and 100 ml/min/kg for rat

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(Ito and Houston, 2004). Where applicable and data available, CL_h was determined by subtracting renal clearance from total CL_b . Where sources provided CL_p and fraction unbound in plasma (f_{up}), CL_b and f_{ub} were calculated using reported blood/plasma concentration ratio (R_b) (CL_p/R_b and f_{up}/R_b respectively). Where R_b was unavailable, it was assumed to be equal to 1 for a basic or neutral compound and 0.55 (1 - haematocrit) for an acidic compound. If data from multiple studies were available for the same compound, the arithmetic means of CL_b and f_{ub} were used in the calculation of $CL_{int,u}$.

In vivo $CL_{int,u}$ was derived using both the well-stirred and parallel tube models of hepatic clearance to assess the impact of liver model on the predictive accuracy of *in vitro* data. As the difference in bias between these two liver models (representing both extremes of drug hepatic dispersion) was found to be marginal, consistent with previous studies (Jones and Houston, 2004; Riley et al., 2005; Brown et al., 2007), data from the well-stirred model (Equation 4) is presented.

$$In\ vivo\ CL_{int,u} = \frac{CL_h}{f_{ub} \cdot \left(1 - \frac{CL_h}{Q_H}\right)} \quad (4)$$

Assessment of accuracy and precision of predictions

The overall bias in predictions was assessed by calculation of the average fold error (AFE) (Equation 5). Root mean squared error (RMSE) (Equation 6) was used as a measure of precision.

$$AFE = 10^{\frac{\sum \log \frac{\text{predicted}}{\text{observed}}}{n}} \quad (5)$$

$$RMSE = \sqrt{\frac{1}{n} \sum (\text{predicted} - \text{observed})^2} \quad (6)$$

where n = number of predictions.

As underprediction yields an AFE below 1, underprediction was also expressed as fold-underprediction (inverse of AFE). The percentage of $CL_{int,u}$ predictions within (and beyond) two-fold of *in vivo* was used as an additional indicator of predictive accuracy, consistent with previous

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publications (Obach, 1999; Naritomi et al., 2001; McGinnity et al., 2004; Stringer et al., 2008; Sohlenius-Sternbeck et al., 2012; Chan et al., 2013).

Calculation of empirical scaling factors

The empirical scaling factors (ESFs) required to equate predicted $CL_{int,u}$ with observed $CL_{int,u}$ for individual compounds within each dataset were calculated using Equation 7.

$$ESF = \frac{\text{observed } CL_{int,u}}{\text{predicted } CL_{int,u}} \quad (7)$$

Average ESF (for $CL_{int,u}$ and f_{up} subsets) was calculated using Equation 8 (log average)

$$\text{Average ESF} = 10^{\frac{\sum \log \frac{\text{observed}}{\text{predicted}}}{n}} \quad (8)$$

Segregation of predictions into subsets

For the human dataset, hepatocyte predictions were segregated into those derived from freshly isolated (n= 52) or cryopreserved cells (n= 93), to enable comparison of predictive accuracy between such preparations. Although several original research studies have performed such a comparison on small numbers of drugs, finding few significant differences (Diener et al., 1995; Li et al., 1999; Hewitt et al., 2000; Lau et al., 2002; Naritomi et al., 2003; McGinnity et al., 2004; Blanchard et al., 2005; Floby et al., 2009), this compilation provided an opportunity to address this question on a larger scale. In order to eliminate any bias associated with the inclusion of different substrates, a dataset comprising only of compounds common to both fresh and cryopreserved hepatocyte predictions (n= 43) was also evaluated.

A recent review by Jones et al. (2015) indicated that the pharmaceutical industry has less confidence in predictions of non-cytochrome P450 (CYP) mediated clearance predictions than their CYP-mediated counterparts. To test this assumption, human hepatocyte predictions of substrates for uridine 5'-diphospho-glucuronosyltransferases (UGTs), the major non-CYP metabolic enzyme family, were segregated from the remainder of the dataset (predominantly CYP substrates). Glucuronidated compounds were identified and categorised by fraction metabolised by UGT (fm_{UGT}) based on published *in vitro* and *in vivo* data (Miners and Mackenzie, 1991; Kaiser et al., 1992; Laethem et al., 1995; Soars et al., 2002).

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Prompted by the recent publication by Bowman and Benet (2016), the relationship between protein binding and accuracy of *in vivo* clearance predictions (from both human hepatocytes and human liver microsomes) was also reassessed. In this study, the f_{up} , as opposed to the f_{ub} , was used as the measure of protein binding to circumvent the influence of drug binding to red blood cells. In addition, consistent with Bowman and Benet (2016), drugs were segregated by BDDCS class to examine potential relationships between drugs identified as substrates of uptake or efflux transporters, protein binding, and underprediction (human). For human hepatocyte and human liver microsome (HLM) predictions respectively, 99/101 and 83/83 drugs were able to be classified by BDDCS according to Benet et al. (2011) and Hosey et al. (2016). In addition, the BDDCS assignments were used to distinguish drug type for the analysis of ESF (both species). For rat, 125/128 (hepatocytes) and 65/71 (RLM) assignments were made including the proprietary compounds based on our assessment of the actual/likely physico-chemical properties. To support the examination of drug properties, drugs (human and rat hepatocyte dataset) were identified as acidic, basic or neutral.

Comparison of human and rat *in vitro* prediction of intrinsic clearance

Using the criteria detailed above, predictions of *in vivo* $CL_{int,u}$ were recorded for 101 drugs from human hepatocyte data (Figure 1A) and for 83 drugs from HLM data (Figure 1B); 66 drugs were common to both systems in human. *In vivo* $CL_{int,u}$ predictions for 128 compounds were made from rat hepatocyte data (Figure 1C) and for 71 compounds from rat liver microsome (RLM) data (Figure 1D); 52 compounds were common to both systems in rat. *In vivo* and *in vitro* clearance data and references for individual compounds are given in the Supplement (tables 1 and 2 for human and rat respectively).

In vivo $CL_{int,u}$ was predominantly underpredicted in hepatocytes and microsomes for both human and rat. In both species underprediction was greatest in hepatocytes with an average fold-underprediction of 4.2 in human and 4.7 in rat; microsomes showed less overall bias with average fold-underprediction of 2.8 in human and 2.3 in rat (Table 1). However, despite evidence of less average bias in microsomes, the percentage of predictions within two-fold of observed was similar across systems and species, ranging between 20 and 30 %. In both species, microsomes showed a greater incidence of overprediction (predicted values more than two-fold above observed) than hepatocytes.

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Precision as represented by RMSE was similar between human hepatocytes and HLM; precision for rat was much less than for human, least of all for RLM (Table 1).

In both species, microsomes appear to display a wider range of $CL_{int,u}$ predictions than hepatocytes. In human, predicted $CL_{int,u}$ in hepatocytes ranged from approximately 1-1,000 ml/min/kg compared to approximately 0.1-10,000 ml/min/kg in microsomes (Figure 1, A and B respectively). In rat, predicted $CL_{int,u}$ appears to be limited to approximately 10,000 ml/min/kg in hepatocytes, but reaches 100,000 ml/min/kg in microsomes (Figure 1, C and D respectively). The greater imprecision implied by RMSE for rat may have at least in part reflected the greater upper range (hence greater error) in this species. An apparent intercept of prediction trend at the line of unity occurred at about 10 ml/min/kg for human and about 100 ml/min/kg for rat, possibly reflecting the general difference in metabolic rate expected between these two species.

Comparison of predictions from fresh and cryopreserved human hepatocytes

Division of the complete human hepatocyte dataset into *in vivo* $CL_{int,u}$ predictions from fresh and cryopreserved preparations revealed no meaningful differences in both prediction bias and precision (Table 2). This finding was consolidated by the similar outcome from analysis of only those drugs (n= 43) common to both types of preparation (Table 2, Figure 2). There was, therefore, clear evidence that the cryopreservation process has no influence on prediction accuracy.

Relationship between empirical scaling factor and *in vivo* clearance

To further characterise the underprediction of *in vivo* $CL_{int,u}$ in human and rat hepatocytes and liver microsomes, ESFs were calculated for individual compounds in each system and species and plotted as a function of observed $CL_{int,u}$ (Figure 3).

A similar trend of increasing ESF with increasing *in vivo* $CL_{int,u}$ was observed for human and rat hepatocytes (Figure 3, A and C), indicating clearance-dependent underprediction. For microsomes, clearance-dependency was also apparent, with comparable magnitude between the two *in vitro* systems (Figure 3, B and D).

Comparison of the average ESF between segregated levels of *in vivo* $CL_{int,u}$ showed an exponential progression of underprediction of a similar magnitude between human hepatocytes and microsomes up to 10,000 ml/min/kg, despite the high variance (Table 3). Above 10,000 ml/min/kg, detailed

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comparison was not justified due to few individual data, although a lower limitation in prediction by hepatocytes, compared to microsomes, was apparent. For rat, there was also quantitative agreement between the systems, although – as for human – there was marginally less underprediction evident for microsomes compared to hepatocytes. In addition, there was a progression of underprediction similar to human, albeit out of step by an order of magnitude (in terms of observed $CL_{int,u}$ subgroup) – possibly reflecting species differences in metabolic rates (as noted above). Together, these quantitative prediction profiles provide clear evidence of strong species-independent and, to a large extent, system-independent trends in clearance-dependent underprediction.

Examining the ESF trends in terms of drug type, as denoted by the BDDCS, there was extensive overlap between permeable drugs cleared by metabolism (Class 1) and similar drugs which were potential hepatic uptake substrates and which might have their clearance limited by this (Class 2) (Figure 3). For human, the trend of increasing ESF with *in vivo* CL_{int} appeared to be independent of drug type, although this was clearer for hepatocytes compared with microsomes; in the latter system, a number of Class 2 or 3 drugs were predicted accurately or overpredicted, possibly reflecting enhanced access to metabolic enzymes in this system (Figure 3, A and B). A similar lack of drug type dependency was evident also for rat, although a number of the most highly cleared drugs were potentially dependent on transport for clearance (Class 2 to 4). The drug type assignment according to BDDCS is necessarily imprecise and some of the drugs designated as BDDCS Class 2 could be otherwise designated as dependent on metabolism, rather than transport, according to the alternative ECCS system (Varma et al., 2015) by virtue of being neutral or basic compounds; this would include a considerable number of the proprietary compounds in the rat datasets. As such, there would be a predominance of compounds dependent on metabolic clearance. Comparing human with rat, the trend in increasing ESF with *in vivo* CL_{int} was generally independent of species and to a large extent, *in vitro* system.

Use of empirical scaling factors between *in vitro* and *in vivo* from pre-clinical species has been suggested as a pragmatic refinement for methodology for human prediction (Naritomi et al., 2003). In the current analysis, similarity in ESF between rat and human might appear to support this, at least in general terms. However, for those drugs common to both rat and human datasets ($n=24$, hepatocytes; $n=17$, microsomes) there was no apparent correlation between the species for either

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system (data not shown), indicating a lack of drug dependency in the scaling factor and hence persistence of considerable uncertainty on an individual drug basis.

Prediction of *in vivo* clearance of UGT substrates

Within the human hepatocyte dataset, drugs subject to glucuronidation were categorised as high (≥ 0.75), medium high (0.50-0.75), medium low (0.25-0.50) or low (<0.25) fm_{UGT} . As a single group, glucuronidated drugs span a large range of *in vivo* $CL_{int,u}$ (<10 - $>1,000$ ml/min/kg) and show comparable underprediction to drugs metabolised predominantly by other enzymes (CYP). There appears to be no relationship between fm_{UGT} and underprediction of *in vivo* $CL_{int,u}$ (Figure 4).

Prediction of *in vivo* clearance of acidic, basic and neutral drugs

There was extensive overlap of prediction accuracy for drugs identified in groups according to ionic character for prediction made for both human and rat hepatocytes (Figure 5) and hence this criterion was considered too simplistic for distinguishing prediction trends.

Assessment of extent of underprediction in relation to f_{up} and BDDCS

To assess the extent of any relationship between binding in blood (assumed to be mostly due to binding to plasma protein), ESF was plotted against f_{up} for both human hepatocyte and microsome datasets (Figure 6, A and B). Visually, there was no trend in prediction accuracy with f_{up} for either dataset, although there was a clear tendency for BDDCS Class 2 drugs to be more highly bound than those of Class 1, as highlighted by Bowman and Benet (2016). The equivalent relationship for total blood binding (f_{ub}) was also examined but, as there were only very minor differences (data not shown) this was not analysed further.

Segregating the drugs by level of f_{up} showed that the majority (90%) of drugs had f_{up} values either within the range 0.01-0.1 (40%) or within the range 0.1-1 (50%). Average ESF was between 3 and 4 for hepatocytes and between 2 and 3 for microsomes, across these two levels of f_{up} (Table 4). There were relatively few drugs with f_{up} in the lower range of 0.001-0.01 and, excluding one/two highly aberrant values for hepatocytes and microsomes, ESF was about 6 (Table 4). For BDDCS Class 1 drugs specifically, ESF was between 3 and 6 for f_{up} across the ranges 0.01-0.1 and 0.1-1 for hepatocytes, and between 2 and 4 across the same ranges, for microsomes (Table 4). For the same f_{up} ranges, ESF for Class 2 drugs was between 4 and 13 for hepatocytes and between 1 and 3 for

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microsomes (Table 4). The greater ESF values obtained for the lowest f_{up} level for all drugs (6.0 and 5.6 for hepatocytes and microsomes, respectively) were largely reflected in Class 2 drugs (8.2 and 6.3). A single compound in the microsome dataset was identified as BDDCS Class 4 but was excluded from the tabular analysis. Taken together, these observations indicate a lack of relationship between f_{up} and prediction accuracy and have no bearing on the trend of clearance-dependent prediction described earlier.

It is recognised that some historical f_{up} values may be biased by lack of assay pH control (Kochansky et al., 2008). An adjustment of f_{up} values for bases (average 2.6-fold increase, Kochansky et al., 2008) in this dataset would cause some skew towards overall greater f_{up} for the values for the range 0.1-1, due to the relatively weak binding of these compounds; however, exclusion of bases would not impact the lack of relationship between f_{up} and ESF for acidic and neutral drugs. Because the data cannot be retrospectively corrected with respect to exact assay conditions, such an adjustment was not made.

Current status of *in vitro* prediction of clearance

Prediction of clearance is a key component of drug discovery but as this report and many others verify, *in vitro* measured CL_{int} (using human/rat hepatocytes/hepatic microsomes) extrapolated to *in vivo* is not reliably quantitative on a physiologically mechanistic basis; a general problem of some 2-5-fold underprediction has been increasingly recognised for more than a decade (Shibata et al., 2002; Hallifax et al., 2005; Ito and Houston, 2005; Riley et al., 2005; Brown et al., 2007; Stringer et al., 2008). Although this problem can be circumvented by empirical corrections based on available datasets across a range of drugs, as has been reported (Sohlenius-Sternbeck et al., 2012; Yamagata et al., 2016), a high level of uncertainty remains for any individual case. This situation cannot be resolved without thorough understanding of the underlying causes of bias and imprecision which characterise prediction of clearance, as highlighted in a report by Bowman and Benet (2016).

The use of *in vitro* systems has progressed from microsomes to hepatocytes and from the rat pre-clinical species to human over several decades, but recognition of the performance and limitations of component processes, let alone the causes of underprediction, has been slow. In 2010, Hallifax et al. showed that predictions from human hepatocytes tended to be increasingly poorer with increasing *in vivo* CL_{int} ; a less distinct trend was observed for human liver microsomes, indicating that the intact cell

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system was inherently unsuitable for handling highly cleared drugs. Whether this implication was specific to human hepatocytes was not resolvable due to the paucity of reported data for rat at that time. However, the position has changed regarding studies in rat and a viable comparison with human has become realistic and hence a key component of this commentary.

This current assessment shows an average underprediction of *in vivo* CL_{int} by human hepatocytes (n=101) of 4.2-fold, which confirms the bias reported by Hallifax et al. (2010) (n=89). But, in addition, this study shows a similar (4.7-fold) underprediction from rat hepatocytes (n=128) and in both cases, despite the anticipated imprecision, there is a strong trend of clearance-dependency in prediction accuracy. Quantitatively, prediction accuracy ranges from unbiased (approximately 1-fold) for drug clearance below 10 ml/min/kg *in vivo* CL_{int} to about either 20- (rat) or 30-fold (human) underprediction for drug clearance between 1,000 and 10,000 ml/min/kg. Since their initial use in clearance experiments, it has been accepted that human hepatocytes have been inherently more variable and subjected to more preparative and experimental variation than their rat counterparts; an additional caveat has been the possible mismatch between enzyme activity in donors and healthy volunteers and clinical patients, with consequential uncertainties (Iwatsubo et al., 1996; Iwatsubo et al., 1997; Wilson et al., 2003; Ito and Houston, 2005; Riley et al., 2005; Hallifax and Houston, 2009). By contrast, early rat hepatocyte studies indicated a lack of bias (Houston and Carlile, 1997; Ito and Houston, 2004) and consequently, underprediction of CL has been considered specific to the human situation. Now, using accumulated data from over a decade, we have shown that this is not the case and that clearance-dependent prediction bias is effectively species independent, implying that the methodology for determining CL_{int} in hepatocytes is inadequate specifically for moderate to highly cleared drugs.

In microsomes, the average underprediction of *in vivo* CL_{int} was less than that in hepatocytes for both species, at 2.3-fold for rat (n=71) and 2.8-fold for human (n=83), although the proportion of predictions within 2-fold was similar to hepatocytes. However, as for hepatocytes, there was a clear trend of clearance-dependency in prediction accuracy with a similar exponential slope of increasing underprediction with *in vivo* CL_{int}. Both of these findings – extent of average underprediction and clearance-dependency in microsomes - were unexpected. Previously, underprediction from microsomes has been reported as greater than from hepatocytes and seemingly explained by

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absence of additional potentially critical clearance pathways (Engtrakul et al., 2005; Riley et al., 2005; Hallifax et al., 2010). Based on the present, most extensive study to date, it must be considered that the negative consequences of selectivity of enzyme pathways in microsomes is offset by other factors such as removal of rate-limiting access of drug to metabolic enzymes. Furthermore, the BDDCS analysis indicates that the trends in bias were apparently independent of the importance of clearance mechanism. Clearance-dependent prediction bias of a similar magnitude by both cellular and subcellular systems suggests influential factors beyond the above.

Aside from experimental limitations, potential problems within the physiological scaling methodology remain pertinent to this analysis. Here, as in most studies, predictions used the well-stirred liver model because the parallel tube liver model (at the other extreme of drug hepatic dispersion) offered only marginal reduction in bias and precision (not shown), consolidating previous conclusions (Hallifax et al., 2010). The discussion is necessarily limited to conventional non-permeability-limited models of hepatic clearance for two main reasons: the historical predominance of drugs not limited by permeability in their hepatic uptake and the lack of distinction of non-metabolic rate-limiting processes within the available *in vitro* data. Extended liver clearance models may be appropriate for an increasing number of drugs due to reliance on uptake transport and, indeed, lack of *in vitro* distinction of such processes has been suggested as a reason for underprediction of clearance (Chiba et al., 2009; Patilea-Vrana and Unadkat, 2016; Korzekwa and Nagar, 2017). This report, however, attempts to focus on more general prediction trends. Beyond liver model selection, binding of drug to plasma protein is another important physiological factor in prediction methodology that has been re-appraised recently. The f_{u_b} is potentially highly influential in determining *in vivo* CL_{int} from clearance, especially where f_{u_b} is low (<0.05); the extent of binding within blood, or at least to plasma proteins, has been reported to correlate with underprediction (Soars et al., 2007b; Ring et al., 2011). There has been a perception that the f_{u_p} is difficult to measure at or below about 0.05 and this may be seen as a source of imprecision if not also bias. Although Riccardi et al. (2015) demonstrated that such low levels can be measured accurately with sufficiently controlled experiments, historical data might still be questioned. In addition, it has been shown that historical data might be erroneous where assay pH was not adequately controlled (Kochansky et al., 2008). This would appear to affect the f_{u_p} of basic compounds more than others, although basic drugs show no bias in prediction relative to acids and neutrals. There is some lack of agreement in the literature on whether extensive binding is related to

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poor prediction of CL; Hallifax and Houston (2012) demonstrated a lack of relation between binding and prediction, based on analysis within the database reported in 2010, in contrast to earlier reports (Chiba et al., 2009). More recently though, Bowman and Benet (2016) found a tendency for drugs categorised as highly bound to have greater prediction bias than lesser bound drugs, particularly for BDDCS Class 2, although their analysis used sub-groups of relatively small numbers. The present study, examining trends among the entire datasets for these most recent databases (human hepatocytes and microsomes), appears not to support substantial involvement of the degree of binding to plasma proteins. Alternatives to fraction unbound ie. mechanistic involvement of bound drug in uptake (Poulin et al., 2012), has been discussed elsewhere (Hallifax and Houston, 2012) and is beyond the scope of this report.

Preparative experimental steps, such as cryopreservation of human hepatocytes, have been seen as potentially detrimental, but within the human hepatocyte dataset in this study, predictions using cryopreserved hepatocytes were not biased compared to predictions from freshly isolated hepatocytes - focussing attention on downstream experimental procedures. This consolidates previous reports by Diener et al. (1995), Li et al. (1999), Hewitt et al. (2000), Lau et al. (2002), Naritomi et al. (2003), McGinnity et al. (2004), Blanchard et al. (2005) and Floby et al. (2009). For hepatocyte assays, false clearance prediction of drugs which rely on uptake transport might be expected either due to unrepresentative transporter activity *in vitro* (Soars et al., 2007a) or absolute loss of activity due to instability *in vitro*. In the datasets presented here, the impact of such substrates on the trend in prediction bias appears to be limited; the majority of drugs included are considered to be cleared predominantly by metabolic (particularly CYP) enzymes without rate limitation by uptake transport. Those compounds considered liable to transport dependence largely followed the same trend which was similar in magnitude between hepatocytes and microsomes (for both human and rat). Predictions for drugs identified as UGT substrates within the hepatocyte dataset were not clearly resolved from others again indicating no particular hepatocyte bias over microsomes for these pathways.

Future prospects to refine *in vitro* prediction of clearance

A number of factors can be speculated to cause the clearance-dependent underprediction now established in both human and rat hepatocytes and microsomes. The increase in prediction bias seen

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with increasing *in vivo* CL_{int} has been previously speculated as due to an increasing incidence of permeation rate limitation among high-turnover drugs (Hallifax et al., 2010). This would be supported by the similar trend in the present study which involves many drugs not limited by active uptake. However, Hallifax et al. (2012) showed no discernible relationship between prediction accuracy and either of several independent measures of passive permeability for a wide range of drugs. Huang et al. (2010) showed that among a number of highly permeable proprietary compounds, those which were also efflux substrates gave greater underprediction. Umehara and Camenisch (2012) suggested using a combination of hepatocytes and microsome assays to distinguish where clearance was rate limited by permeation. While the phenomenon of permeation rate limitation to hepatic clearance *in vitro* may nevertheless occur, the present analysis provides no definite evidence of a permeation rate limitation and, indeed, the similar trend in underprediction with increasing clearance between hepatocytes and microsomes suggests involvement of an alternative, common explanation.

Cofactor depletion has been suggested as a possible cause of clearance-dependent underprediction when using hepatocytes with high-turnover drugs (Swales et al., 1996; Swales and Utesch, 1998; Steinberg et al., 1999; Hengstler et al., 2000; Hewitt et al., 2000; Hewitt and Utesch, 2004; Hallifax et al., 2010; Foster et al., 2011). But this would not explain the similar trends in underprediction between hepatocytes and microsomes without considerable co-incidence given the different media used (cell culture medium vs. NADPH or regenerating system). Cofactor depletion has been a suggested consequence of cryopreservation and human hepatocytes (often cryopreserved) tend to be used with lower viability (typically not exceeding 80-90% in the studies included here, where reported) than rat hepatocytes, implying greater potential loss of cofactors in these preparations (potentially reflected in cells deemed viable or not). But overall, the lack of any prediction bias among numerous studies for cells prepared this way does not support a significant role for cofactor loss associated with cryopreservation.

For typical drug depletion assays, sub-optimal substrate concentration would be a source of error (imprecision) among predictions and, if there was a tendency to use concentrations approaching or greater than K_M values for key pathways, this might manifest as a source of bias. In a review by Klopff and Worboys (2010), the widely practiced experimental expediency of a fixed substrate concentration of the order of 1 μM for all drugs screened for metabolic stability was highlighted. For some drugs, this

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practice might exclude otherwise influential high affinity, low capacity enzyme sites with resultant poor predictions. Highly metabolically cleared drugs are often substrates of the CYP- 2D and 3A families for which K_M values at around or below 1 μM are not uncommon (Lewis and Ito, 2010); such cases, if ignored, could lead to clearance dependent prediction.

The phenomenon of the unstirred water layer (UWL) has been extensively discussed with respect to intestinal permeability *in vitro* systems (Karlsson and Artursson, 1991; Naruhashi et al., 2003; Avdeef et al., 2004; Korjamo et al., 2009) but has been little discussed with regard to metabolic clearance methodology, possibly due to segregation of drug discovery functions within companies. However, the UWL should be considered for hepatocyte assays at least, considering the present problem with high turnover drugs. Diffusion through the UWL might be rate limiting for such compounds, although, again, a degree of coincidence between the external environment of the whole cell and that of its microsomal fraction would be implied.

Ultimately, the *in vitro* causes of underprediction are likely to be multi-factorial. Further investigation into possible experimental causes of underprediction of clearance – and its clearance-dependence – is highly desirable in the light of the findings of this report. As the trend towards larger molecules with multiple charges and increasing involvement of dispositional transporters continues, more variable factors are added to the situation. While a broader view of the role of transporters and their interrelationship with enzymes will be important, detailed and systematic investigation needs to be focussed on certain other fundamental factors such as passive permeation mechanism, integrity of membrane and unstirred water barriers and their relative impact on rates. Other basic assay parameters such as substrate concentration dependence in CL_{int} also need to be considered. Some of these potentially instrumental phenomena have recently been investigated in our laboratory and the findings will be reported in due course.

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Author contributions

Participated in research design: Wood, Hallifax and Houston.

Performed data analysis: Wood and Hallifax.

Wrote or contributed to the writing of the manuscript: Wood, Hallifax and Houston.

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References

- Akabane T, Gerst N, Masters JN, and Tamura K (2012a) A quantitative approach to hepatic clearance prediction of metabolism by aldehyde oxidase using custom pooled hepatocytes. *Xenobiotica* 42:863-871.
- Akabane T, Gerst N, Naritomi Y, Masters JN, and Tamura K (2012b) A practical and direct comparison of intrinsic metabolic clearance of several non-CYP enzyme substrates in freshly isolated and cryopreserved hepatocytes. *Drug Metab Pharmacokinet* 27:181-191.
- Avdeef A, Nielsen PE, and Tsinman O (2004) PAMPA - a drug absorption in vitro model 11. Matching the in vivo unstirred water layer thickness by individual-well stirring in microtitre plates. *Eur J Pharm Sci* 22:365-374.
- Bayliss MK, Bell JA, Jenner WN, Park GR, and Wilson K (1999) Utility of hepatocytes to model species differences in the metabolism of loxidine and to predict pharmacokinetic parameters in rat, dog and man. *Xenobiotica* 29:253-268.
- Benet LZ, Broccatelli F, and Oprea TI (2011) BDDCS applied to over 900 drugs. *The AAPS journal* 13:519-547.
- Blanchard N, Alexandre E, Abadie C, Lave T, Heyd B, Mantion G, Jaeck D, Richert L, and Coassolo P (2005) Comparison of clearance predictions using primary cultures and suspensions of human hepatocytes. *Xenobiotica* 35:1-15.
- Bowman CM and Benet LZ (2016) Hepatic Clearance Predictions from In Vitro-In Vivo Extrapolation and the Biopharmaceutics Drug Disposition Classification System. *Drug Metab Dispos* 44:1731-1735.
- Brown HS, Griffin M, and Houston JB (2007) Evaluation of cryopreserved human hepatocytes as an alternative in vitro system to microsomes for the prediction of metabolic clearance. *Drug Metab Dispos* 35:293-301.
- Chan TS, Yu HB, Moore A, Khetani SR, and Tweedie D (2013) Meeting the challenge of predicting hepatic clearance of compounds slowly metabolized by cytochrome P450 using a novel hepatocyte model, HepatoPac. *Drug Metab Dispos* 41:2024-2032.

DMD # 77040

- Chiba M, Ishii Y and Sugiyama Y (2009) Prediction of hepatic clearance in human from in vitro data for successful drug development. *AAPS J* 11: 262-276.
- Cubitt HE, Houston JB, and Galetin A (2009) Relative importance of intestinal and hepatic glucuronidation-impact on the prediction of drug clearance. *Pharm Res* 26:1073-1083.
- Davies B and Morris T (1993) Physiological-parameters in laboratory-animals and humans. *Pharm Res* 10:1093-1095.
- De Buck SS, Sinha VK, Fenu LA, Nijsen MJ, Mackie CE, and Gilissen RAHJ (2007) Prediction of human pharmacokinetics using physiologically based modeling: A retrospective analysis of 26 clinically tested drugs. *Drug Metab Dispos* 35: 1766-1780.
- Diener B, Abdellatif H, Arand M, and Oesch F (1995) Xenobiotic-metabolizing enzyme-activities and viability are well preserved in EDTA-isolated rat-liver parenchymal-cells after cryopreservation. *Tox App Pharmacol* 130:1161-1166.
- Engtrakul JJ, Foti RS, Strelevitz TJ, and Fisher MB (2005) Altered AZT (3 '-azido-3 '-deoxythymidine) glucuronidation kinetics in liver microsomes as an explanation for underprediction of in vivo clearance: comparison to heptacytes and effect of incubation environment. *Drug Metab Dispos* 33: 1621-1627.
- Floby E, Johansson J, Hoogstraate J, Hewitt NJ, Hill J, and Sohlenius-Sternbeck AK (2009) Comparison of intrinsic metabolic clearance in fresh and cryopreserved human hepatocytes. *Xenobiotica* 39:656-662.
- Foster JA, Houston JB, and Hallifax D (2011) 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* 41:124-136.
- Gertz M, Harrison A, Houston JB, and Galetin A (2010) Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos* 38:1147-1158.
- Hakooz N, Ito K, Rawden H, Gill H, Lemmers L, Boobis AR, Edwards RJ, Carlile DJ, Lake BG, and Houston JB (2006) Determination of a human hepatic microsomal scaling factor for predicting in vivo drug clearance. *Pharm Res* 23:533-539.
- Hallifax D, Foster JA, and Houston JB (2010) Prediction of human metabolic clearance from in vitro systems: Retrospective analysis and prospective view. *Pharm Res* 27:2150-2161.

DMD # 77040

- Hallifax D, Galetin A, and Houston JB (2008) Prediction of metabolic clearance using fresh human hepatocytes: Comparison with cryopreserved hepatocytes and hepatic microsomes for five benzodiazepines. *Xenobiotica* 38:353-367.
- Hallifax D and Houston JB (2006) Binding of drugs to hepatic microsomes: Comment and assessment of current prediction methodology with recommendation for improvement. *Drug Metab Dispos* 34:724-726.
- Hallifax D and Houston JB (2009) Methodological uncertainty in quantitative prediction of human hepatic clearance from in vitro experimental systems. *Curr Drug Metab* 10:307-321.
- Hallifax D and Houston JB (2012) Evaluation of hepatic clearance prediction using in vitro data: Emphasis on fraction unbound in plasma and drug ionisation using a database of 107 drugs. *J Pharm Sci* 101:2645-2652.
- Hallifax D, Rawden HC, Hakooz N, and Houston JB (2005) Prediction of metabolic clearance using cryopreserved human hepatocytes: Kinetic characteristics for five benzodiazepines. *Drug Metab Dispos* 33:1852-1858.
- Hallifax D, Turlizzi E, Zanelli U, and Houston JB (2012) Clearance-dependent underprediction of in vivo intrinsic clearance from human hepatocytes: Comparison with permeabilities from artificial membrane (PAMPA) assay, in silico and caco-2 assay, for 65 drugs. *Eur J Pharm Sci* 45:570-574.
- Hengstler JG, Utesch D, Steinberg P, Platt K, Diener B, Ringel M, Swales N, Fischer T, Biefang K, Gerl M, Bottger T, and Oesch F (2000) Cryopreserved primary hepatocytes as a constantly available in vitro model for the evaluation of human and animal drug metabolism and enzyme induction. *Drug Metab Revs* 32:81-118.
- Hewitt NJ, Fischer T, Zuehlke U, Oesch F, and Utesch D (2000) Metabolic activity of fresh and cryopreserved cynomolgus monkey (*Macaca fascicularis*) hepatocytes. *Xenobiotica* 30:665-681.
- Hewitt NJ and Utesch D (2004) Cryopreserved rat, dog and monkey hepatocytes: measurement of drug metabolizing enzymes in suspensions and cultures. *Hum Exp Toxicol* 23:307-316.
- Hosey CM, Chan R, and Benet LZ (2016) BDDCS Predictions, Self-Correcting Aspects of BDDCS Assignments, BDDCS Assignment Corrections, and Classification for more than 175 Additional Drugs. *The AAPS journal* 18:251-260.

DMD # 77040

- Houston JB (1994) Utility of in-vitro drug-metabolism data in predicting in-vivo metabolic-clearance. *Bioch Pharmacol* 47:1469-1479.
- Houston JB and Carlile DJ (1997) Prediction of hepatic clearance from microsomes, hepatocytes, and liver slices. *Drug Metab Rev* 29:891-922.
- Huang L, Berry L, Ganga S, Janosky B, Chen A, Roberts J, Colletti AE, and Lin M-HJ (2010) Relationship between passive permeability, efflux, and predictability of clearance from in vitro metabolic intrinsic clearance. *Drug Metab Dispos* 38:223-231.
- Ito K and Houston JB (2004) Comparison of the use of liver models for predicting drug clearance using in vitro kinetic data from hepatic microsomes and isolated hepatocytes. *Pharm Res* 21:785-792.
- Ito K and Houston JB (2005) Prediction of human drug clearance from in vitro and preclinical data using physiologically based and empirical approaches. *Pharm Res* 22:103-112.
- Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, Ishizaki T, Green CE, Tyson CA, and Sugiyama Y (1997) Prediction of in vivo drug metabolism in the human liver from in vitro metabolism data. *Pharmacol Ther* 73:147-171.
- Iwatsubo T, Hirota N, Ooie T, Suzuki H, and Sugiyama Y (1996) Prediction of in vivo drug disposition from in vitro data based on physiological pharmacokinetics. *Biopharm Drug Dispos* 17:273-310.
- Jones HM, Chen Y, Gibson C, Heimbach T, Parrott N, Peters SA, Snoeys J, Upreti VV, Zheng M, and Hall SD (2015) Physiologically Based Pharmacokinetic Modeling in Drug Discovery and Development: A Pharmaceutical Industry Perspective. *Clin Pharmacol Ther* 97:247-262.
- Jones HM and Houston JB (2004) Substrate depletion approach for determining in vitro metabolic clearance: Time dependencies in hepatocyte and microsomal incubations. *Drug Metab Dispos* 32:973-982.
- Kaiser G, Ackermann R, Dieterle W, Reimann IW, and Bieck PR (1992) Pharmacokinetics of the antidepressant levoprotiline after intravenous and peroral administration in healthy-volunteers. *Biopharm Drug Dispos* 13:83-93.
- Karlsson J and Artursson P (1991) A Method for the Determination of Cellular Permeability Coefficients and Aqueous Boundary-Layer Thickness in Monolayers of Intestinal Epithelial (Caco-2) Cells Grown in Permeable Filter Chambers. *Int J Pharm* 71:55-64.

DMD # 77040

- Kilford PJ, Gertz M, Houston JB, and Galetin A (2008) Hepatocellular binding of drugs: Correction for unbound fraction in hepatocyte incubations using microsomal binding or drug lipophilicity data. *Drug Metab Dispos* 36:1194-1197.
- Klopf W and Worboys P (2010) Scaling in vivo pharmacokinetics from in vitro metabolic stability data in drug discovery. *Combinatorial Chemistry & High Throughput Screening* 13:159-169.
- Kochansky CJ, McMasters DR, Lu P, Koeplinger KA, Kerr HH, Shou M and Korzekwa KR (2008) Impact of pH on plasma protein binding in equilibrium analysis. *Mol Pharm* 5: 438-448.
- Korjamo T, Heikkinen AT, and Monkkonen J (2009) Analysis of unstirred water layer in in vitro permeability experiments. *J Pharm Sci* 98:4469-4479.
- Korzekwa K and Nagar S (2017) On the nature of physiologically-based pharmacokinetic models-a priori or a posteriori? mechanistic or empirical?. *Pharm Res* 34: 529-534.
- Laethem ME, Lefebvre RA, Belpaire FM, Vanhoe HL, and Bogaert MG (1995) Stereoselective pharmacokinetics of oxprenolol and its glucuronides in humans. *Clin Pharmacol Ther* 57:419-424.
- Lau YY, Sapidou E, Cui XM, White RE, and Cheng KC (2002) Development of a novel in vitro model to predict hepatic clearance using fresh, cryopreserved, and sandwich-cultured hepatocytes. *Drug Metab and Dispos* 30:1446-1454.
- Lewis DFV and Ito Y (2010) Human CYPs involved in drug metabolism: structures, substrates and binding affinities. *Expert Opin Drug Metab Toxicol* 6: 661-674.
- Li AP, Lu C, Brent JA, Pham C, Fackett A, Ruegg CE, and Silber PM (1999) Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug-drug interaction potential. *Chem Biol Interact* 121:17-35.
- McGinnity DF, Soars MG, Urbanowicz RA, and Riley RJ (2004) Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for the prediction of metabolic clearance. *Drug Metab Dispos* 32:1247-1253.
- Miners JO and Mackenzie PI (1991) Drug glucuronidation in humans. *Pharmacol Ther* 51:347-369.
- Naritomi Y, Terashita S, Kagayama A, and Sugiyama Y (2003) Utility of hepatocytes in predicting drug metabolism: Comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab Dispos* 31:580-588.

DMD # 77040

- Naritomi Y, Terashita S, Kimura S, Suzuki A, Kagayama A, and Sugiyama Y (2001) Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans. *Drug Metab Dispos* 29:1316-1324.
- Naruhashi K, Tamai I, Li Q, Sai Y, and Tsujii A (2003) Experimental demonstration of the unstirred water layer effect on drug transport in Caco-2 cells. *J Pharm Sci* 92:1502-1508.
- Obach RS (1999) 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* 27:1350-1359.
- Paixao P, Gouveia LF, and Morais JAG (2010) Prediction of the in vitro intrinsic clearance determined in suspensions of human hepatocytes by using artificial neural networks. *Eur J Pharm Sci* 39:310-321.
- Patilea-Vrana G and Unadkat JD (2016) Transport vs. metabolism: what determines the pharmacokinetics and pharmacodynamics of drugs? Insights from the extended clearance model. *Clin Pharm Ther* 100: 413-418.
- Poulin P and Haddad S (2013) Toward a new paradigm for the efficient in vitro-in vivo extrapolation of metabolic clearance in humans from hepatocyte data. *J Pharm Sci* 102:3239-3251.
- Poulin P, Kenny JR, Hop CECA, and Haddad S (2012) In vitro-in vivo extrapolation of clearance: Modeling hepatic metabolic clearance of highly bound drugs and comparative assessment with existing calculation methods. *J Pharm Sci* 101:838-851.
- Riccardi K, Cawley S, Yates PD, Chang C, Funk C, Niosi M, Lin J and Di L (2015) Plasma Protein Binding of Challenging Compounds. *J Pharm Sci* 104:2627-2636.
- Riley RJ, McGinnity DF, and Austin RP (2005) A unified model for predicting human hepatic, metabolic clearance from in vitro intrinsic clearance data in hepatocytes and microsomes. *Drug Metab Dispos* 33:1304-1311.
- Ring BJ, Chien JY, Adkison KK, Jones HM, Rowland M, Jones RD, Yates JW, Ku MS, Gibson CR, He H, Vuppugalla R, Marathe P, Fischer V, Dutta S, Sinha VK, Bjornsson T, Lave T, and Poulin P (2011) PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: comparative assessment of prediction methods of human clearance. *J Pharm Sci* 100:4090-4110.

DMD # 77040

- Shibata Y, Takahashi H, Chiba M, and Ishii Y (2002) Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: An application of serum incubation method. *Drug Metab Dispos* 30:892-896.
- Soars MG, Burchell B, and Riley RJ (2002) In vitro analysis of human drug glucuronidation and prediction of in vivo metabolic clearance. *J Pharmacol Expl Ther* 301:382-390.
- Soars MG, Grime K, Sproston JL, Webborn PJH, and Riley RJ (2007a) Use of hepatocytes to assess the contribution of hepatic uptake to clearance in vivo. *Drug Metab Dispos* 35:859-865.
- Soars MG, McGinnity DF, Grime K, and Riley RJ (2007b) The pivotal role of hepatocytes in drug discovery. *Chem Biol Interact* 168:2-15.
- Sohlenius-Sternbeck AK, Afzelius L, Prusis P, Neelissen J, Hoogstraate J, Johansson J, Floby E, Bengtsson A, Gissberg O, Sternbeck J, and Petersson C (2010) Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. *Xenobiotica* 40:637-649.
- Sohlenius-Sternbeck AK, Jones C, Ferguson D, Middleton BJ, Projean D, Floby E, Bylund J, and Afzelius L (2012) Practical use of the regression offset approach for the prediction of in vivo intrinsic clearance from hepatocytes. *Xenobiotica* 42:841-853.
- Steinberg P, Fischer T, Kiulies S, Biefang K, Platt KL, Oesch F, Bottger T, Bulitta C, Kempf P, and Hengstler J (1999) Drug metabolizing capacity of cryopreserved human, rat, and mouse liver parenchymal cells in suspension. *Drug Metab Dispos* 27:1415-1422.
- Stringer R (2006) Evaluation of higher-throughput in vitro methods for the reliable prediction of in vivo metabolic clearance in humans, in: *School of Pharmacy and Pharmaceutical Science*, pp 191, University of Manchester.
- Stringer R, Nicklin PL, and Houston JB (2008) Reliability of human cryopreserved hepatocytes and liver microsomes as in vitro systems to predict metabolic clearance. *Xenobiotica* 38:1313-1329.
- Swales NJ, Johnson T, and Caldwell J (1996) Cryopreservation of rat and mouse hepatocytes: 2. Assessment of metabolic capacity using testosterone metabolism. *Drug Metab Dispos* 24:1224-1230.
- Swales NJ and Utesch D (1998) Metabolic activity of fresh and cryopreserved dog hepatocyte suspensions. *Xenobiotica* 28:937-948.

DMD # 77040

Umehara K-I and Camenisch G (2012) Novel in vitro-in vivo extrapolation (IVIVE) method to predict hepatic organ clearance in rat. *Pharm Res* 29: 603-617.

Varma MV, Steyn SJ, Allerton C and El-Kattan AF (2015) Predicting clearance mechanism in drug discovery: extended clearance classification system (ECCS). *Pharm Res* 32: 3785-3802.

Wilson ZE, Rostami-Hodjegan A, Burn JL, Tooley A, Boyle J, Ellis SW, and Tucker GT (2003) Inter-individual variability in levels of human microsomal protein and hepatocellularity per gram of liver. *Br J Clin Pharmacol* 56:433-440.

Yamagata T, Zanelli U, Gallemann D, Perrin D, Dolgos H, and Petersson C (2016) Comparison of methods for the prediction of human clearance from hepatocyte intrinsic clearance for a set of reference compounds and an external evaluation set. *Xenobiotica*: Early Online 1-11.

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Footnote

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Figure legends

Figure 1. Prediction of *in vivo* $CL_{int,u}$ in hepatocytes (A and C) and microsomes (B and D) in human (A and B) and rat (C and D). Dashed lines represent unity and dotted lines a two-fold margin of error. A single data point above predicted $CL_{int,u}$ of 100,000 ml/min/kg in RLM has been omitted for the purpose of comparable graphical representation.

Figure 2. Comparison of predicted *in vivo* $CL_{int,u}$ in fresh and cryopreserved human hepatocytes (drugs common to both preparations only). Dashed lines represent unity and dotted line represents unity.

Figure 3. Relationship between empirical scaling factor and observed $CL_{int,u}$ according to BDDCS classification for hepatocytes (A and C) and microsomes (B and D) in human (A and B) and rat (C and D). Compounds are represented as BDDCS Class 1 (◆), Class 2 (□), Class 3 (▲), Class 4 (+) or unclassified (●). BDDCS assignments for propriety compounds (n= 88, rat hepatocytes; n= 37 rat microsomes) were judged on available physico-chemical information provided with source publications.

Figure 4. Comparison of predicted $CL_{int,u}$ in human hepatocytes with observed $CL_{int,u}$. Drugs subject to glucuronidation are identified as high (≥ 0.75) (●), medium high (0.50-0.75) (+), medium low (0.25-0.50) (▲) and low (<0.25) (■) $f_{m_{UGT}}$; all other compounds are represented as (◇). Dashed line represents unity and dotted lines a two-fold margin of error.

Figure 5. Relationship between empirical scaling factor (ESF) and observed $CL_{int,u}$ according to drug ionic character for human (A) and rat (B) hepatocytes. Compounds are represented as acidic (◆), basic (▲), neutral (□) or unclassified (x).

Figure 6. Relationship between f_{up} , empirical scaling factor (ESF) and BDDCS class in (A) human hepatocytes and (B) HLM. Drugs are represented as BDDCS Class 1 (◆), Class 2 (□) and Class 3 (▲).

DMD # 77040

Table 1. Accuracy and precision of *in vivo* CL_{int,u} predictions in human and rat hepatocytes and liver microsomes as represented by AFE, average fold-underprediction, RMSE and percentage of predictions that fall within-, above- and below two-fold of observed *in vivo* CL_{int,u}; n = number of drugs.

	Human		Rat	
	Hepatocytes	Microsomes	Hepatocytes	Microsomes
n	101	83	128	71
AFE	0.24	0.36	0.21	0.43
Average fold-underprediction	4.2	2.8	4.7	2.3
RMSE	3548	3524	36203	63280
% predictions within two-fold of observed	24	25	20	30
% predictions more than two- fold above observed	7	15	9	14
% predictions more than two- fold below observed	69	60	71	56

DMD # 77040

Table 2. Accuracy and precision of *in vivo* CL_{int,u} predictions in fresh and cryopreserved human hepatocytes as represented by AFE, average fold-underprediction, RMSE and percentage of predictions that fall within-, above- and below two-fold of observed *in vivo* CL_{int,u}. Analyses of the complete dataset and of a reduced dataset of common drugs only are presented; n = number of compounds.

	Complete Dataset		Common Drugs	
	Fresh	Cryopreserved	Fresh	Cryopreserved
n	52	93	43	43
AFE	0.25	0.23	0.25	0.22
Average fold-underprediction	3.9	4.3	4.0	4.5
RMSE	2891	3013	390	395
% predictions within two-fold of observed	29	22	26	26
% predictions more than two-fold above observed	8	6	7	2
% predictions more than two-fold below observed	63	72	67	72

DMD # 77040

Table 3. Average individual empirical scaling factor for predicted $CL_{int,u}$ according to level of observed $CL_{int,u}$ for human and rat hepatocytes and liver microsomes.

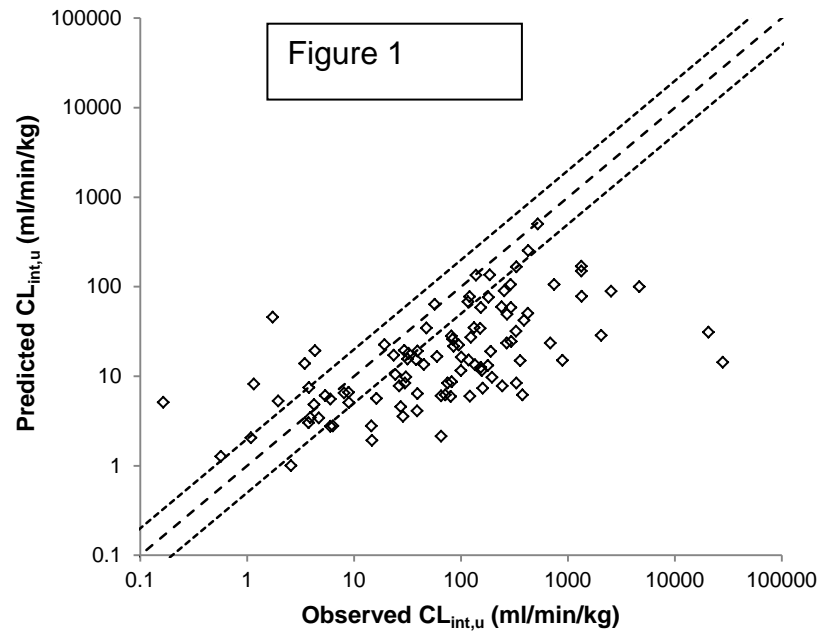
Observed $CL_{int,u}$ (ml/min/kg)	Empirical Scaling Factor (log average [n])			
	Human		Rat	
	Hepatocytes	Microsomes	Hepatocytes	Microsomes
< 10	0.61 [21]	0.70 [17]	0.13 [3]	0.086 [3]
10-100	3.9 [32]	1.8 [20]	1.6 [12]	0.83 [8]
100-1000	7.1 [40]	4.6 [34]	3.2 [67]	1.7 [34]
1000-10000	22 [6]	7.5 [10]	7.2 [37]	2.5 [20]
>10000	1200 [2]	58 [2]	180 [9]	230 [6]

DMD # 77040

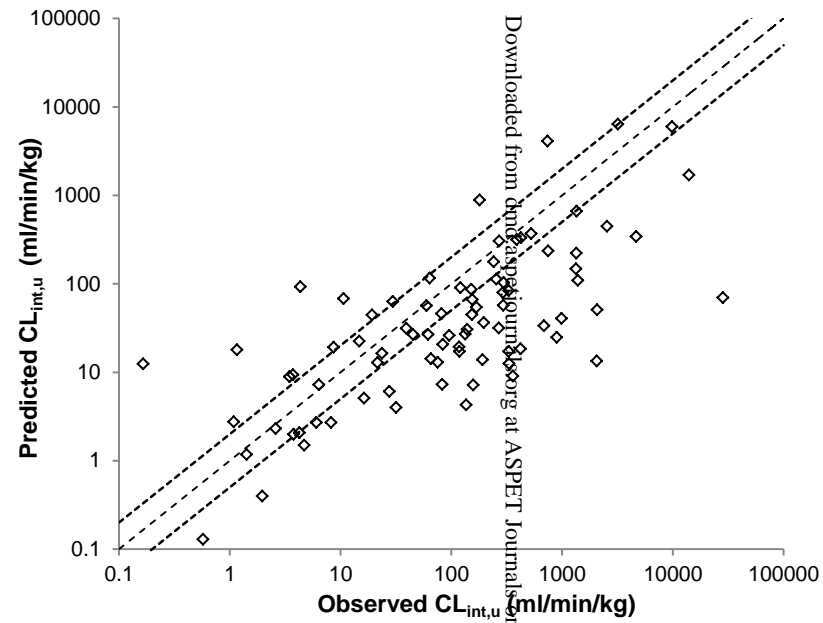
Table 4. Average individual empirical scaling factor for predicted $CL_{int,u}$ according to fu_p and BDDCS classification for human hepatocytes and liver microsomes. Numbers in italic indicate the exclusion of a single compound in this class, numbers in bold indicate exclusion of two compounds from this group.

Category		Empirical Scaling Factor		
		(log average [n])		
		fu_p		
		0.001-0.01	0.01-0.1	0.1-1
Hepatocytes				
	total	6.0 [9]	4.0 [37]	3.3 [53]
BDDCS	Class 1	2.1 [2]	5.8 [13]	3.5 [42]
	Class 2	8.2 [7]	4.1 [23]	13 [4]
	Class 3	[0]	0.032 [1]	0.61 [5]
Microsomes				
	total	5.6 [7]	2.1 [35]	2.6 [39]
BDDCS	Class 1	4.3 [2]	2.7 [13]	3.8 [31]
	Class 2	6.3 [5]	2.3 [20]	1.0 [4]
	Class 3	[0]	0.013 [1]	0.22 [3]

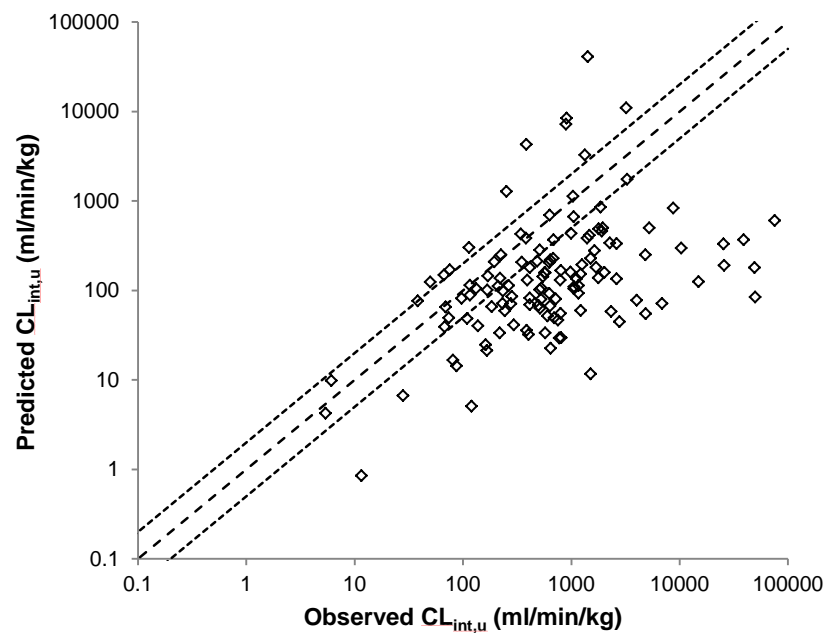
A



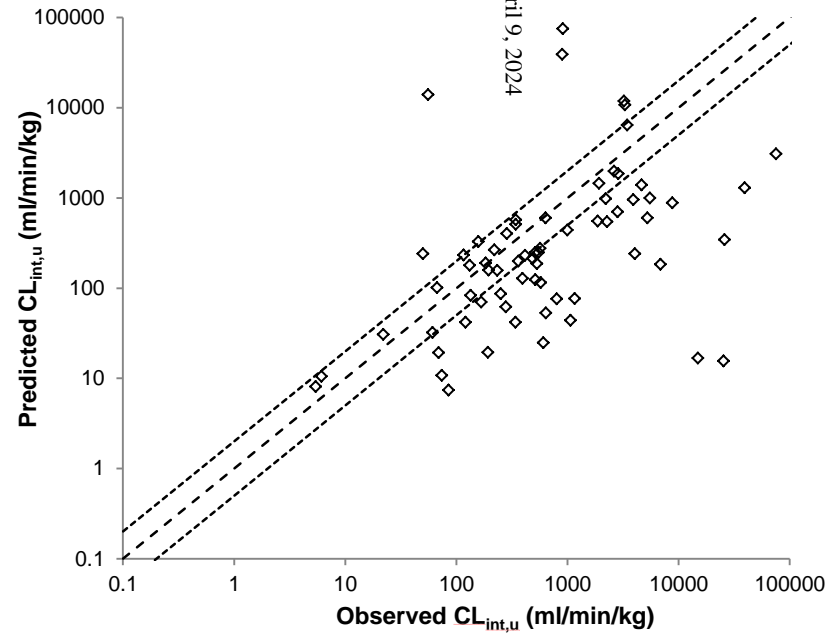
B



C



D



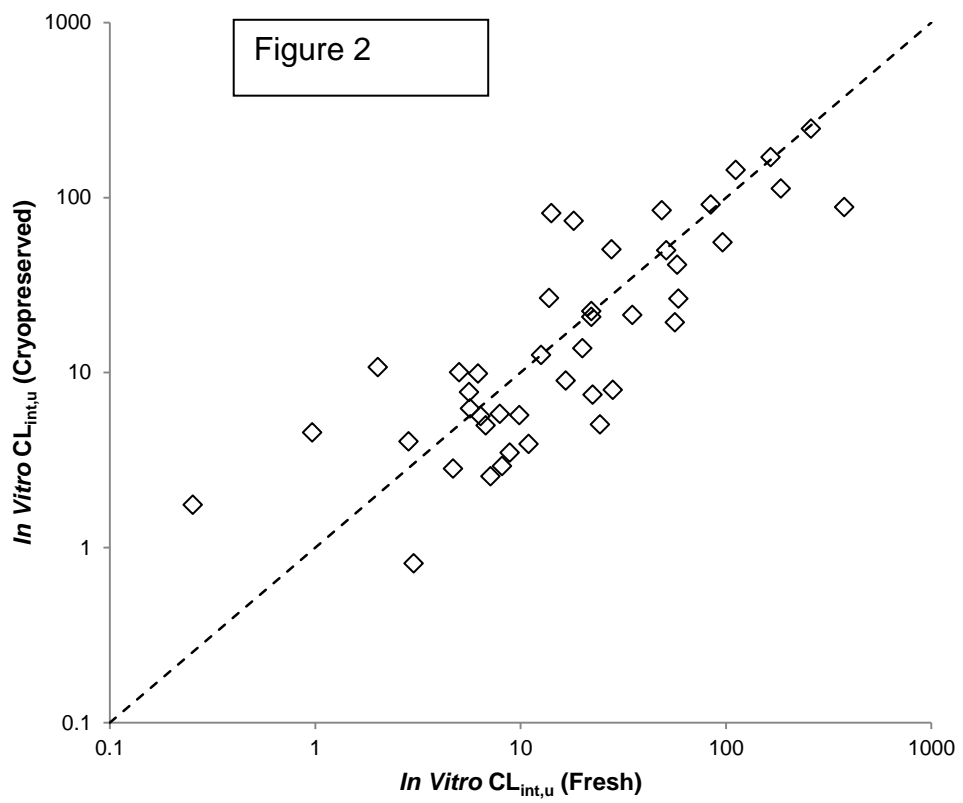
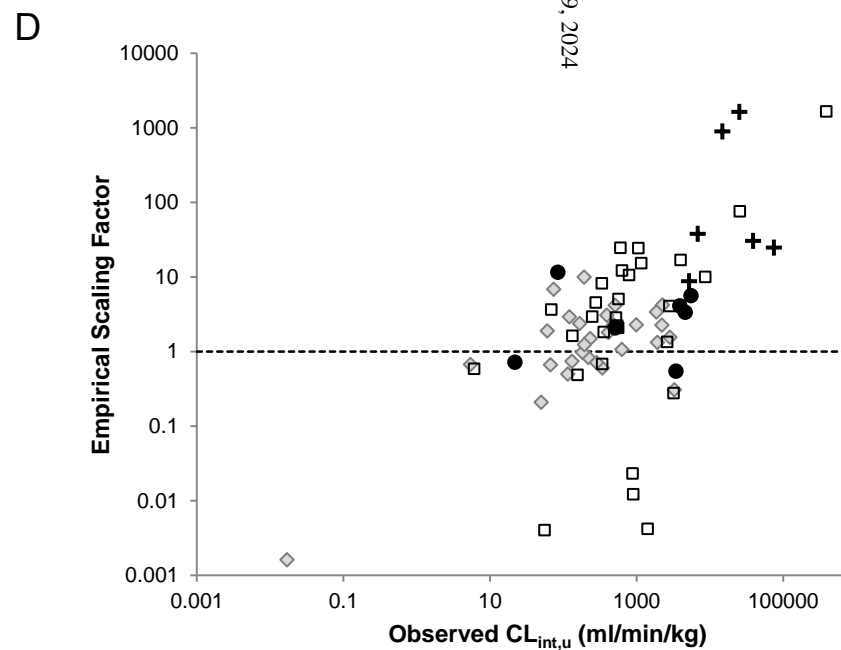
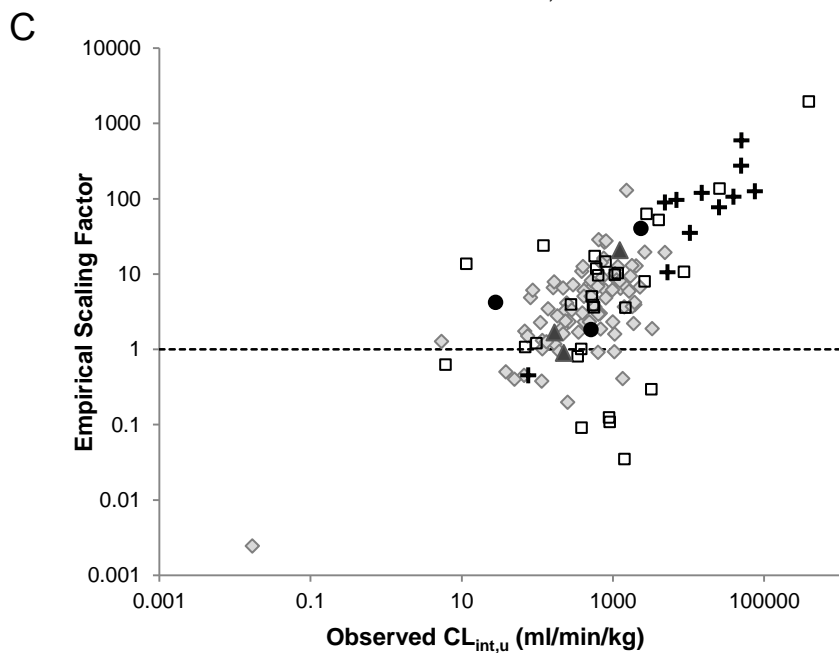
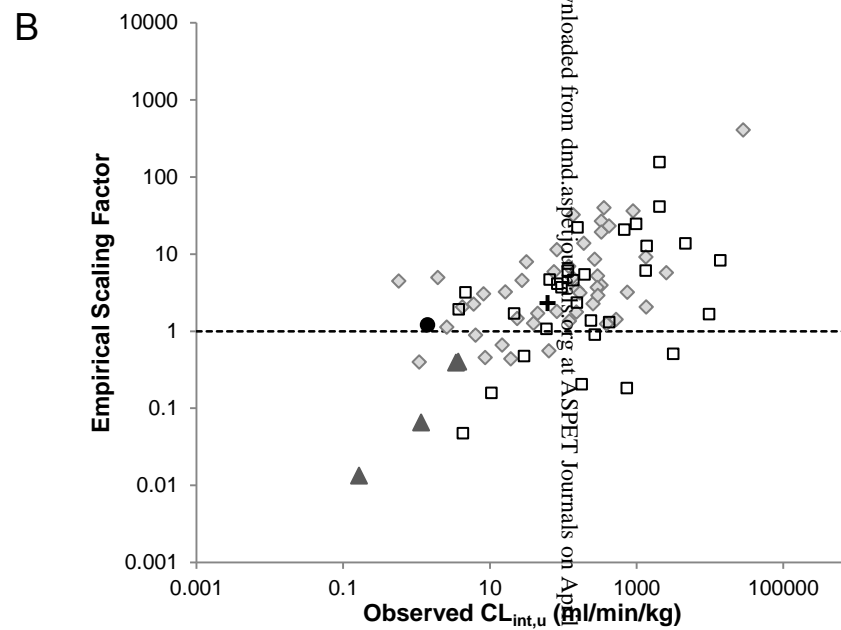
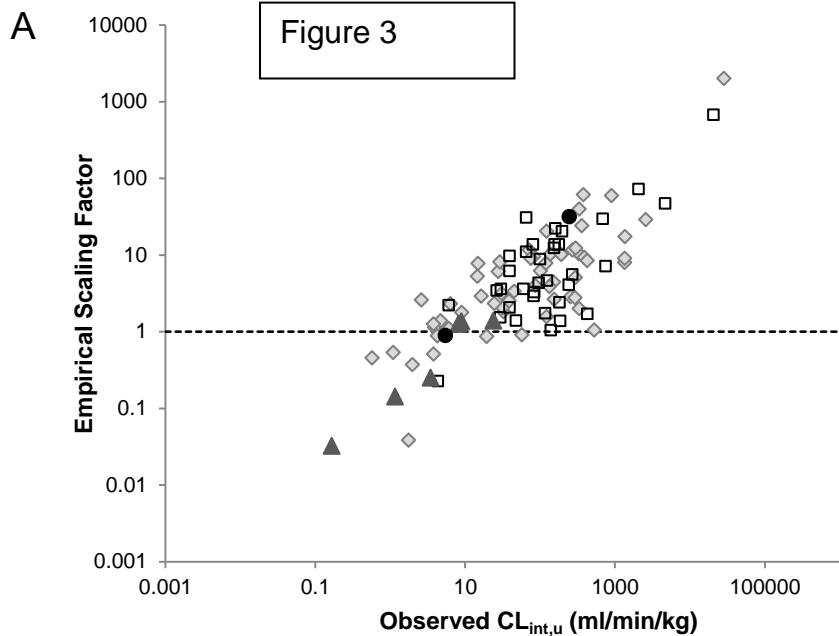
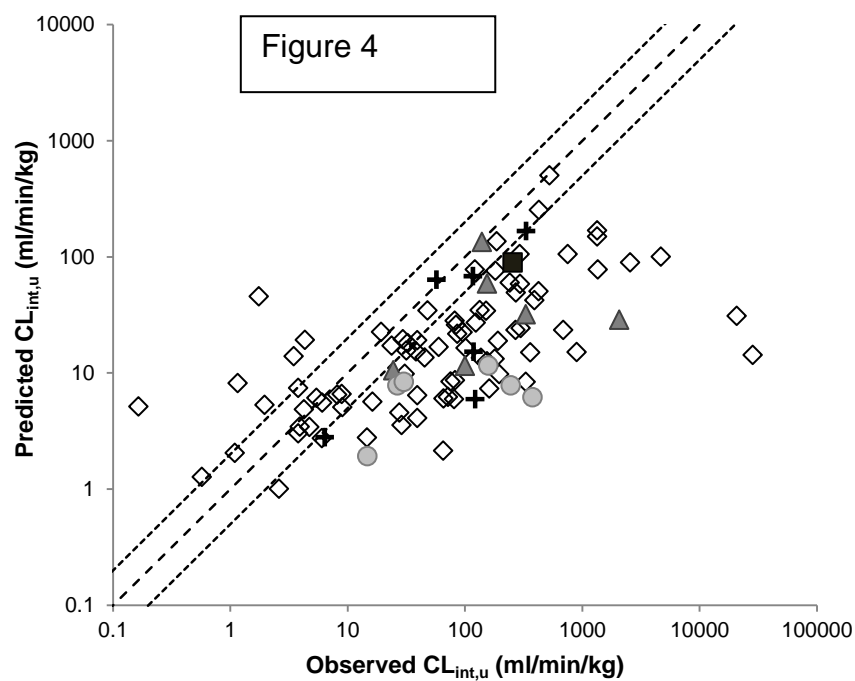
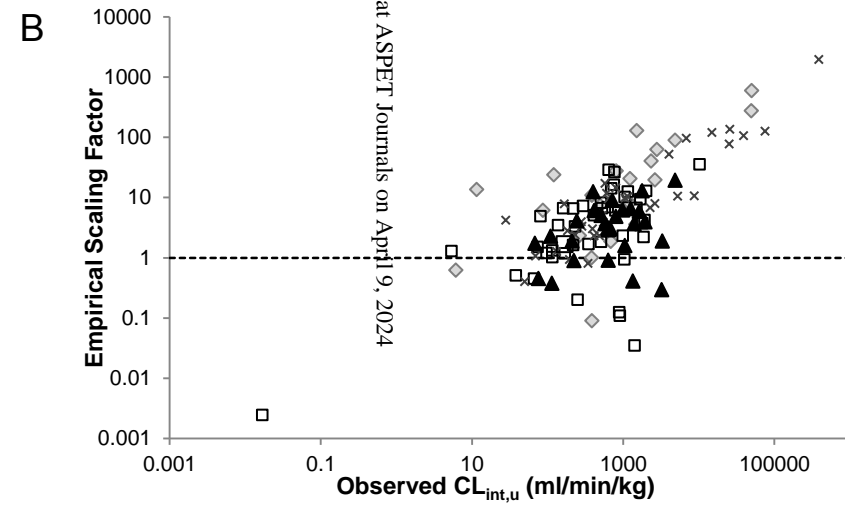
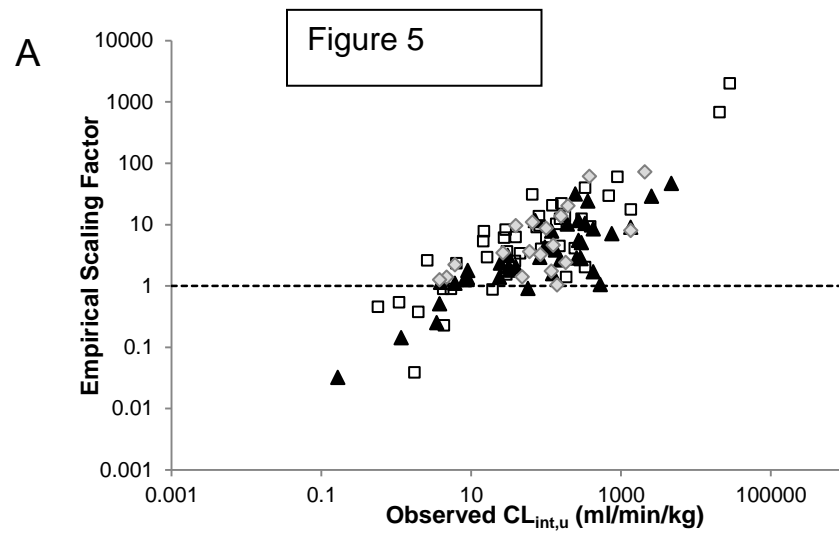
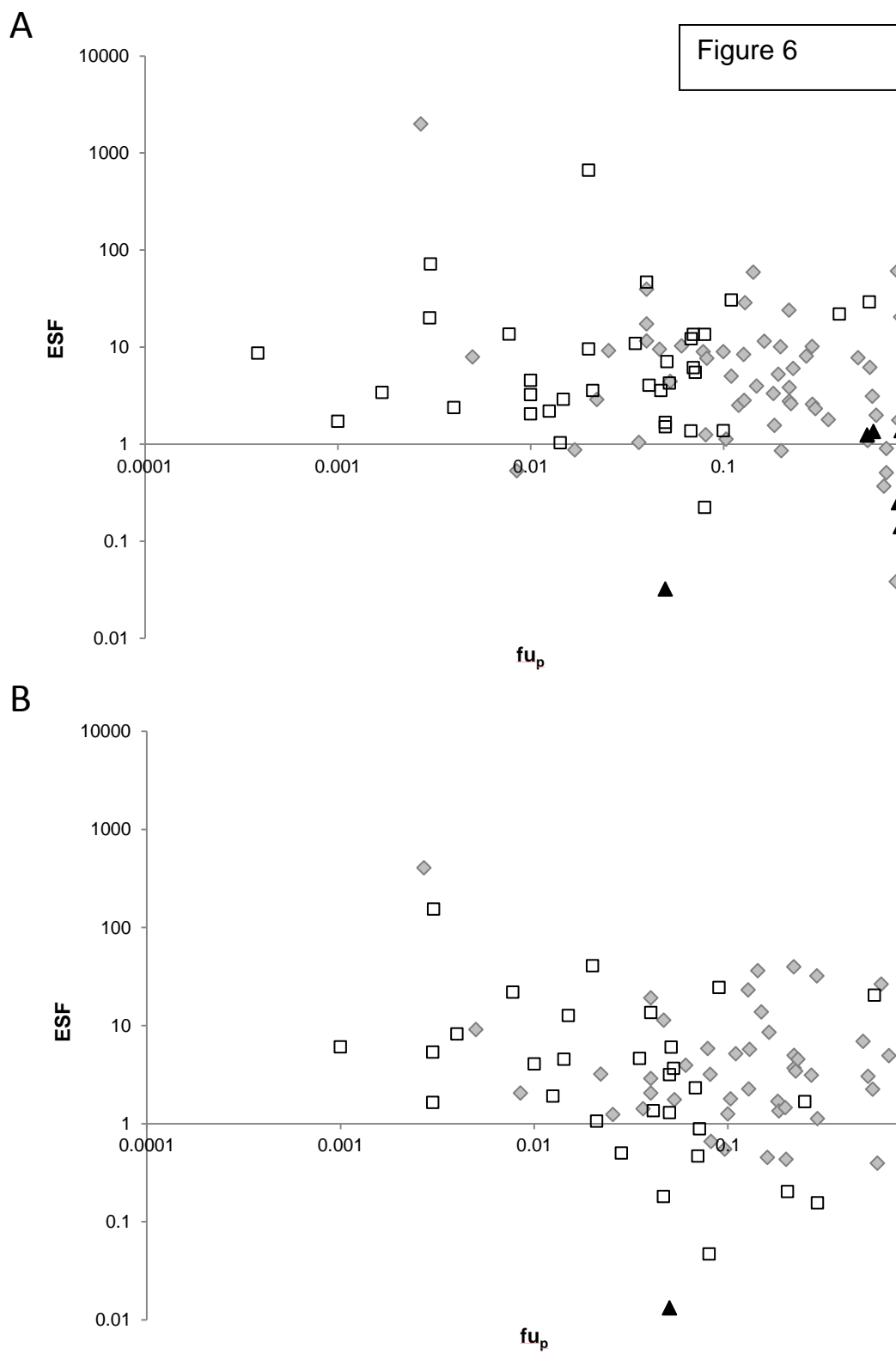


Figure 3









Drug Metabolism and Disposition: Commentary

Clearance prediction methodology needs fundamental improvement: trends common to rat and human hepatocytes/microsomes and implications for experimental methodology

Wood FL, Houston JB and Hallifax D

Supplemental Table 1. Collated literature human *in vivo* and *in vitro* parameter values for drug intrinsic clearance prediction

	In Vivo					In Vitro						
						Hepatocytes				Microsomes		
	n	CL _h (ml/min/kg)	f _{ub}	f _{up}	CL _{int,u} (ml/min/kg)	n	Donors (n)	f _{u_{hep}} *	CL _{int,u} (ml/min/kg)	n	f _{u_{mic}} *	CL _{int,u} (ml/min/kg)
Acebutolol	3	5.4 [1-3]	0.81 [1, 3]	0.81	9.0	1	I(4)	0.91	5.1 [5]			
Acetaminophen	4	4.4 [6-9]	0.88 [6, 10]	0.88	6.4	3	I(5-7), P(2-5), P(5)	0.93 [7]	2.8 [7, 11, 12]	1	0.92	7.2 [11]
Alfentanil	5	6.9 [4, 13-16]	0.16 [4, 13- 15]	0.096	64					1	a	116 [14]
Alprazolam	4	0.78 [4, 6, 14, 18]	0.31 [4, 6, 14]	0.29	2.6	2	P(5), P(5)	0.93	1.0 [20, 21]	1	0.91 [19]	2.3 [19]
Alprenolol	2	14 [6, 22]	0.27 [6]	0.22	154	1	P(6)	0.85	59 [6]	1	0.79	45 [6]
Amitriptyline	4	9.6 [4, 6, 23, 24]	0.054 [4, 6, 23, 25]	0.061	330	1	P(2-5)	0.15	32 [11]	2	0.10	84 [11, 19]
Amobarbital	2	0.37 [26, 27]	0.26 [27]	0.39	1.4					1	0.97 [19]	1.2 [19]
Antipyrine	2	0.55 [6, 28]	0.99 [6, 28]	0.99	0.57	1	P(5+)	0.95	1.3 [29]	1	0.93	0.13 [30]
Atenolol	3	0.13 [4, 6, 31]	0.79 [4, 6]	0.05	0.16	1	P(2-5)	0.95	5.1 [11]	1	0.93	12 [11]
Atorvastatin	1	16 [14]	0.036 [14]	0.02	2071					1	a	51 [14]
Betaxolol	2	2.9 [6, 32]	0.56 [6]	0.56	6.1	2	I(6), P(2)	0.79	5.5 [5, 33]	1	0.70	2.7 [11]
Bosentan	2	3.5 [34, 35]	0.064 [34]	0.035	66	1	I(2), I(3)	0.81	6.0 [36]	1	0.73	14 [11]
Bupivacaine	3	7.1 [4, 6, 37]	0.071 [4, 6, 37]	0.053	152	1	P(6)	0.55	34 [6]	1	0.43	87 [6]
Buprenorphine	3	15.0 [4, 6, 38]	0.040 [4, 6, 38]	0.040	1354	1	P(6)	0.35	78 [6]	2	0.10 [39]	661 [6, 39]
Caffeine	2	1.2 [29, 40]	0.65 [40, 41]	0.68	2.0	3	I(1), P(5), P(5+)	0.96	5.3 [5, 12, 29]	1	0.93	0.40 [42]
Carbamazepine	1	1.6 [41]	0.31 [41]	0.26	5.4	1	I(1)	0.84	6.1 [5]			
Carvedilol	3	10 [4, 43, 44]	0.050 [4,	0.050	427	4	I(5),	0.38	253 [5, 11,	1	0.28	330 [11]

	<i>In Vivo</i>					<i>In Vitro</i>						
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes				Microsomes		
						n	Donors (n)	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
			43]				P(2-5), P(5), P(5)		12, 43]			
Chlorpheniramine	2	1.6 [6, 45]	0.44 [6, 45]	0.70	3.8	2	I(4), P(2-5)	0.66	7.4 [5, 11]			
Chlorpromazine	3	11 [6, 40, 46]	0.043 [6, 12, 40, 41]	0.037	525	4	P(2-5), P(5), P(6), P(5+)	0.068	501 [6, 11, 12, 29]	3	0.11 [19]	371 [6, 11, 19]
Cimetidine	4	2.6 [4, 43, 47, 48]	0.87 [4, 43]	0.81	3.5	3	I(9), P(2-5), P(5)	0.95	14 [5, 11, 43]	1	0.92	8.9 [11]
Clozapine	2	4.1 [4, 49]	0.054 [4, 49]	0.053	96	4	I(1), P(2-5), P(5), P(2)	0.70	22 [5, 11, 12, 33]	2	0.59	26 [11, 19]
Codeine	1	13.8 [50]	0.73 [50]	0.70	57	1	I(2)	0.93	63 [5]			
Cyclosporine A	3	5.4 [14, 43, 52]	0.047 [14, 43, 52]	0.068	154	2	I(8), P(5)	0.77	13 [5, 43]	1	a	66 [14]
Desipramine	1	11 [53]	0.21 [53]	0.18	121	5	I(1), P(6), P(2-5), P(5+), P(5+)	0.16	77 [5, 6, 11, 12, 29]	2	0.10	90 [11, 19]
Dexamethasone	2	5.7 [54, 55]	0.29 [55]	0.23	28	1	P(2-5)	0.90	4.6 [11]	2	0.85	6.1 [11, 19]
Diazepam	5	0.57 [19, 29, 56-58]	0.036 [19, 29, 56, 58]	0.022	16	8	I(1), I(5-7), P(2-5), P(5), P(5), P(5), P(5+), I(7)	0.54 [7]	5.6 [5, 7, 11, 12, 20, 21, 29, 59]	2	0.66	5.1 [11, 19]
Diclofenac	1	7.7 [60]	0.0091 [60]	0.0050	1342	10	I(5), P(6), P(2-5), P(5), I(3), I(7), I(3), I(11)	0.94	168 [5, 6, 11, 12, 40, 59, 61, 62]	4	0.84 [39]	147 [6, 11, 19, 39]
Diflusal	2	0.14 [43, 63]	0.0053 [43]	0.0017	27	2	P(5), P(5), I(88), I(5), P(6),	0.95	7.8 [12, 43]			
Diltiazem	4	12 [4, 29, 64, 65]	0.22 [4, 19]	0.22	134	9	I(5-7), P(2-5), P(5), P(5+), P(2)	0.360 [7]	35 [5-7, 11, 12, 29, 33]	3	0.72	27 [6, 11, 19]
Diphenhydramine	3	18 [66-68]	0.34 [66]	0.22	360	2	I(1), P(2-5)	0.69	15 [5, 11]	2	0.58	11 [11, 19]
Domperidone	2	12 [1, 13]	0.097 [1, 13]	0.072	271	1	P(6)	0.48	49 [6]	1	0.37	306 [6]
Felodipine	2	16 [14, 69]	0.0057 [14]	0.0040	14012					1	a	1703 [14]

	In Vivo					In Vitro						
						Hepatocytes				Microsomes		
	n	CL _h (ml/min/kg)	f _{u_b}	f _{u_p}	CL _{int,u} (ml/min/kg)	n	Donors (n)	f _{u_{hep}} *	CL _{int,u} (ml/min/kg)	n	f _{u_{mic}} *	CL _{int,u} (ml/min/kg)
Fenoprofen	1	7.3 [43]	0.0055 [43]	0.0030	2063	4	P(6), P(2-5), P(5), P(5)	0.95	29 [6, 11, 12, 43]	1	0.92	13 [6]
Flumazenil	4	15 [4, 70-72]	0.60 [4, 70-72]	0.58	101	1	P(6)	0.94	16 [6]			
Flunitrazepam	2	3.1 [1, 40]	0.25 [1, 40]	0.19	15	2	P(5), P(5)	0.89	2.8 [20, 21]			
Fluphenazine	1	0.58 [4]	0.14 [4]	0.080	4.3	1	P(6)	0.63	19 [6]	1	0.51	92 [6]
Furosemide	4	1.2 [4, 40, 73, 74]	0.020 [4, 11, 40]	0.013	62					1	0.92	27 [11]
Gemfibrozil	2	3.1 [40, 43]	0.026 [6, 40, 43]	0.014	139	10	I(5), P(6), P(2-5), P(5), P(5), I(3), I(11)	0.93	134 [5, 6, 11, 12, 43, 61, 62]	3	0.91 [39]	31 [6, 11, 39]
Glimepiride	2	1.0 [1, 4]	0.0055 [1, 4]	0.0030	196	1	P(6)	0.90	9.8 [6]	1	0.84	37 [6]
Glipizide	2	0.75 [1, 43]	0.020 [1, 43]	0.011	39	3	P(2-5), P(5), P(5)	0.96	4.1 [11, 12, 43]			
Glyburide	2	2.0 [4, 75]	0.038 [76]	0.021	60	1	P(6)	0.93	17 [6]	1	0.89	57 [6]
Granisetron	2	11 [43, 77]	0.70 [43]	0.35	33	4	I(4), P(2-5), P(5), P(5)	0.87	18 [5, 11, 12, 43]			
Haloperidol	3	9.6 [78-80]	0.10 [78]	0.080	179	2	I(3)	0.56	13 [61]			
Hexobarbital	1	3.6 [19]	0.53 [19]	0.53	8.2					1	0.96 [19]	2.7 [19]
Hydrocortisone	2	3.3 [81, 82]	0.20 [81]	0.20	19	1	P(2-5) P(6),	0.93	22 [11]	1	0.89	45 [11]
Ibuprofen	2	1.4 [29, 83]	0.018 [83]	0.010	84	4	P(2-5), P(5), P(5+)	0.94	26 [6, 11, 12, 29]	2	0.91	21 [11, 19]
Imipramine	5	13 [5, 40, 41, 53, 84]	0.13 [25, 40, 53]	0.13	255	8	I(2), I(3), I(5), P(6), P(2-5), P(5), P(5+), P(2)	0.18	90 [5, 6, 11, 12, 29, 33]	2	0.12	113 [11, 19]
Indomethacin	3	2.1 [4, 43, 85]	0.019 [4, 43, 85]	0.010	124	1	P(5)	0.95	27 [43]			
Irbesartan	3	3.8 [4, 43, 86]	0.10 [4, 43]	0.057	48	4	P(2-5), P(5), P(2), P(5)	0.94	35 [11, 12, 33, 43]			
Ketanserin	4	9.7 [87-90]	0.097 [87,	0.068	187	4	I(3), I(11)	0.87	136 [61, 62]			

	<i>In Vivo</i>					<i>In Vitro</i>						
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes				Microsomes		
						n	Donors (n)	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
Ketoprofen	4	2.2 [6, 40, 43, 91]	88, 90] 0.015 [6, 12, 40, 43, 92]	0.0078	158	6	I(5), P(6), P(2-5), P(5), P(5)	0.96	12 [5, 6, 11, 12, 43]	1	0.93	7.2 [11]
Labetalol	3	14 [93-95]	0.38 [94, 95]	0.50	119	1	P(6)	0.93	15 [6]	1	0.89	17 [6]
Levoprotiline	1	14 [96]	0.19 [6]	0.19	246	1	P(6)	0.66	7.8 [6]			
Lidocaine	7	14 [4, 40, 97-99]	0.34 [4, 11, 40, 100]	0.29	137	2	P(6), P(2-5)	0.90	13 [6, 11]	1	0.85	4.3 [101]
Lorazepam	4	1.1 [29, 40, 102, 103]	0.080 [4, 11, 40, 104]	0.082	15	2	I(1), P(5+)	0.85	1.9 [5, 29]	1	0.78	22 [11]
Lorcainide	3	20 [11, 105, 106]	0.17 [11, 106]	0.13	2559	1	P(2-5)	0.17	89 [11]	2	0.52 [19]	449 [11, 19]
Lovastatin	2	15 [1, 41]	0.082 [1, 41]	0.047	740					1	a	4095 [14]
Methadone	4	1.7 [4, 14, 107, 108]	0.21 [4, 14]	0.16	8.7					1	a	19 [14]
Methohexital	1	16 [19]	0.39 [19]	0.27	169					1	0.86 [19]	54 [19]
Methoxsalen	1	18 [109]	0.13 [110]	0.090	989					1	0.89 [19]	40 [19]
Methylprednisolone	4	5.9 [11, 40, 111, 112]	0.18 [11, 40]	0.18	45	3	P(2-5), P(5), P(5+)	0.87	14 [11, 12, 29]	1	0.81	27 [11]
Metoclopramide	1	4.3 [113]	0.60 [113]	0.60	8.9	1	P(6)	0.82	6.6 [6]			
Metoprolol	4	12 [4, 40, 114, 115]	0.83 [4, 12, 40, 114]	0.89	32	7	I(5), P(6), P(2-5), P(5), P(2)	0.90	16 [5, 6, 11, 12, 33]	1	0.85	4.0 [11]
Mianserin	1	18 [116]	0.14 [117]	0.14	897	1	P(6)	0.83	15 [6]	1	0.76	25 [6]
Midazolam	5	9.2 [14, 40, 41, 118, 119]	0.043 [4, 12, 14, 40, 41, 118]	0.025	390	11	I(5), P(6), P(2-5), P(5), P(5), P(5+), (3), I(2), I(7)	0.92	42 [5, 6, 11, 12, 20, 21, 29, 36, 59]	4	0.88 [19]	315 [6, 11, 14, 19]
Montelukast	6	1.1 [4, 43, 120-122]	0.0062 [122]	0.0040	182	2	P(2-5), P(5)	0.41	76 [11, 43]			
Nadolol	3	0.92 [4, 5, 123]	0.83 [4, 124]	0.83	1.2	1	P(2-5)	0.94	8.2 [11]	1	0.91	18 [11]

	In Vivo					In Vitro						
						Hepatocytes				Microsomes		
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	n	Donors (n)	fu _{hep} [*]	CL _{int,u} (ml/min/kg)	n	fu _{mic} [*]	CL _{int,u} (ml/min/kg)
Naloxone	2	18 [29, 125]	0.51 [4, 126]	0.62	333	11	I(2), P(6), P(2-5), P(5), P(5+), I(3), I(2), I(3), I(11)	0.93	167 [5, 6, 11, 12, 29, 36, 61, 62]	2	0.87 [39]	13 [11, 39]
Naltrexone	1	1.4 [127]	0.83 [127]	0.79	1.8	4	I(3), I(11)	0.94	46 [61, 62]			
Naproxen	2	0.12 [4, 40]	0.0010 [11]	0.0010	117	5	I(5), P(2-5), I(3)	0.91	68 [5, 11, 61]	1	0.86	19 [11]
Nifedipine	6	5.4 [5, 29, 40, 43, 128-131]	0.030 [4, 40, 128, 129]	0.041	242	4	I(5), P(2-5), P(5+), I(3)	0.79	60 [5, 11, 29, 40]	2	0.70	178 [11, 14]
Nisoldipine	3	12 [14, 132, 133]	0.0030 [14]	0.0030	9837					1	a	5993 [14]
Nitrendipine	1	20 [5]	0.029 [4]	0.020	20700	1	I(7)	0.61	31 [5]			
Omeprazole	2	11 [5, 134]	0.067 [134]	0.040	331	3	I(2), P(2-5), P(5)	0.87	8.4 [5, 11, 12]	1	0.81	17 [11]
Ondansetron	3	6.5 [4, 5, 135]	0.33 [4]	0.27	29	5	I(5), P(2-5), P(5), P(2), P(5)	0.88	3.6 [5, 11, 12, 33, 43]			
Oxaprozin	1	0.07 [43]	0.0007 [43]	0.00039	100	3	P(2-5), P(5), P(5)	0.94	12 [11, 12, 43]			
Oxazepam	2	1.2 [40, 136]	0.043 [40, 136]	0.048	30	3	P(2-5), P(5), P(5+)	0.87	8.4 [11, 12, 29]			
Oxprenolol	1	5.4 [137]	0.30 [6]	0.30	24	2	P(6), P(2)	0.88	11 [6, 33]			
Phenacetin	2	20 [4, 138]	0.57 [4, 11, 139]	0.57	687	4	P(2-5), P(2), P(6), I(7)	0.91	24 [6, 11, 33, 59]	2	0.87	34 [6, 11]
Phenytoin	2	5.3 [4, 41]	0.11 [4]	0.11	65	1	P(2)	0.84	2.1 [33]			
Pindolol	3	3.7 [1, 4, 41]	0.56 [1, 4, 41]	0.56	8.1	3	I(4), P(2-5), P(5)	0.91	6.5 [5, 11, 12]			
Prazosin	3	4.4 [4, 5, 140]	0.067 [4, 11, 141]	0.047	83	6	I(7), P(6), P(2-5), P(5), P(2), P(5)	0.94	8.6 [5, 6, 11, 12, 33, 43]	1	0.91	7.3 6[11]
Prednisolone	4	4.9 [29, 40, 43, 54]	0.17 [11, 40, 43]	0.12	38	3	P(2-5), P(5), P(5+)	0.92	15 [11, 12, 29]			
Prednisone	1	4.9 [19]	0.30 [19]	0.25	22					1	0.56 [19]	13 [19]

	In Vivo					In Vitro						
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes				Microsomes		
						n	Donors (n)	fu _{hep} [*]	CL _{int,u} (ml/min/kg)	n	fu _{mic} [*]	CL _{int,u} (ml/min/kg)
Prochlorperazine	1	16 [142]	0.0027 [102]	0.0027	28402	1	P(6)	0.76	14 [6]	1	0.66	70 [6]
Promazine	1	12 [1]	0.092 [143]	0.11	295	1	P(6)	0.26	58 [6]	1	0.18	57 [6]
Promethazine	1	16 [144]	0.22 [144]	0.22	293	1	P(6)	0.18	106 [6]	1	0.12	79 [6]
Propafenone	2	19 [19, 145]	0.057 [19]	0.040	4672	2	P(6), P(2-5)	0.50	100 [6, 11]	3	0.15 [19]	343 [6, 11, 19]
Propranolol	11	15 [4, 5, 40, 95, 146-152]	0.15 [4, 40, 150]	0.13	333	8	I(90), I(5), P(6), P(2-5), P(5), P(5+), P(2)	0.63	50 [5, 6, 11, 12, 29, 33]	2	0.51	18 [6, 11]
(-)-Propranolol	3	13 [150-152]	0.17 [150, 151]	0.15	191	1	P(6)	0.63	19 [6]	1	0.51	14 [6]
(+)-Propranolol	3	15 [150-152]	0.19 [150, 151]	0.16	270	1	P(6)	0.63	23 [6]	1	0.51	32 [6]
Quinidine	5	4.1 [4, 14, 40, 43, 153]	0.21 [4, 14, 40, 43, 153, 154]	0.20	24	3	P(2-5), P(5), I(3)	0.64	17 [11, 12, 40]	3	0.70 [19]	16 [11, 19, 155]
Ranitidine	4	2.7 [4, 43, 156, 157]	0.80 [4, 43]	0.84	3.9	4	I(5), P(2-5), P(5), P(5)	0.95	3.4 [5, 11, 12, 43]			
Repaglinide	2	13 [1, 14]	0.025 [14]	0.015	1388					1	a	110 [14]
Rifabutin	2	4.1 [14, 158]	0.48 [14]	0.29	11					1	a	68 [14]
Risperidone	3	7.9 [4, 13, 159]	0.15 [4, 13]	0.10	82	2	P(6), P(2-5)	0.74	28 [6, 11]	2	0.64	46 [6, 11]
Ritonavir	1	1.2 [43]	0.015 [43]	0.015	86	3	I(7), P(5), P(5)	0.39	22 [5, 12, 43]			
Salbutamol	2	3.0 [160, 161]	0.93 [160, 161]	0.93	3.7					1	a	9.3 [39]
Saquinavir	2	18 [14, 162]	0.038 [14]	0.028	3199					1	a	6386 [14]
Scopolamine	3	13 [4, 5, 163]	0.90 [4]	0.90	39	1	I(1)	0.94	19 [5]			
Sildenafil	2	7.6 [164, 165]	0.040 [164]	0.040	298	4	P(6), P(2-5), P(5), P(5+)	0.92	24 [6, 11, 12, 29]	3	0.88	103 [6, 11, 14]
Sumatriptan	1	15 [166]	0.81 [166]	0.83	72	4	I(3), I(6)	0.94	6.2 [61, 62]			
Tacrolimus	1	0.71 [167]	0.0040 [14, 168, 169]	0.20	181					1	a	889 [14]

	In Vivo					In Vitro						
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes				Microsomes		
						n	Donors (n)	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
Temazepam	2	1.9 [5, 170]	0.017 [170]	0.017	122	2	I(2), P(6)	0.91	6.0 [5, 6]			
Tenoxicam	1	0.054 [171]	0.013 [171]	0.0085	4.2	2	P(2-5), P(5+)	0.96	4.8 [11, 29]	1	0.97 [19]	2.1 [19]
Theophylline	3	0.47 [29, 172, 173]	0.45 [4, 92]	0.59	1.1	2	P(5), P(5+)	0.96	2.0 [12, 29]	1	0.93	2.8 [11]
Timolol	3	9.7 [4, 41, 174]	0.59 [4]	0.90	31	2	P(2-5), P(5), P(6),	0.90	9.8 [11, 12]			
Tolbutamide	2	0.35 [29, 175]	0.076 [40, 175]	0.050	4.7	5	P(2-5), P(5), P(5+), I(3)	0.83	3.4 [6, 11, 12, 29, 40]	2	0.76	1.5 [11, 19]
Trazodone	3	2.3 [4, 14, 176]	0.086 [4, 14]	0.070	30	1	P(6)	0.82	19 [6]	2	0.74	63 [6, 14]
Triazolam	4	4.3 [1, 5, 40, 177]	0.14 [1, 40]	0.10	39	4	I(2), P(6), P(5), P(5)	0.91	6.4 [5, 6, 20, 21]	2	0.78 [19]	31 [6, 19]
Trimipramine	1	16 [178]	0.051 [178]	0.051	1344	1	P(6)	0.098	150 [6]	1	0.063	222 [6]
Verapamil	5	16 [5, 14, 41, 179, 180]	0.096 [14, 19, 41, 179, 180]	0.081	750	8	I(86), I(5), P(6), P(2-5), P(5), P(5+), P(2)	0.52	106 [5, 6, 11, 12, 29, 33]	4	0.27 [19]	236 [6, 11, 14, 19]
Warfarin	2	0.086 [29, 181]	0.023 [4, 181]	0.013	3.8	1	P(5+)	0.94	3.0 [29]	1	0.90	2.0 [11]
Wafarin (S-)	1	0.11 [40]	0.018 [40]	0.010	6.0	1	I(3)	0.94	2.7 [40]			
Zaleplon	1	16 [182]	0.40 [4]	0.40	161	5	P(6), I(3), I(11)	0.92	7.3 [6, 61, 62]			
Zidovudine	1	19 [183]	0.82 [184]	0.80	377	3	I(5-7), I(3)	1.00 [7]	6.2 [7, 61]			
Zileuton	1	6 [5]	0.10 [185]	0.070	81	1	I(16)	0.91	5.9 [5]			
Zolpidem	4	5.7 [1, 4, 14, 186]	0.10 [1, 4, 14, 186]	0.079	76	3	P(6), P(2-5), P(5)	0.86	8.4 [6, 11, 12]	4	0.79	13 [6, 11, 14, 19]

n, number of studies; (n) number of donors; P, Pooled; I, Individual. * fu_{hep} values are normalised to 1 x 10⁶ cells/ml and fu_{mic} values are normalised to 1 mg microsomal protein/ml. Experimentally determined values are followed by the accompanying reference; all other values were determined using lipophilicity relationship algorithms (Equations 2.2 and 2.1, fu_{hep} and fu_{mic} respectively). a, only CL_{int,u} or equivalent reported, no value for fu_{mic} given.

Supplemental Table 1 References

1. Obach RS, Lombardo F, and Waters NJ (2008) Trend analysis of a database of intravenous pharmacokinetic parameters in humans for 670 drug compounds. *Drug Metab Dispos* 36: 1385-1405.
2. Roux A, Flouvat B, Chau NP, Letac B, and Lucsko M (1980) Pharmacokinetics of acebutolol after intravenous bolus administration. *Br J Clin Pharmacol* 9: 215-217.
3. Singh BN, Thoden WR, and Wahl J (1986) Acebutolol - A review of its pharmacology, pharmacokinetics, clinical uses, and adverse-effects. *Pharmacotherapy* 6: 45-63.
4. Paixao P, Gouveia LF, and Morais JAG (2010) Prediction of the in vitro intrinsic clearance determined in suspensions of human hepatocytes by using artificial neural networks. *Eur J Pharm Sci* 39: 310-321.
5. McGinnity DF, Soars MG, Urbanowicz RA, and Riley RJ (2004) Evaluation of fresh and cryopreserved hepatocytes as in vitro drug metabolism tools for the prediction of metabolic clearance. *Drug Metab Dispos* 32: 1247-1253.
6. Stringer R (2006) Evaluation of higher-throughput in vitro methods for the reliable prediction of in vivo metabolic clearance in humans (Unpublished Doctoral Thesis). University of Manchester, Manchester, UK.
7. Naritomi Y, Terashita S, Kagayama A, and Sugiyama Y (2003) Utility of hepatocytes in predicting drug metabolism: Comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab Dispos* 31: 580-588.
8. Sonne J, Poulsen HE, Loft S, Dossing M, Vollmerlarsen A, Simonsen K, Thyssen H, and Lundstrom K (1988) Therapeutic doses of codeine have no effect on acetaminophen clearance or metabolism. *Eur J Clin Pharmacol* 35: 109-111.
9. Rawlins MD, Henderson DB, and Hijab AR (1977) Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral-administration. *Eur J Clin Pharmacol* 11: 283-286.
10. Gazzard BG, Ford-Hutchinson AW, Smith MJ, and Williams R (1973) The binding of paracetamol to plasma proteins of man and pig. *J Pharm Pharmacol* 25: 964-7.
11. Sohlenius-Sternbeck AK, Afzelius L, Prusis P, Neelissen J, Hoogstraate J, Johansson J, Floby E, Bengtsson A, Gissberg O, Sternbeck J, and Petersson C (2010) Evaluation of the human prediction of clearance from hepatocyte and microsome intrinsic clearance for 52 drug compounds. *Xenobiotica* 40: 637-649.
12. Sohlenius-Sternbeck AK, Jones C, Ferguson D, Middleton BJ, Projean D, Floby E, Bylund J, and Afzelius L (2012) Practical use of the regression offset approach for the prediction of in vivo intrinsic clearance from hepatocytes. *Xenobiotica* 42: 841-853.
13. De Buck SS, Sinha VK, Fenu LA, Nijssen MJ, Mackie CE, and Gilissen RAHJ (2007) Prediction of human pharmacokinetics using physiologically based modeling: A retrospective analysis of 26 clinically tested drugs. *Drug Metab Dispos* 35: 1766-1780.
14. Gertz M, Harrison A, Houston JB, and Galetin A (2010) Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos* 38: 1147-1158.
15. Bower S and Hull CJ (1982) Comparative pharmacokinetics of fentanyl and alfentanil. *Brit J Anaesthet* 54: 871-7.
16. McDonnell CG, Malkin D, Van Pelt FD, and Shorten GD (2003) Elimination of alfentanil delivered by infusion is not altered by the chronic administration of atorvastatin. *Eur J Anaesthesiol* 20: 662-667.
17. Paixao P, Gouveia LF, and Morais JAG (2009) Prediction of drug distribution within blood. *Eur J Pharm Sci* 36: 544-554.
18. Smith RB, Kroboth PD, Vanderlugt JT, Phillips JP, and Juhl RP (1984) Pharmacokinetics and pharmacodynamics of alprazolam after oral and iv administration. *Psychopharmacology* 84: 452-456.
19. Obach RS (1999) 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* 27: 1350-1359.

20. Hallifax D, Rawden HC, Hakooz N, and Houston JB (2005) Prediction of metabolic clearance using cryopreserved human hepatocytes: Kinetic characteristics for five benzodiazepines. *Drug Metab Dispos* 33: 1852-1858.
21. Hallifax D, Galetin A, and Houston JB (2008) Prediction of metabolic clearance using fresh human hepatocytes: Comparison with cryopreserved hepatocytes and hepatic microsomes for five benzodiazepines. *Xenobiotica* 38: 353-367.
22. Bodin NO, Borg KO, Johansson R, Obianwu H, and Svensson R (1974) Absorption, distribution and excretion of alprenolol in man, dog and rat. *Acta Pharmacol Tox* 35: 261-269.
23. Schulz P, Turnertamiyasu K, Smith G, Giacomini KM, and Blaschke TF (1983) Amitriptyline disposition in young and elderly normal men. *Clin Pharmacol Ther* 33: 360-366.
24. Jorgensen A and Hansen V (1976) Pharmacokinetics of amitriptyline infused intravenously in man. *Eur J Clin Pharmacol* 10: 337-341.
25. Nyberg G and Martensson E (1984) Determination of free fractions of tricyclic antidepressants. *Naunyn-Schmiedeberg's Arch Pharmacol* 327: 260-265.
26. Bachmann K (1987) Estimating the Clearance of Amylobarbitone from a Single Plasma Measurement. *Br J Clin Pharmacol* 23: 94-98.
27. Sawada Y, Hanano M, Sugiyama Y, and Iga T (1985) Prediction of the disposition of 9 weakly acidic and 6 weakly basic drugs in humans from pharmacokinetic parameters in rats. *J Pharmacokinet Biop* 13: 477-492.
28. Lave T, Dupin S, Schmitt C, Chou RC, Jaeck D, and Coassolo P (1997) Integration of in vitro data into allometric scaling to predict hepatic metabolic clearance in man: Application to 10 extensively metabolized drugs. *J Pharm Sci* 86: 584-590.
29. Lau YY, Sapidou E, Cui XM, White RE, and Cheng KC (2002) Development of a novel in vitro model to predict hepatic clearance using fresh, cryopreserved, and sandwich-cultured hepatocytes. *Drug Metab Dispos* 30: 1446-1454.
30. Engel G, Hofmann U, Heidemann H, Cosme J, and Eichelbaum M (1996) Antipyrine as a probe for human oxidative drug metabolism: Identification of the cytochrome P450 enzymes catalyzing 4-hydroxyantipyrine, 3-hydroxymethylantipyrine, and norantipyrine formation. *Clin Pharmacol Ther* 59: 613-623.
31. Mason WD, Winer N, Kochak G, Cohen I, and Bell R (1979) Kinetics and Absolute Bioavailability of Atenolol. *Clin Pharmacol Ther* 25: 408-415.
32. Ludden TM, Boyle DA, Gieseke D, Kennedy GT, Crawford MH, Ludden LK, and Clementi WA (1988) Absolute bioavailability and dose proportionality of betaxolol in normal healthy-subjects. *J Pharm Sci* 77: 779-783.
33. Jacobson L, Middleton B, Holmgren J, Eirefelt S, Frijd M, Blomgren A, and Gustavsson L (2007) An optimized automated assay for determination of metabolic stability using hepatocytes: Assay validation, variance component analysis, and in vivo relevance. *Assay Drug Dev Technol* 5: 403-415.
34. Weber C, Schmitt R, Birnboeck H, Hopfgartner G, Eggers H, Meyer J, van Marle S, Viischer HW, and Jonkman JHG (1999) Multiple-dose pharmacokinetics, safety, and tolerability of bosentan, an endothelin receptor antagonist, in healthy male volunteers. *J Clin Pharmacol* 39: 703-714.
35. Weber C, Schmitt R, Birnboeck H, Hopfgartner G, vanMarle SP, Peeters PAM, Jonkman JHG, and Jones CR (1996) Pharmacokinetics and pharmacodynamics of the endothelin-receptor antagonist bosentan in healthy human subjects. *Clin Pharmacol Ther* 60: 124-137.
36. Blanchard N, Alexandre E, Abadie C, Lave T, Heyd B, Mantion G, Jaeck D, Richert L, and Coassolo P (2005) Comparison of clearance predictions using primary cultures and suspensions of human hepatocytes. *Xenobiotica* 35: 1-15.
37. Burm AGL, Vandermeer AD, Vankleef JW, Zeijlmans PWM, and Groen K (1994) Pharmacokinetics of the enantiomers of bupivacaine following intravenous administration of the racemate. *Br J Clin Pharmacol* 38: 125-129.
38. Kuhlman JJ, Lalani S, Maglullo J, Levine B, Darwin WD, Johnson RE, and Cone EJ (1996) Human pharmacokinetics of intravenous, sublingual, and buccal buprenorphine. *J Anal Toxicol* 20: 369-378.
39. Cubitt HE, Houston JB, and Galetin A (2009) Relative importance of intestinal and hepatic glucuronidation-impact on the prediction of drug clearance. *Pharmaceut Res* 26: 1073-1083.

40. Brown HS, Griffin M, and Houston JB (2007) Evaluation of cryopreserved human hepatocytes as an alternative in vitro system to microsomes for the prediction of metabolic clearance. *Drug Metab Dispos* 35: 293-301.
41. Chao P, Uss AS, and Cheng KC (2010) Use of intrinsic clearance for prediction of human hepatic clearance. *Expert Opin Drug Metab Toxicol* 6: 189-198.
42. Bloomer JC, Clarke SE, and Chenery RJ (1995) Determination of P4501A2 activity in human liver-microsomes using [3-C-14-Methyl] caffeine. *Xenobiotica* 25: 917-927.
43. Riley RJ, McGinnity DF, and Austin RP (2005) A unified model for predicting human hepatic, metabolic clearance from in vitro intrinsic clearance data in hepatocytes and microsomes. *Drug Metab Dispos* 33: 1304-1311.
44. Neugebauer G, Akpan W, V ME, Neubert P, and Reiff K (1987) Pharmacokinetics and disposition of carvedilol in humans. *J Cardiovasc Pharm* 10: S85-S88.
45. Huang SM, Athanikar NK, Sridhar K, Huang YC, and Chiou WL (1982) Pharmacokinetics of Chlorpheniramine after Intravenous and Oral-Administration in Normal Adults. *Eur J Clin Pharmacol* 22: 359-365.
46. Yeung PKF, Hubbard JW, Korchinski ED, and Midha KK (1993) Pharmacokinetics of chlorpromazine and key metabolites. *Eur J Clin Pharmacol* 45: 563-569.
47. Gugler R, Mullerliebenau B, and Somogyi A (1982) Altered disposition and availability of cimetidine in liver cirrhotic-patients. *Br J Clin Pharmacol* 14: 421-429.
48. Bauer LA, Wareingtran C, Edwards WAD, Raisys V, Ferreri L, Jack R, Dellinger EP, and Simonowitz D (1985) Cimetidine clearance in the obese. *Clin Pharmacol Ther* 37: 425-430.
49. Cheng YF, Lundberg T, Bondesson U, Lindstrom L, and Gabrielsson J (1988) Clinical pharmacokinetics of clozapine in chronic-schizophrenic patients. *Eur J Clin Pharmacol* 34: 445-449.
50. Guay DRP, Awni WM, Findlay JWA, Halstenson CE, Abraham PA, Opsahl JA, Jones EC, and Matzke GR (1988) Pharmacokinetics and pharmacodynamics of codeine in end-stage renal-disease. *Clin Pharmacol Ther* 43: 63-71.
51. Mohammed SS, Christopher MM, Mehta P, Kedar A, Gross S, and Derendorf H (1993) Increased Erythrocyte and Protein-Binding of Codeine in Patients with Sickle-Cell Disease. *J Pharm Sci* 82: 1112-1117.
52. Legg B, Gupta SK, Rowland M, Johnson RWG, and Solomon LR (1988) Cyclosporin - Pharmacokinetics and detailed studies of plasma and erythrocyte binding during intravenous and oral-administration. *Eur J Clin Pharmacol* 34: 451-460.
53. Ciraulo DA, Barnhill JG, and Jaffe JH (1988) Clinical pharmacokinetics of imipramine and desipramine in alcoholics and normal volunteers. *Clin Pharmacol Ther* 43: 509-518.
54. Rose JQ, Yurchak AM, Meikle AW, and Jusko WJ (1981) Effect of smoking on prednisone, prednisolone, and dexamethasone pharmacokinetics. *J Pharmacokinetic Biopharm* 9: 1-14.
55. Tsuei SE, Moore RG, Ashley JJ, and McBride WG (1979) Disposition of synthetic glucocorticoids .1. Pharmacokinetics of dexamethasone in healthy-adults. *J Pharmacokinetic Biopharm* 7: 249-264.
56. Klotz U, Avant GR, Hoyumpa A, Schenker S, and Wilkinson GR (1975) Effects of age and liver-disease on disposition and elimination of diazepam in adult man. *J Clin Invest* 55: 347-359.
57. Andersson T, Andren K, Cederberg C, Edvardsson G, Heggelund A, and Lundborg P (1990) Effect of omeprazole and cimetidine on plasma diazepam levels. *Eur J Clin Pharmacol* 39: 51-54.
58. Herman RJ and Wilkinson GR (1996) Disposition of diazepam in young and elderly subjects after acute and chronic dosing. *Br J Clin Pharmacol* 42: 147-155.
59. Floby E, Johansson J, Hoogstraate J, Hewitt NJ, Hill J, and Sohlenius-Sternbeck AK (2009) Comparison of intrinsic metabolic clearance in fresh and cryopreserved human hepatocytes. *Xenobiotica* 39: 656-662.
60. Willis JV, Kendall MJ, Flinn RM, Thornhill DP, and Welling PG (1979) Pharmacokinetics of diclofenac sodium following intravenous and oral-administration. *Eur J Clin Pharmacol* 16: 405-410.
61. Akabane T, Gerst N, Masters JN, and Tamura K (2012) A quantitative approach to hepatic clearance prediction of metabolism by aldehyde oxidase using custom pooled hepatocytes. *Xenobiotica* 42: 863-871.

62. Akabane T, Gerst N, Naritomi Y, Masters JN, and Tamura K (2012) A practical and direct comparison of intrinsic metabolic clearance of several non-CYP enzyme substrates in freshly isolated and cryopreserved hepatocytes. *Drug Metab Pharmacokinet* 27: 181-191.
63. Nuernberg B and Brune K (1989) Buffering the stomach content enhances the absorption of diflunisal in man. *Biopharm Drug Dispos* 10: 377-387.
64. Smith MS, Verghese CP, Shand DG, and Pritchett ELC (1983) Pharmacokinetic and pharmacodynamic effects of diltiazem. *Am J Cardiol* 51: 1369-1374.
65. Hoglund P and Nilsson LG (1988) Physiological disposition of intravenously administered C-14-labeled diltiazem in healthy-volunteers. *Ther Drug Monit* 10: 401-409.
66. Meredith CG, Christian CD, Johnson RF, Madhavan SV, and Schenker S (1984) Diphenhydramine disposition in chronic liver-disease. *Clin Pharmacol Ther* 35: 474-479.
67. Spector R, Choudhury AK, Chiang CK, Goldberg MJ, and Ghoneim MM (1980) Diphenhydramine in orientals and caucasians. *Clin Pharmacol Ther* 28: 229-234.
68. Blyden GT, Greenblatt DJ, Scavone JM, and Shader RI (1986) Pharmacokinetics of diphenhydramine and a demethylated metabolite following intravenous and oral-administration. *J Clin Pharmacol* 26: 529-533.
69. Edgar B, Regardh CG, Johnsson G, Johansson L, Lundborg P, Lofberg I, and Ronn O (1985) Felodipine kinetics in healthy-men. *Clin Pharmacol Ther* 38: 205-211.
70. Janssen U, Walker S, Maier K, Vongaisberg U, and Klotz U (1989) Flumazenil disposition and elimination in cirrhosis. *Clin Pharmacol Ther* 46: 317-323.
71. Pomierlayrargues G, Giguere JF, Lavoie J, Willems B, and Butterworth RF (1989) Pharmacokinetics of benzodiazepine antagonist Ro 15-1788 in cirrhotic-patients with moderate or severe liver dysfunction. *Hepatology* 10: 969-972.
72. Roncari G, Ziegler WH, and Guentert TW (1986) Pharmacokinetics of the new benzodiazepine antagonist Ro 15-1788 in man following intravenous and oral-administration. *Br J Clin Pharmacol* 22: 421-428.
73. Hammarlund MM, Paalzow LK, and Odling B (1984) Pharmacokinetics of furosemide in man after intravenous and oral-administration - application of moment analysis. *Eur J Clin Pharmacol* 26: 197-207.
74. Smith DE, Lin ET, and Benet LZ (1980) Absorption and Disposition of Furosemide in Healthy-Volunteers, Measured with a Metabolite-Specific Assay. *Drug Metab Dispos* 8: 337-342.
75. Jonsson A, Chan JCN, Rydberg T, Vaaler S, Hallengren B, Cockram CS, Critchley JAJH, and Melander A (2000) Pharmacodynamics and pharmacokinetics of intravenous glibenclamide in Caucasian and Chinese patients with type-2 diabetes. *Eur J Clin Pharmacol* 55: 721-727.
76. George S, Mcburney A, and Cole A (1990) Possible protein-binding displacement interaction between glibenclamide and metolazone. *Eur J Clin Pharmacol* 38: 93-95.
77. Allen A, Asgill CC, Pierce DM, Upward J, and Zussman BD (1994) Pharmacokinetics and tolerability of ascending intravenous doses of granisetron, a novel 5-HT₃ antagonist, in healthy human subjects. *Eur J Clin Pharmacol* 46: 159-62.
78. Cheng YF, Paalzow LK, Bondesson U, Ekblom B, Eriksson K, Eriksson SO, Lindberg A, and Lindstrom L (1987) Pharmacokinetics of haloperidol in psychotic patients. *Psychopharmacology (Berl)* 91: 410-4.
79. Chang WH, Lam YW, Jann MW, and Chen H (1992) Pharmacokinetics of haloperidol and reduced haloperidol in Chinese schizophrenic patients after intravenous and oral administration of haloperidol. *Psychopharmacology (Berl)* 106: 517-22.
80. Holley FO, Magliozzi JR, Stanski DR, Lombrozo L, and Hollister LE (1983) Haloperidol kinetics after oral and intravenous doses. *Clin Pharmacol Ther* 33: 477-84.
81. Derendorf H, Mollmann H, Barth J, Mollmann C, Tunn S, and Krieg M (1991) Pharmacokinetics and oral bioavailability of hydrocortisone. *J Clin Pharmacol* 31: 473-476.

82. Toothaker RD and Welling PG (1982) Effect of dose size on the pharmacokinetics of intravenous hydrocortisone during endogenous hydrocortisone suppression. *J Pharmacokinet Biop* 10: 147-156.
83. Martin W, Koselowske G, Toberich H, Kerkmann T, Mangold B, and Augustin J (1990) Pharmacokinetics and absolute bioavailability of ibuprofen after oral-administration of ibuprofen lysine in man. *Biopharm Drug Dispos* 11: 265-278.
84. Brosen K and Gram LF (1988) First-pass metabolism of imipramine and desipramine - impact of the sparteine oxidation phenotype. *Clin Pharmacol Ther* 43: 400-406.
85. Yeh KC, Berger ET, Breault GO, Lei BW, and McMahon FG (1982) Effect of sustained-release on the pharmacokinetic profile of indomethacin in man. *Biopharm Drug Dispos* 3: 219-230.
86. Vachharajani NN, Shyu WC, Chando TJ, Everett DW, Greene DS, and Barbhuiya RH (1998) Oral bioavailability and disposition characteristics of irbesartan, an angiotensin antagonist, in healthy volunteers. *J Clin Pharmacol* 38: 702-707.
87. Heykants J, Vanpeer A, Woestenborghs R, Gould S, and Mills J (1986) Pharmacokinetics of ketanserin and its metabolite ketanserin-ol in man after intravenous, intramuscular and oral-administration. *Eur J Clin Pharmacol* 31: 343-350.
88. Trenk D, Mosler A, Kirch W, Meinertz T, and Jahnchen E (1983) Pharmacokinetics and Pharmacodynamics of the 5-HT₂ Receptor Antagonist Ketanserin in Man. *J Cardiovasc Pharm* 5: 1034-1039.
89. Persson B, Pettersson A, and Hedner T (1987) Pharmacokinetics of ketanserin in patients with essential-hypertension. *Eur J Clin Pharmacol* 32: 259-265.
90. Reimann IW, Okonkwo PO, and Klotz U (1983) Pharmacokinetics of ketanserin in man. *Eur J Clin Pharmacol* 25: 73-76.
91. Debruyne D, Deligny BH, Ryckelynck JP, Albessard F, and Moulin M (1987) Clinical pharmacokinetics of ketoprofen after single intravenous administration as a bolus or infusion. *Clin Pharmacokinet* 12: 214-221.
92. Lee KJ, Mower R, Hollenbeck T, Castelo J, Johnson N, Gordon P, Sinko PJ, Holme K, and Lee YH (2003) Modulation of nonspecific binding in ultrafiltration protein binding studies. *Pharmaceut Res* 20: 1015-1021.
93. Nyberg G, Hansson R, and Tietz F (1982) Single-dose pharmacokinetics of labetalol in healthy-young men. *Acta Med Scand* 212: 67-73.
94. Lalonde RL, Orear TL, Wainer IW, Drda KD, Herring VL, and Bottorff MB (1990) Labetalol pharmacokinetics and pharmacodynamics - evidence of stereoselective disposition. *Clin Pharmacol Ther* 48: 509-519.
95. Cheymol G, Poirier JM, Carrupt PA, Testa B, Weissenburger J, Levron JC, and Snoeck E (1997) Pharmacokinetics of beta-adrenoceptor blockers in obese and normal volunteers. *Br J Clin Pharmacol* 43: 563-570.
96. Kaiser G, Ackermann R, Dieterle W, Reimann IW, and Bieck PR (1992) Pharmacokinetics of the antidepressant levoprotiline after intravenous and peroral administration in healthy-volunteers. *Biopharm Drug Dispos* 13: 83-93.
97. Bennett PN, Aarons LJ, Bending MR, Steiner JA, and Rowland M (1982) Pharmacokinetics of lidocaine and its de-ethylated metabolite - dose and time dependency studies in man. *J Pharmacokinet Biop* 10: 265-281.
98. Bauer LA, Brown T, Gibaldi M, Hudson L, Nelson S, Raisys V, and Shea JP (1982) Influence of long-term infusions on lidocaine kinetics. *Clin Pharmacol Ther* 31: 433-437.
99. Rowland M, Thomson PD, Guichard A, and Melmon KL (1971) Disposition kinetics of lidocaine in normal subjects. *Ann N Y Acad Sci* 179: 383-&.
100. Grossman SH, Davis D, Kitchell BB, Shand DG, and Routledge PA (1982) Diazepam and lidocaine plasma-protein binding in renal-disease. *Clin Pharmacol Ther* 31: 350-357.
101. Parker RJ, Collins JM, and Strong JM (1996) Identification of 2,6-xylydine as a major lidocaine metabolite in human liver slices. *Drug Metab Dispos* 24: 1167-1173.
102. Stringer R, Nicklin PL, and Houston JB (2008) Reliability of human cryopreserved hepatocytes and liver microsomes as in vitro systems to predict metabolic clearance. *Xenobiotica* 38: 1313-1329.

103. Wermeling DPH, Miller JL, Archer SM, Manaligod JM, and Rudy AC (2001) Bioavailability and pharmacokinetics of lorazepam after intranasal, intravenous, and intramuscular administration. *J Clin Pharmacol* 41: 1225-1231.
104. Desmond PV, Roberts RK, Wood AJJ, Dunn GD, Wilkinson GR, and Schenker S (1980) Effect of heparin administration on plasma-binding of benzodiazepines. *Br J Clin Pharmacol* 9: 171-175.
105. Klotz U, Mullerseydlitz P, and Heimbürg P (1979) Disposition and anti-arrhythmic effect of lorcanide. *Int J Clin Pharmacol Biopharm* 17: 152-158.
106. Klotz U, Mullerseydlitz P, and Heimbürg P (1978) Pharmacokinetics of lorcanide in man - a new antiarrhythmic agent. *Clin Pharmacokinet* 3: 407-418.
107. Kristensen K, Blemmer T, Angelo HR, Christrup LL, Drenck NE, Rasmussen SN, and Sjogren P (1996) Stereoselective pharmacokinetics of methadone in chronic pain patients. *Ther Drug Monit* 18: 221-227.
108. Nilsson MI, Gronbladh L, Widerlov E, and Anggard E (1983) Pharmacokinetics of methadone in methadone-maintenance treatment - characterization of therapeutic failures. *Eur J Clin Pharmacol* 25: 497-501.
109. Billard V, Gambus PL, Barr J, Minto CF, Corash L, Tessman JW, Stickney JL, and Shafer SL (1995) The Pharmacokinetics of 8-Methoxypsoralen Following Iv Administration in Humans. *Br J Clin Pharmacol* 40: 347-360.
110. Pibouin M, Zini R, Nguyen P, Renouard A, and Tillement JP (1987) Binding of 8-Methoxypsoralen to Human-Serum Proteins and Red-Blood-Cells. *Br J Dermatol* 117: 207-215.
111. Booker BM, Magee MH, Blum RA, Lates CD, and Jusko WJ (2002) Pharmacokinetic and pharmacodynamic interactions between diltiazem and methylprednisolone in healthy volunteers. *Clin Pharmacol Ther* 72: 370-382.
112. Alhabet SMH and Rogers HJ (1989) Methylprednisolone pharmacokinetics after intravenous and oral-administration. *Br J Clin Pharmacol* 27: 285-290.
113. Magueur E, Hagege H, Attali P, Singlas E, Etienne JP, and Taburet AM (1991) Pharmacokinetics of metoclopramide in patients with liver-cirrhosis. *Br J Clin Pharmacol* 31: 185-187.
114. Schaaf LJ, Campbell SC, Mayersohn MB, Vagedes T, and Perrier DG (1987) Influence of smoking and gender on the disposition kinetics of metoprolol. *Eur J Clin Pharmacol* 33: 355-361.
115. Jordo L, Attman PO, Aurell M, Johansson L, Johnsson G, and Regardh CG (1980) Pharmacokinetic and pharmacodynamic properties of metoprolol in patients with impaired renal-function. *Clin Pharmacokinet* 5: 169-180.
116. Shami M, Elliott HL, Kelman AW, and Whiting B (1983) The pharmacokinetics of mianserin. *Br J Clin Pharmacol* 15: S313-S322.
117. Torres I, Suarez E, Rodriguezsasiain JM, Aguirre C, and Calvo R (1995) Differential effect of cancer on the serum-protein binding of mianserin and imipramine. *Eur J Drug Metab Pharmacokinet* 20: 107-111.
118. Pentikainen PJ, Valisalmi L, Himberg JJ, and Crevoisier C (1989) Pharmacokinetics of midazolam following intravenous and oral-administration in patients with chronic liver-disease and in healthy-subjects. *J Clin Pharmacol* 29: 272-277.
119. Heizmann P, Eckert M, and Ziegler WH (1983) Pharmacokinetics and bioavailability of midazolam in man. *Br J Clin Pharmacol* 16: S43-S49.
120. Cheng HY, Leff JA, Amin R, Gertz BJ, DeSmet M, Noonan N, Rogers JD, Malbecq W, Meisner D, and Somers G (1996) Pharmacokinetics, bioavailability, and safety of montelukast sodium (MK-0476) in healthy males and females. *Pharmaceut Res* 13: 445-448.
121. Zhao JJ, Rogers JD, Holland SD, Larson P, Amin RD, Haesen R, Freeman A, Seiberling M, Merz M, and Cheng HY (1997) Pharmacokinetics and bioavailability of montelukast sodium (MK-0476) in healthy young and elderly volunteers. *Biopharm Drug Dispos* 18: 769-777.
122. Filppula AM, Laitila J, Neuvonen PJ, and Backman JT (2011) Reevaluation of the microsomal metabolism of montelukast: major contribution by CYP2C8 at clinically relevant concentrations. *Drug Metab Dispos* 39: 904-911.
123. Morrison RA, Singhvi SM, Creasey WA, and Willard DA (1988) Dose proportionality of nadolol pharmacokinetics after intravenous administration to healthy-subjects. *Eur J Clin Pharmacol* 33: 625-628.

124. Patel L, Johnston A, and Turner P (1984) Nadolol binding to human-serum proteins. *J Pharm Pharmacol* 36: 414-415.
125. Ngai SH, Berkowitz BA, Yang JC, Hempstead J, and Spector S (1976) Pharmacokinetics of naloxone in rats and in man - basis for its potency and short duration of action. *Anesthesiology* 44: 398-401.
126. Asali LA and Brown KF (1984) Naloxone protein-binding in adult and fetal plasma. *Eur J Clin Pharmacol* 27: 459-463.
127. Wall ME, Brine DR, and Perezreyes M (1981) Metabolism and disposition of naltrexone in man after oral and intravenous administration. *Drug Metab Dispos* 9: 369-375.
128. Kleinbloesem CH, Vanbrummelen P, Vandelinde JA, Voogd PJ, and Breimer DD (1984) Nifedipine - kinetics and dynamics in healthy-subjects. *Clin Pharmacol Ther* 35: 742-749.
129. Kleinbloesem CH, Vanharten J, Wilson JPH, Danhof M, Vanbrummelen P, and Breimer DD (1986) Nifedipine - kinetics and hemodynamic-effects in patients with liver-cirrhosis after intravenous and oral-administration. *Clin Pharmacol Ther* 40: 21-28.
130. Robertson DRC, Waller DG, Renwick AG, and George CF (1988) Age-related-changes in the pharmacokinetics and pharmacodynamics of nifedipine. *Br J Clin Pharmacol* 25: 297-305.
131. Rashid TJ, Martin U, Clarke H, Waller DG, Renwick AG, and George CF (1995) Factors affecting the absolute bioavailability of nifedipine. *Br J Clin Pharmacol* 40: 51-58.
132. Vanharten J, Vanbrummelen P, Lodewijks MTM, Danhof M, and Breimer DD (1988) Pharmacokinetics and hemodynamic-effects of nisoldipine and its interaction with cimetidine. *Clin Pharmacol Ther* 43: 332-341.
133. Vanharten J, Vanbrummelen P, Wilson JHP, Lodewijks MTM, and Breimer DD (1988) Nisoldipine - kinetics and effects on blood-pressure and heart-rate in patients with liver-cirrhosis after intravenous and oral-administration. *Eur J Clin Pharmacol* 34: 387-394.
134. Regardh CG, Gabrielsson M, Hoffman KJ, Lofberg I, and Skanberg I (1985) Pharmacokinetics and metabolism of omeprazole in animals and man - an overview. *Scand J Gastroenterol* 20: 79-94.
135. Pritchard JF, Bryson JC, Kernodle AE, Benedetti TL, and Powell JR (1992) Age and gender effects on ondansetron pharmacokinetics - evaluation of healthy aged volunteers. *Clin Pharmacol Ther* 51: 51-55.
136. Sonne J, Loft S, Dossing M, Vollmerlarsen A, Olesen KL, Victor M, Andreasen F, and Andreasen PB (1988) Bioavailability and pharmacokinetics of oxazepam. *Eur J Clin Pharmacol* 35: 385-389.
137. Mason WD and Winer N (1976) Pharmacokinetics of oxprenolol in normal subjects. *Clin Pharmacol Ther* 20: 401-412.
138. Raaflaub J and Dubach UC (1975) Pharmacokinetics of phenacetin in man. *Eur J Clin Pharmacol* 8: 261-265.
139. Kurz H, Mauseganshorn A, and Stickel HH (1977) Differences in binding of drugs to plasma-proteins from newborn and adult man .1. *Eur J Clin Pharmacol* 11: 463-467.
140. Meredith PA, Elliott HL, Kelman AW, and Reid JL (1985) Application of pharmacokinetic-pharmacodynamic modeling for the comparison of quinazoline alpha-adrenoceptor agonists in normotensive volunteers. *J Cardiovasc Pharm* 7: 532-537.
141. Vanholder R, Vanlandschoot N, Desmet R, Schoots A, and Ringoir S (1988) Drug protein-binding in chronic renal-failure - evaluation of 9 drugs. *Kidney Int* 33: 996-1004.
142. Isah AO, Rawlins MD, and Bateman DN (1991) Clinical-pharmacology of prochlorperazine in healthy-young males. *Br J Clin Pharmacol* 32: 677-684.
143. Hu OYP and Curry SH (1989) Stability, human-blood distribution and rat-tissue localization of promazine and desmonomethylpromazine. *Biopharm Drug Dispos* 10: 537-548.
144. Taylor G, Houston JB, Shaffer J, and Mawer G (1983) Pharmacokinetics of promethazine and its sulfoxide metabolite after intravenous and oral-administration to man. *Br J Clin Pharmacol* 15: 287-293.

145. Arboix M, Puigdemont A, Moya A, and Cinca J (1985) Pharmacokinetics of intravenous propafenone in patients with episodes of paroxysmal supraventricular tachycardia. *Methods Find Exp Clin Pharmacol* 7: 435-438.
146. Watson RGP, Bastain W, Larkin KA, Hayes JR, Mcainsh JA, and Shanks RG (1987) A comparative pharmacokinetic study of conventional propranolol and a long-acting preparation of propranolol in patients with cirrhosis and normal controls. *Br J Clin Pharmacol* 24: 527-535.
147. Regardh CG, Johnsson G, Jordo L, Lungborg P, Persson BA, and Ronn O (1980) Plasma-concentrations and beta-blocking effects in normal volunteers after intravenous doses of metoprolol and propranolol. *J Cardiovasc Pharm* 2: 715-723.
148. Cid E, Mella F, Lucchini L, Carcamo M, and Monasterio J (1986) Plasma-concentrations and bioavailability of propranolol by oral, rectal and intravenous administration in man. *Biopharm Drug Dispos* 7: 559-566.
149. Bowman SL, Hudson SA, Simpson G, Munro JF, and Clements JA (1986) A comparison of the pharmacokinetics of propranolol in obese and normal volunteers. *Br J Clin Pharmacol* 21: 529-532.
150. Sowinski KM, Lima JJ, Burlew BS, Massie JD, and Johnson JA (1996) Racial differences in propranolol enantiomer kinetics following simultaneous iv and oral administration. *Br J Clin Pharmacol* 42: 339-346.
151. Olanoff LS, Walle T, Walle UK, Cowart TD, and Gaffney TE (1984) Stereoselective clearance and distribution of intravenous propranolol. *Clin Pharmacol Ther* 35: 755-761.
152. Bleske BE, Welage LS, Rose S, Amidon GL, and Shea MJ (1995) The effect of dosage release formulations on the pharmacokinetics of propranolol stereoisomers in humans. *J Clin Pharmacol* 35: 374-378.
153. Fremstad D, Nilsen OG, Storstein L, Amlie J, and Jacobsen S (1979) Pharmacokinetics of quinidine related to plasma-protein binding in man. *Eur J Clin Pharmacol* 15: 187-192.
154. Woo E and Greenblatt DJ (1979) Pharmacokinetic and Clinical Implications of Quinidine Protein-Binding. *J Pharm Sci* 68: 466-470.
155. Otton SV, Brinn RU, and Gram LF (1988) In vitro evidence against the oxidation of quinidine by the sparteine/debrisoquine monooxygenase of human liver. *Drug Metab Dispos* 16: 15-7.
156. Vanhecken AM, Tjandramaga TB, Mullie A, Verbesselt R, and Deschepper PJ (1982) Ranitidine - single dose pharmacokinetics and absolute bioavailability in man. *Br J Clin Pharmacol* 14: 195-200.
157. Chau NP, Zech PY, Pozet N, and Hadjaissa A (1982) Ranitidine kinetics in normal subjects. *Clin Pharmacol Ther* 31: 770-774.
158. Skinner MH, Hsieh M, Torseth J, Pauloin D, Bhatia G, Harkonen S, Merigan TC, and Blaschke TF (1989) Pharmacokinetics of rifabutin. *Antimicrob Agents Chemother* 33: 1237-1241.
159. Huang ML, Vanpeer A, Woestenborghs R, Decoster R, Heykants J, Jansen AAI, Zyllicz Z, Visscher HW, and Jonkman JHG (1993) Pharmacokinetics of the novel antipsychotic agent risperidone and the prolactin response in healthy-subjects. *Clin Pharmacol Ther* 54: 257-268.
160. Boulton DW and Fawcett JP (1996) Enantioselective disposition of salbutamol in man following oral and intravenous administration. *Br J Clin Pharmacol* 41: 35-40.
161. Morgan DJ, Paull JD, Richmond BH, Wilson-evered E, and Ziccone SP (1986) Pharmacokinetics of Intravenous and Oral Salbutamol and Its Sulfate Conjugate. *Br J Clin Pharmacol* 22: 587-593.
162. Kupferschmidt HHT, Fattinger KE, Ha HR, Follath F, and Krahenbuhl S (1998) Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. *Br J Clin Pharmacol* 45: 355-359.
163. Putcha L, Cintron NM, Tsui J, Vanderploeg JM, and Kramer WG (1989) Pharmacokinetics and oral bioavailability of scopolamine in normal subjects. *Pharmaceut Res* 6: 481-485.
164. Walker DK, Ackland MJ, James GC, Muirhead GJ, Rance DJ, Wastall P, and Wright PA (1999) Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. *Xenobiotica* 29: 297-310.

165. Nichols DJ, Muirhead GJ, and Harness JA (2002) Pharmacokinetics of sildenafil citrate after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. *Br J Clin Pharmacol* 53: 5S-12S.
166. Cosson VF, Fuseau E, Efthymiopoulos C, and Bye A (1997) Mixed effect modeling of sumatriptan pharmacokinetics during drug development. I: Interspecies allometric scaling. *J Pharmacokinet Biop* 25: 149-67.
167. Mancinelli LM, Frassetto L, Floren LC, Dressler D, Carrier S, Bekersky I, Benet LZ, and Christians U (2001) The pharmacokinetics and metabolic disposition of tacrolimus: A comparison across ethnic groups. *Clin Pharmacol Ther* 69: 24-31.
168. Zahir H, McCaughan G, Gleeson M, Nand RA, and McLachlan AJ (2004) Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *Br J Clin Pharmacol* 57: 298-309.
169. Iwasaki K, Miyazaki Y, Teramura Y, Kawamura A, Tozuka Z, Hata T, and Undre N (1996) Binding of tacrolimus (FK506) with human plasma proteins re-evaluation and effect of mycophenolic acid. *Res Commun Mol Pathol Pharmacol* 94: 251-257.
170. Vansteveninck AL, Schoemaker HC, Denhartigh J, Pieters MSM, Breimer DD, and Cohen AF (1994) Effects of intravenous temazepam .2. a study of the long-term reproducibility of pharmacokinetics, pharmacodynamics, and concentration-effect parameters. *Clin Pharmacol Ther* 55: 546-555.
171. Heintz RC, Guentert TW, Enrico JF, Dubach UC, Brandt R, and Jeunet FS (1984) Pharmacokinetics of tenoxicam in healthy human volunteers. *Eur J Rheumatol Inflamm* 7: 33-44.
172. Lombardi TP, Bertino JS, Goldberg A, Middleton E, and Slaughter RL (1987) The effects of a beta-2 selective adrenergic agonist and a beta-nonselective antagonist on theophylline clearance. *J Clin Pharmacol* 27: 523-529.
173. Kraan J, Borgstrom L, Koeter GH, Laseur M, Jonkman JHG, and Denoord OE (1988) Creation of four consecutive instantaneous steady-state plasma-concentration plateaus of theophylline and enprofylline by repeated infusions with exponentially decreasing delivery rates. *Eur J Clin Pharmacol* 35: 657-661.
174. Vermeij P, el-Sherbini-Schepers M, and van Zwieten PA (1978) The disposition of timolol in man. *J Pharm Pharmacol* 30: 53-5.
175. Zilly W, Breimer DD, and Richter E (1975) Induction of drug-metabolism in man after rifampicin treatment measured by increased hexobarbital and tolbutamide clearance. *Eur J Clin Pharmacol* 9: 219-227.
176. Nilsen OG and Dale O (1992) Single dose pharmacokinetics of trazodone in healthy-subjects. *Pharmacol Toxicol* 71: 150-153.
177. Smith RB, Kroboth PD, and Varner PD (1987) Pharmacodynamics of triazolam after intravenous administration. *J Clin Pharmacol* 27: 971-979.
178. Abernethy DR, Greenblatt DJ, and Shader RI (1984) Trimipramine kinetics and absolute bioavailability - use of gas-liquid-chromatography with nitrogen-phosphorus detection. *Clin Pharmacol Ther* 35: 348-353.
179. Abernethy DR, Wainer IW, Longstreth JA, and Andrawis NS (1993) Stereoselective verapamil disposition and dynamics in aging during racemic verapamil administration. *J Pharmacol Exp Ther* 266: 904-911.
180. Eichelbaum M, Mikus G, and Vogelgesang B (1984) Pharmacokinetics of (+)-verapamil, (-)-verapamil and (+/-)-verapamil after intravenous administration. *Br J Clin Pharmacol* 17: 453-458.
181. Bjornsson TD, Meffin PJ, Swezey S, and Blaschke TF (1979) Clofibrate displaces warfarin from plasma-proteins in man - example of a pure displacement interaction. *J Pharmacol Exp Ther* 210: 316-321.
182. Rosen AS, Fournie P, Darwish M, Danjou P, and Troy SM (1999) Zaleplon pharmacokinetics and absolute bioavailability. *Biopharm Drug Dispos* 20: 171-175.
183. Aweeka FT, Gambertoglio JG, Kramer F, Vanderhorst C, Polsky B, Jayewardene A, Lizak P, Emrick L, Tong W, and Jacobson MA (1995) Foscarnet and ganciclovir pharmacokinetics during concomitant or alternating maintenance therapy for aids-related cytomegalovirus retinitis. *Clin Pharmacol Ther* 57: 403-412.
184. Kilford PJ, Stringer R, Sohal B, Houston JB, and Galetin A (2009) Prediction of drug clearance by glucuronidation from in vitro data: use of combined cytochrome P450 and UDP-glucuronosyltransferase cofactors in alamethicin-activated human liver microsomes. *Drug Metab Dispos* 37: 82-89.

185. Machinist JM, Kukulka MJ, and Bopp BA (1995) In vitro plasma protein binding of zileuton and its N-dehydroxylated metabolite. *Clin Pharmacokinet* 29 Suppl 2: 34-41.
186. Durand A, Thenot JP, Bianchetti G, and Morselli PL (1992) Comparative pharmacokinetic profile of two imidazopyridine drugs: zolpidem and alpidem. *Drug Metab Rev* 24: 239-66.

Supplemental Table 2. Collated literature rat *in vivo* and *in vitro* parameter values for drug intrinsic clearance prediction

Compound	In Vivo					In Vitro					
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes			Microsomes		
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
Acetaminophen	1	24 [1]	0.82 [1]	0.82	39	1	0.93 [1]	76 [1]			
Alfentanil	1	45 [2]	0.24 [2]	0.16	342				1	0.97 [2]	572 [2]
Alprazolam	1	19 [3]	0.35 [3]	0.56	67	1	0.93	149 [4]	1	1.00 [4]	101 [4]
Antipyrine	5	5.1 [5-7]	1.00 [5]	1.00	5.4	2	1.0 [8]	4.3 [8]	1	0.93	8.1 [9]
Atorvastatin	3	35 [10-12]	0.036 [10, 12]	0.044	1470	3	0.92	413 [12-14]			
Bosentan	5	30 [15-17]	0.015 [15, 17]	0.016	2791	2	0.81	45 [15]			
Caffeine	1	0.017 [18]	1.00 [18]	0.80	0.017	1	0.97	7.0 [18]	1	0.93	11 [18]
Cerivastatin	1	39 [12]	0.041 [12]	0.029	1516	1	0.94	11 [12]			
Chlordiazepoxide	1	10 [3]	0.15 [3]	0.15	74	1	0.87	49 [4]	1	1.00 [4]	11 [4]
Chlorpromazine	1	61 [5]	0.068 [5]	0.067	2264						
Clobazam	1	32 [3]	0.21 [3]	0.21	220	1	0.90	137 [4]	1	1.00 [4]	264 [4]
Clonazepam	1	20 [3]	0.21 [3]	0.21	116	1	0.85	89 [4]	1	0.76 [4]	233 [4]
Dextromethorphan	1	62 [19]	0.26 [20]	0.45	635	5	0.52	696 [15, 20-22]	2	0.92 [20]	596 [20, 23]
Diazepam	5	51 [5, 24-27]	0.10 [5, 25-27]	0.13	999	7	0.54 [1]	432 [1, 4, 21, 22, 28, 29]	2	0.93 [4]	438 [4, 29]
Diclofenac	6	22 [30-33]	0.041 [30, 31]	0.022	687	3	0.94	369 [15, 28, 31]			
Diltiazem	2	71 [34, 35]	0.18 [34-36]	0.17	1342	2	0.36 [1]	3277 [1, 21]			
Domperidone	1	67 [2]	0.070 [2]	0.092	2826				1	0.34 [2]	698 [2]
Erythromycin	2	32 [34, 37]	0.60 [34, 37]	0.78	76	1	0.74	169 [14]			
Ethoxycoumarin	2	54 [3, 38]	0.22 [3]	0.22	515	5	0.86	282 [15, 22, 39]	3	0.79	248 [23, 39, 40]
Felodipine	1	3.8 [41]	0.070 [41]	0.10	56				1	0.09	13904 [41]
Fexofenadine	8	38 [42-45]	0.34 [42]	0.34	168	1	0.81	101 [14]			

Compound	<i>In Vivo</i>					<i>In Vitro</i>					
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes			Microsomes		
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
FK079	1	2.6 [1]	0.095 [1]	0.060	28	1	0.91 [1]	6.7 [1]			
Galantamine	1	32 [2]	0.76 [2]	0.76	61				1	0.86 [2]	32 [2]
Granisetron	1	41 [31]	0.61 [31]	0.61	114	1	0.87	302 [31]			
Ibuprofen	5	4.9 [46, 47]	0.038 [46]	0.023					1	0.91	83 [47]
Indinavir	2	51 [48, 49]	0.65 [48, 49]	0.40					2	0.77	326 [48, 50]
Indomethacin	1	0.60 [46]	0.0050 [46]	0.0030	121	1	0.95	5.1 [12]			
Ketanserin	1	5.9 [2]	0.018 [2]	0.012	339				1	0.32 [2]	42 [2]
Lorcainide	1	86 [2]	0.22 [2]	0.26	2862				1	0.45 [2]	1849 [2]
Lubeluzole	1	33 [2]	0.010 [2]	0.0080	4657				1	0.050 [2]	1387 [2]
Mazapertine	1	62 [2]	0.047 [2]	0.030	3459				1	0.13 [2]	6390 [2]
Metoprolol	2	73 [6, 51]	0.53 [6]	0.81	513	1	0.90	101 [21]	1	0.85	124 [52]
Midazolam	7	54 [3, 31, 53-55]	0.062 [31, 54, 56]	0.051	1925	7	0.98 [8]	460 [4, 8, 28, 31, 39]	2	0.68 [4, 39]	1447 [4, 39]
Naloxone	1	59 [57]	0.57 [57]	0.62	253	2	1.00 [8]	1273 [8]			
Nebivolol	1	41 [2]	0.013 [2]	0.015	5534				1	0.12 [2]	990 [2]
Nelfinavir	1	37 [49]	0.041 [49]	0.035	1426	1	0.26	41070 [58]	1	0.022 [58]	342857 [58]
Norcisapride	1	27 [2]	0.43 [2]	0.65	85				1	0.79 [2]	7.4 [2]
Oxodipine	1	18 [3]	1.00 [3]	1.00	22				1	0.58	31 [59]
Phenytoin	6	18 [5, 60-63]	0.23 [5, 60-63]	0.23	97	4	0.84	82 [21, 22, 60]			
Pindolol	1	59 [31]	0.64 [31]	0.64	225	1	0.91	251 [31]			
Prazosin	2	49 [31, 64]	0.33 [31]	0.33	296	1	0.94	41 [31]			
Propafenone	3	42 [65]	0.023 [65]	0.022	3227	1	0.50	10977 [65]	1	0.39	11753 [65]
Propranolol	8	74 [3, 5, 6, 15, 17, 51]	0.088 [5, 6, 15, 66]	0.091	3295	7	0.62	1746 [15, 21, 22, 39, 67, 68]	3	0.57 [68]	10705 [39, 68, 69]
Quinidine	3	28 [5, 70, 71]	0.20 [5, 70, 71]	0.31	193				1	0.53	19 [23]
Quintoloast	2	54 [72]	0.051 [72]	0.033	2341	1	0.88 [1]	58 [1]			
Repaglinide	2	8.8 [73, 74]	0.025 [75] [#]	0.015	384	1	0.63	382 [14]			

Compound	In Vivo					In Vitro					
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes			Microsomes		
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
Risperidone	1	76 [2]	0.14 [2]	0.12	2214				1	0.34 [2]	980 [2]
Ritonavir	2	30 [31, 49]	0.048 [31, 49]	0.040	894	2	0.39	7225 [31, 58]	1	0.23 [58]	38764 [58]
Rosuvastatin	5	51 [15, 43, 76-78]	0.084 [15, 78, 79]	0.064	1228	3	0.96	60 [14, 15]			
Sabeluzole	1	43 [2]	0.019 [2]	0.016	3912				1	0.06 [2]	956 [2]
Saquinavir	1	36 [49]	0.062 [49]	0.051	911	2	0.66	8428 [14, 58]	2	0.09 [58]	37818 [58, 80]
Tolbutamide	4	0.81 [5, 7, 81, 82]	0.13 [5, 81, 82]	0.10	6.1	4	0.83	9.8 [15, 22, 60]	3	0.76	11 [60, 83, 84]
Triazolam	1	84 [3]	0.28 [3]	0.19	1875	1	0.91	854 [4]	1	0.84 [4]	552 [4]
Troglitazone	2	37 [85]	0.16 [85]	0.092	386	1	0.12 [1]	4277 [1]			
Verapamil	4	43 [34, 35, 86, 87]	0.071 [34, 35, 86]	0.063	1056	2	0.52	664 [15, 21]			
S-Warfarin	4	0.24 [88-91]	0.021 [88-90]	0.012	12	2	0.94	0.85 [22]			
Zidovudine	1	41 [1]	0.79 [1]	0.79	87	1	1.00 [1]	14 [1]			
AZ1	1	11 [31]	0.025 [31]	0.014	499	1	0.82 [31]	68 [31]			
AZ2	1	16 [31]	0.071 [31]	0.039	265	1	0.85 [31]	113 [31]			
AZ3	1	12 [31]	0.035 [31]	0.019	388	1	0.89 [31]	36 [31]			
AZ4	1	36 [31]	0.032 [31]	0.032	1781	1	0.71 [31]	138 [31]			
AZ5	1	57 [31]	0.16 [31]	0.16	805	1	0.70 [31]	167 [31]			
AZ6	1	9.3 [31]	0.016 [31]	0.016	641	1	0.64 [31]	220 [31]			
AZ7	1	13 [31]	0.038 [31]	0.038	404	1	0.61 [31]	32 [31]			
AZ8	1	57 [31]	0.14 [31]	0.14	987	1	0.90 [31]	160 [31]			
AZ9	1	46 [31]	0.050 [31]	0.050	1697	1	0.75 [31]	182 [31]			
AZ10	1	23 [31]	0.029 [31]	0.029	1042	1	0.051 [31]	1126 [31]			
AZ11	1	40 [31]	0.086 [31]	0.086	775	1	0.82 [31]	29 [31]			
AZ12	1	50 [31]	0.086 [31]	0.086	1167	1	0.72 [31]	93 [31]			
AZ13	1	33 [31]	0.076 [31]	0.076	648	1	0.76 [31]	23 [31]			
AZ14	1	21 [31]	0.036 [31]	0.036	756	1	0.71 [31]	47 [31]			
AZ15	1	32 [31]	0.089 [31]	0.089	529	1	0.85 [31]	80 [31]			
AZ16	1	17 [31]	0.030 [31]	0.030	697	1	0.73 [31]	49 [31]			

Compound	In Vivo					In Vitro					
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes		Microsomes			
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
AZ17	1	13 [31]	0.19 [31]	0.19	81	1	0.95 [31]	17 [31]			
AZ18	1	47 [31]	0.085 [31]	0.085	1060	1	0.77 [31]	105 [31]			
AZ19	1	53 [31]	0.090 [31]	0.090	1258	1	0.91 [31]	194 [31]			
AZ20	1	39 [31]	0.013 [31]	0.013	4856	1	0.44 [31]	250 [31]			
AZ21	1	41 [31]	0.10 [31]	0.10	682	1	0.86 [31]	228 [31]			
AZ22	1	18 [31]	0.011 [31]	0.011	1955	1	0.36 [31]	497 [31]			
AZ23	1	8.7 [31]	0.0036 [31]	0.0020	2629	1	0.60 [31]	135 [31]			
AZ24	1	7.9 [31]	0.062 [31]	0.062	138	1	0.60 [31]	40 [31]			
AZ25	1	17 [31]	0.13 [31]	0.13	162	1	0.90 [31]	25 [31]			
AZ26	1	7.3 [31]	0.036 [31]	0.036	219	1	0.85 [31]	33 [31]			
AZ27	1	19 [31]	0.017 [31]	0.017	1407	1	0.51 [31]	385 [31]			
AZ28	1	17 [31]	0.084 [31]	0.084	244	1	0.79 [31]	59 [31]			
AZ29	1	22 [31]	0.045 [31]	0.045	630	1	0.55 [31]	205 [31]			
AZ30	1	50 [31]	0.056 [31]	0.056	1800	1	0.50 [31]	486 [31]			
AZ31	1	56 [31]	0.062 [31]	0.062	2028	1	0.78 [31]	159 [31]			
AZ32	1	25 [31]	0.064 [31]	0.064	515	1	0.79 [31]	63 [31]			
AZ33	1	51 [31]	0.13 [31]	0.13	792	1	0.77 [31]	130 [31]			
AZ34	1	32 [31]	0.076 [31]	0.076	631	1	0.61 [31]	92 [31]			
AZ35	1	1.5 [31]	0.0018 [31]	0.0010	812	1	0.46 [31]	29 [31]			
AZ36	1	16 [31]	0.083 [31]	0.083	233	1	0.59 [31]	71 [31]			
AZ37	1	2.0 [31]	0.0018 [31]	0.001	1122	1	0.44 [31]	135 [31]			
AZ38	1	40 [31]	0.19 [31]	0.19	349	1	0.74 [31]	207 [31]			
AZ39	1	42 [31]	0.047 [31]	0.047	1516	1	0.63 [31]	228 [31]			
AZ40	1	13 [31]	0.14 [31]	0.14	110	1	0.88 [31]	48 [31]			
AZ41	1	8.7 [31]	0.14 [31]	0.14	68	1	0.90 [31]	39 [31]			
AZ42	1	40 [31]	0.16 [31]	0.16	415	1	0.88 [31]	69 [31]			
AZ43	1	14 [31]	0.022 [31]	0.022	722	1	0.84 [31]	80 [31]			
AZ44	1	19 [31]	0.11 [31]	0.11	212	1	0.72 [31]	112 [31]			
AZ45	1	25 [31]	0.28 [31]	0.28	117	1	0.59 [31]	114 [31]			
AZ46	1	2.2 [31]	0.0018	0.0010	1227	1	0.43 [31]	152 [31]			

Compound	In Vivo					In Vitro					
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes			Microsomes		
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
AZ47	1	3.6 [31]	[31] 0.0090	0.0090	415	1	0.61 [31]	82 [31]			
AZ48	1	33 [31]	[31] 0.29 [31]	0.29	171	1	0.77 [31]	145 [31]			
AZ49	1	4.4 [31]	0.0080 [31]	0.008	575	1	0.82 [31]	157 [31]			
AZ50	1	15 [31]	0.011 [31]	0.011	1642	1	0.83 [31]	278 [31]			
H1	1	44 [92]	0.035 [92]	0.035	2275	1	0.60 [92]	338 [92]	1	0.46 [92]	542 [92]
H2	1	4.9 [92]	0.042 [92]	0.042	121				1	0.74 [92]	42 [92]
H3	1	0.050 [92]	0.0010 [92]	0.0010	50	1	0.73 [92]	124 [92]	1	0.48 [92]	240 [92]
H4	1	0.17 [92]	0.0010 [92]	0.0010	167	1	0.85 [92]	21 [92]	1	0.67 [92]	70 [92]
H5	1	1.4 [92]	0.011 [92]	0.011	132	1	0.81 [92]	105 [92]	1	0.89 [92]	178 [92]
H6	1	0.55 [92]	0.003 [92]	0.003	184	1	0.71 [92]	66 [92]	1	0.59 [92]	189 [92]
H7	1	0.58 [92]	0.003 [92]	0.003	196	1	0.69 [92]	209 [92]	1	0.53 [92]	158 [92]
H8	1	3.8 [92]	0.010 [92]	0.010	393	1	0.78 [92]	130 [92]	1	0.69 [92]	128 [92]
H9	1	4.2 [92]	0.0090 [92]	0.0090	487	1	0.57 [92]	212 [92]	1	0.48 [92]	213 [92]
H10	1	0.23 [92]	0.0010 [92]	0.0010	234	1	0.75 [92]	98 [92]	1	0.63 [92]	157 [92]
H11	1	15 [92]	0.041 [92]	0.041	416	1	0.29 [92]	180 [92]	1	0.23 [92]	231 [92]
H12	1	5.4 [92]	0.020 [92]	0.020	284	1	0.63 [92]	85 [92]	1	0.46 [92]	402 [92]
H13	1	100 [92]	0.051 [92]	0.051	390196	1	0.56 [92]	201 [92]	1	0.47 [92]	237 [92]
H14	1	5.0 [92]	0.0020 [92]	0.0020	2632	1	0.38 [92]	333 [92]	1	0.25 [92]	1966 [92]
H15	1	2.0 [92]	0.0060 [92]	0.0060	343	1	0.20 [92]	429 [92]	1	0.23 [92]	507 [92]
H16	1	0.72 [92]	0.0020 [92]	0.0020	361				1	0.32 [92]	199 [92]
H17	1	3.1 [92]	0.0060 [92]	0.0060	530	1	0.79 [92]	106 [92]	1	0.59 [92]	186 [92]
H18	1	4.9 [92]	0.0080	0.0080	639	1	0.70 [92]	67 [92]	1	0.46 [92]	53 [92]

Compound	<i>In Vivo</i>					<i>In Vitro</i>					
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes			Microsomes		
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *	CL _{int,u} (ml/min/kg)
H19	1	6.5 [92]	[92] 0.0060	0.0060	1165	1	0.89 [92]	114 [92]	1	0.89 [92]	77 [92]
H20	1	31 [92]	0.0050 [92]	0.0050	8777	1	0.63 [92]	828 [92]	1	0.69 [92]	880 [92]
H22	1	9.0 [92]	0.018 [92]	0.018	549	1	0.45 [92]	143 [92]	1	0.24 [92]	249 [92]
H23	1	0.25 [92]	0.0010 [92]	0.0010	251				1	0.74 [92]	86 [92]
H24	1	44 [92]	0.0030 [92]	0.0030	25873	1	0.69 [92]	191 [92]	1	0.48 [92]	343 [92]
H25	1	14 [92]	0.0040 [92]	0.0040	4042	1	0.57 [92]	78 [92]	1	0.63 [92]	240 [92]
H26	1	2.2 [92]	0.0080 [92]	0.0080	279	1	0.82 [92]	71 [92]	1	0.77 [92]	62 [92]
H27	1	7.4 [92]	0.014 [92]	0.014	567	1	0.63 [92]	158 [92]	1	0.69 [92]	275 [92]
H28	1	4.1 [92]	0.0040 [92]	0.0040	1064	1	0.88 [92]	110 [92]	1	0.27 [92]	44 [92]
H29	1	13 [92]	0.025 [92]	0.025	575	1	0.79 [92]	33 [92]	1	0.74 [92]	115 [92]
H30	1	6.3 [92]	0.097 [92]	0.097	70	1	0.94 [92]	65 [92]	1	1.00 [92]	19 [92]
H31	1	9.4 [92]	0.013 [92]	0.013	801	1	0.81 [92]	55 [92]	1	0.92 [92]	76 [92]
H32	1	1.8 [92]	0.0030 [92]	0.0030	605	1	0.89 [92]	52 [92]	1	0.63 [92]	25 [92]
H33	1	88 [92]	0.019 [92]	0.019	39214	1	0.21 [92]	368 [92]	1	0.07 [92]	1287 [92]
H34	1	29 [92]	0.0060 [92]	0.0060	6891	1	0.81 [92]	72 [92]	1	0.65 [92]	183 [92]
H35	1	47 [92]	0.017 [92]	0.017	5251	1	0.17 [92]	499 [92]	1	0.30 [92]	598 [92]
H37	1	60 [92]	0.0020 [92]	0.0020	75576	1	0.47 [92]	602 [92]	1	0.19 [92]	3071 [92]
H39	1	79 [92]	0.015 [92]	0.015	25436	1	0.75 [92]	330 [92]	1	0.46 [92]	16 [92]
H40	1	54 [92]	0.0080 [92]	0.0080	14952	1	0.63 [92]	125 [92]	1	0.61 [92]	17 [92]
H2a	1	79 [15]	0.036 [15]	0.025	10383	1	0.63 [15]	296 [15]			

Compound	<i>In Vivo</i>					<i>In Vitro</i>				
	n	CL _h (ml/min/kg)	fu _b	fu _p	CL _{int,u} (ml/min/kg)	Hepatocytes		Microsomes		
						n	fu _{hep} *	CL _{int,u} (ml/min/kg)	n	fu _{mic} *
H12a	1	77 [15]	0.0069 [15]	0.0040	49471	1	0.23 [15]	180 [15]		
H13a	1	8.0 [15]	0.0018 [15]	0.0010	4870	1	0.62 [15]	55 [15]		
H15a	1	64 [15]	0.0035 [15]	0.0020	49940	1	0.64 [15]	84 [15]		

n, number of studies; * fu_{hep} values are normalised to 1 x 10⁶ cells/ml and fu_{mic} values are normalised to 1 mg microsomal protein/ml. Experimentally determined values are followed by the accompanying reference; all other values were determined using lipophilicity relationship algorithms (Equations 2.2 and 2.1, fu_{hep} and fu_{mic} respectively). #Human fu_b value used in the absence of rat data.

Supplemental Table 2 References

1. Naritomi Y, Terashita S, Kagayama A, and Sugiyama Y (2003) Utility of hepatocytes in predicting drug metabolism: Comparison of hepatic intrinsic clearance in rats and humans in vivo and in vitro. *Drug Metab Dispos* 31: 580-588.
2. De Buck SS, Sinha VK, Fenu LA, Nijssen MJ, Mackie CE, and Gilissen RAHJ (2007) Prediction of human pharmacokinetics using physiologically based modeling: A retrospective analysis of 26 clinically tested drugs. *Drug Metab Dispos* 35: 1766-1780.
3. Ito K and Houston JB (2004) Comparison of the use of liver models for predicting drug clearance using in vitro kinetic data from hepatic microsomes and isolated hepatocytes. *Pharmaceut Res* 21: 785-792.
4. Jones HM and Houston JB (2004) Substrate depletion approach for determining in vitro metabolic clearance: Time dependencies in hepatocyte and microsomal incubations. *Drug Metab Dispos* 32: 973-982.
5. Sawada Y, Hanano M, Sugiyama Y, and Iga T (1985) Prediction of the disposition of 9 weakly acidic and 6 weakly basic drugs in humans from pharmacokinetic parameters in rats. *J Pharmacokinet Biop* 13: 477-492.
6. Belpaire FM, Desmet F, Vynckier LJ, Vermeulen AM, Rosseel MT, Bogaert MG, and Chauvelotmoachon L (1990) Effect of aging on the pharmacokinetics of atenolol, metoprolol and propranolol in the rat. *J Pharmacol Exp Ther* 254: 116-122.
7. Matthew DE and Houston JB (1990) Drug-metabolizing capacity in vitro and in vivo II. Correlations between hepatic microsomal monooxygenase markers in phenobarbital-induced rats. *Biochem Pharmacol* 40: 751-758.
8. Blanchard N, Richert L, Notter B, Delobel F, David P, Coassolo P, and Lave T (2004) Impact of serum on clearance predictions obtained from suspensions and primary cultures of rat hepatocytes. *Eur J Pharm Sci* 23: 189-199.
9. Buters JT and Reichen J (1990) Sex difference in antipyrine 3-hydroxylation. An in vivo-in vitro correlation of antipyrine metabolism in two rat strains. *Biochem Pharmacol* 40: 771-7.
10. Watanabe T, Kusuha H, Maeda K, Kanamaru H, Saito Y, Hu Z, and Sugiyama Y (2010) Investigation of the rate-determining process in the hepatic elimination of HMG-CoA reductase inhibitors in rats and humans. *Drug Metab Dispos* 38: 215-222.
11. Lau YY, Okochi H, Huang Y, and Benet LZ (2006) Pharmacokinetics of atorvastatin and its hydroxy metabolites in rats and the effects of concomitant rifampicin single doses: Relevance of first- pass effect from hepatic uptake transporters, and intestinal and hepatic metabolism. *Drug Metab Dispos* 34: 1175-1181.
12. Paine SW, Parker AJ, Gardiner P, Webborn PJH, and Riley RJ (2008) Prediction of the pharmacokinetics of atorvastatin, cerivastatin, and indomethacin using kinetic models applied to isolated rat hepatocytes. *Drug Metab Dispos* 36: 1365-1374.
13. Nordell P, Svanberg P, Bird J, and Grime K (2013) Predicting metabolic clearance for drugs that are actively transported into hepatocytes: incubational binding as a consequence of in vitro hepatocyte concentration is a key factor. *Drug Metab Dispos* 41: 836-843.
14. Jigorel E and Houston JB (2012) Utility of drug depletion-time profiles in isolated hepatocytes for accessing hepatic uptake clearance: Identifying rate-limiting steps and role of passive processes. *Drug Metab Dispos* 40: 1596-1602.
15. Huang LY, Chen A, Roberts J, Janosky B, Be XH, Berry L, and Lin MHJ (2012) Use of uptake intrinsic clearance from attached rat hepatocytes to predict hepatic clearance for poorly permeable compounds. *Xenobiotica* 42: 830-840.
16. Horiuchi I, Mori Y, Taguchi M, Ichida F, Miyawaki T, and Hashimoto Y (2009) Mechanisms responsible for the altered pharmacokinetics of bosentan: Analysis utilizing rats with bile duct ligation-induced liver dysfunction. *Biopharm Drug Dispos* 30: 326-333.
17. Lave T, Dupin S, Schmitt C, Chou RC, Jaeck D, and Coassolo P (1997) Integration of in vitro data into allometric scaling to predict hepatic metabolic clearance in man: Application to 10 extensively metabolized drugs. *J Pharm Sci* 86: 584-590.
18. Hayes KA, Brennan B, Chenery R, and Houston JB (1995) In-vivo disposition of caffeine predicted from hepatic-microsomal and hepatocyte data. *Drug Metab Dispos* 23: 349-353.

19. Roos FJ, Zysset T, and Reichen J (1991) Differential effect of biliary and micronodular cirrhosis on oxidative drug-metabolism – in vivo in vitro correlations of dextromethorphan metabolism in rat models. *Biochem Pharmacol* 41: 1513-1519.
20. Witherow LE and Houston JB (1999) Sigmoidal kinetics of CYP3A substrates: An approach for scaling dextromethorphan metabolism in hepatic microsomes and isolated hepatocytes to predict in vivo clearance in rat. *J Pharmacol Exp Ther* 290: 58-65.
21. Jacobson L, Middleton B, Holmgren J, Eirefelt S, Frjd M, Blomgren A, and Gustavsson L (2007) An optimized automated assay for determination of metabolic stability using hepatocytes: Assay validation, variance component analysis, and in vivo relevance. *Assay Drug Dev Technol* 5: 403-415.
22. Griffin SJ and Houston JB (2004) Comparison of fresh and cryopreserved rat hepatocyte suspensions for the prediction of in vitro intrinsic clearance. *Drug Metab Dispos* 32: 552-558.
23. Axelsson H, Granhall C, Floby E, Jaksch Y, Svedling M, and Sohlenius-Sternbeck AK (2003) Rates of metabolism of chlorzoxazone, dextromethorphan, 7-ethoxycoumarin, imipramine, quinidine, testosterone and verapamil by fresh and cryopreserved rat liver slices, and some comparisons with microsomes. *Toxicol In Vitro* 17: 481-488.
24. Igari Y, Sugiyama Y, Sawada Y, Iga T, and Hanano M (1984) In vitro and in vivo assessment of hepatic and extrahepatic metabolism of diazepam in the rat. *J Pharm Sci* 73: 826-828.
25. Tsang CFC and Wilkinson GR (1982) Diazepam disposition in mature and aged rabbits and rats. *Drug Metab Dispos* 10: 413-416.
26. Klotz U, Antonin KH, and Bieck PR (1976) Pharmacokinetics and plasma binding of diazepam in man, dog, rabbit, guinea-pig and rat. *J Pharmacol Exp Ther* 199: 67-73.
27. Diazgarcia JM, Oliverbotana J, and Fosgalve D (1992) Pharmacokinetics of diazepam in the rat - influence of a carbon tetrachloride-induced hepatic injury. *J Pharm Sci* 81: 768-772.
28. Lundquist P, Loof J, Fagerholm U, Sjogren I, Johansson J, Briem S, Hoogstraate J, Afzelius L, and Andersson TB (2014) Prediction of in vivo rat biliary drug clearance from an in vitro hepatocyte efflux model. *Drug Metab Dispos* 42: 459-468.
29. Zomorodi K, Carlile DJ, and Houston JB (1995) Kinetics of diazepam metabolism in rat hepatic microsomes and hepatocytes and their use in predicting in-vivo hepatic clearance. *Xenobiotica* 25: 907-916.
30. Kim YC, Oh EY, Kim SH, and Lee MG (2006) Pharmacokinetics of diclofenac in rat model of diabetes mellitus induced by alloxan or streptozotocin. *Biopharm Drug Dispos* 27: 85-92.
31. Sohlenius-Sternbeck AK, Jones C, Ferguson D, Middleton BJ, Projean D, Floby E, Bylund J, and Afzelius L (2012) Practical use of the regression offset approach for the prediction of in vivo intrinsic clearance from hepatocytes. *Xenobiotica* 42: 841-853.
32. Perisribera JE, Torresmolina F, Garciacarbonell MC, Aristorena JC, and Pladelfina JM (1991) Pharmacokinetics and bioavailability of diclofenac in the rat. *J Pharmacokinet Biop* 19: 647-665.
33. Zhang J, Li P, Guo HF, Liu L, and Liu XD (2012) Pharmacokinetic-pharmacodynamic modeling of diclofenac in normal and Freund's complete adjuvant-induced arthritic rats. *Acta Pharmacologica Sinica* 33: 1372-1378.
34. Yamano K, Yamamoto K, Kotaki H, Takedomi S, Matsuo H, Sawada Y, and Iga T (2000) Quantitative prediction of metabolic inhibition of midazolam by erythromycin, diltiazem, and verapamil in rats: Implication of concentrative uptake of inhibitors into liver. *J Pharmacol Exp Ther* 292: 1118-1126.
35. Yamano K, Yamamoto K, Kotaki H, Takedomi S, Matsuo H, Sawada Y, and Iga T (1999) Correlation between in vivo and in vitro hepatic uptake of metabolic inhibitors of cytochrome P-450 in rats. *Drug Metab Dispos* 27: 1225-1231.
36. Naritomi Y, Terashita S, Kimura S, Suzuki A, Kagayama A, and Sugiyama Y (2001) Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans. *Drug Metab Dispos* 29: 1316-1324.

37. Lam JL, Okochi H, Huang Y, and Benet LZ (2006) In vitro and in vivo correlation of hepatic transporter effects on erythromycin metabolism: Characterizing the importance of transporter-enzyme interplay. *Drug Metab Dispos* 34: 1336-1344.
38. Ashforth EIL (1991) Determination of in vivo clearance from in vitro hepatocyte and microsomal data (Unpublished Doctoral Thesis). Victoria University of Manchester, Manchester, UK.
39. Lu C, Li P, Gallegos R, Uttamsingh V, Xia CQ, Miwa GT, Balani SK, and Gan LS (2006) Comparison of intrinsic clearance in liver microsomes and hepatocytes from rats and humans: Evaluation of free fraction and uptake in hepatocytes. *Drug Metab Dispos* 34: 1600-1605.
40. Boobis AR, Kahn GC, Whyte C, Brodie MJ, and Davies DS (1981) Biphasic O-deethylation of phenacetin and 7-ethoxycoumarin by human and rat liver microsomal fractions. *Biochem Pharmacol* 30: 2451-2456.
41. Baarnhielm C, Dahlback H, and Skanberg I (1986) In vivo pharmacokinetics of felodipine predicted from in vitro studies in rat, dog and man. *Acta Pharmacol Tox* 59: 113-122.
42. Poirier A, Funk C, Scherrmann JM, and Lave T (2009) Mechanistic modeling of hepatic transport from cells to whole body: Application to napsagatran and fexofenadine. *Mol Pharm* 6: 1716-1733.
43. Lundquist P, Loof J, Sohlenius-Sternbeck A-K, Floby E, Johansson J, Bylund J, Hoogstraate J, Afzelius L, and Andersson TB (2014) The impact of solute carrier (SLC) drug uptake transporter loss in human and rat cryopreserved hepatocytes on clearance predictions. *Drug Metab Dispos* 42: 469-480.
44. Matsuda Y, Konno Y, Hashimoto T, Nagai M, Taguchi T, Satsukawa M, and Yamashita S (2013) In vivo assessment of the impact of efflux transporter on oral drug absorption using portal vein-cannulated rats. *Drug Metab Dispos* 41: 1514-1521.
45. Jaisue S, Gerber JP, and Davey AK (2010) Pharmacokinetics of fexofenadine following LPS administration to rats. *Xenobiotica* 40: 743-750.
46. Gardiner P and Paine SW (2011) The impact of hepatic uptake on the pharmacokinetics of organic anions. *Drug Metab Dispos* 39: 1930-1938.
47. Satterwhite JH and Boudinot FD (1991) Effects of age and dose on the pharmacokinetics of ibuprofen in the rat. *Drug Metab Dispos* 19: 61-67.
48. Lin JH, Chiba M, Balani SK, Chen IW, Kwei GYS, Vastag KJ, and Nishime JA (1996) Species differences in the pharmacokinetics and metabolism of indinavir, a potent human immunodeficiency virus protease inhibitor. *Drug Metab Dispos* 24: 1111-1120.
49. De Bruyn T (2014) Transporter-based boosting of HIV protease inhibitors (Unpublished Doctoral Thesis). KU Leuven, Leuven, Belgium.
50. Chiba M, Hensleigh M, and Lin JH (1997) Hepatic and intestinal metabolism of indinavir, an HIV protease inhibitor, in rat and human microsomes - major role of CYP3A. *Biochem Pharmacol* 53: 1187-1195.
51. Komura H, Kawase A, and Iwaki M (2005) Application of substrate depletion assay for early prediction of nonlinear pharmacokinetics in drug discovery: Assessment of nonlinearity of metoprolol, timolol, and propranolol. *J Pharm Sci* 94: 2656-2666.
52. Barham HM, Lennard MS, and Tucker GT (1994) An evaluation of cytochrome-P450 isoform activities in the female dark agouti (DA) rat - relevance to its use as a model of the CYP2D6 poor metabolizer phenotype. *Biochem Pharmacol* 47: 1295-1307.
53. Lau CE, Ma F, Wang YX, and Smith C (1996) Pharmacokinetics and bioavailability of midazolam after intravenous, subcutaneous, intraperitoneal and oral administration under a chronic food-limited regimen: Relating DRL performance to pharmacokinetics. *Psychopharmacology* 126: 241-248.
54. Kotegawa T, Laurijssens BE, Von Moltke LL, Cotreau MM, Perloff MD, Venkatakrishnan K, Warrington JS, Granda BW, Harmatz JS, and Greenblatt DJ (2002) In vitro, pharmacokinetic, and pharmacodynamic interactions of ketoconazole and midazolam in the rat. *J Pharmacol Exp Ther* 302: 1228-1237.
55. Vuppugalla R, Zhang YP, Chang S, Rodrigues AD, and Marathe PH (2012) Impact of nonlinear midazolam pharmacokinetics on the magnitude of the midazolam-ketoconazole interaction in rats. *Xenobiotica* 42: 1058-1068.

56. Cleton A, Mazee D, Voskuyl RA, and Danhof M (1999) Rate of change of blood concentrations is a major determinant of the pharmacodynamics of midazolam in rats. *Br J Pharmacol* 127: 227-235.
57. Mistry M and Houston JB (1987) Glucuronidation in vitro and in vivo - comparison of intestinal and hepatic conjugation of morphine, naloxone, and buprenorphine. *Drug Metab Dispos* 15: 710-717.
58. Parker AJ and Houston JB (2008) Rate-limiting steps in hepatic drug clearance: Comparison of hepatocellular uptake and metabolism with microsomal metabolism of saquinavir, nelfinavir, and ritonavir. *Drug Metab Dispos* 36: 1375-1384.
59. Flinois JP, Chabin M, Egros F, Dufour A, Dewaziers I, Maschamberlin C, and Beaune PH (1992) Metabolism rate of oxodipine in rats and humans - comparison of in vivo and in vitro data. *J Pharmacol Exp Ther* 261: 381-386.
60. Ashforth EIL, Carlile DJ, Chenery R, and Houston JB (1995) Prediction of in-vivo disposition from in-vitro systems - clearance of phenytoin and tolbutamide using rat hepatic-microsomal and hepatocyte data. *J Pharmacol Exp Ther* 274: 761-766.
61. Colburn WA and Gibaldi M (1977) Plasma-protein binding and metabolic clearance of phenytoin in rat. *J Pharmacol Exp Ther* 203: 500-506.
62. Bowdle TA, Neal GD, Levy RH, and Heimbach DM (1980) Phenytoin pharmacokinetics in burned rats and plasma-protein binding of phenytoin in burned patients. *J Pharmacol Exp Ther* 213: 97-99.
63. Itoh T, Sawada Y, Lin TH, Iga T, and Hanano M (1988) Kinetic analysis of phenytoin disposition in rats with experimental renal and hepatic diseases. *J Pharmacobio-dyn* 11: 289-308.
64. Ohkura T, Yamada S, Deguchi Y, and Kimura R (1999) Comparative study on pharmacokinetics and in vivo alpha(1)-adrenoceptor binding of H-3 tamsulosin and H-3 prazosin in rats. *Biol Pharm Bull* 22: 412-417.
65. Komura H and Iwaki M (2005) Nonlinear pharmacokinetics of propafenone in rats and humans: Application of a substrate depletion assay using hepatocytes for assessment of nonlinearity. *Drug Metab Dispos* 33: 726-732.
66. Rodgers T, Leahy D, and Rowland M (2005) Tissue distribution of basic drugs: Accounting for enantiomeric, compound and regional differences amongst beta-blocking drugs in rat. *J Pharm Sci* 94: 1237-1248.
67. Hallifax D and Houston JB (2006) Uptake and intracellular binding of lipophilic amine drugs by isolated rat hepatocytes and implications for prediction of in vivo metabolic clearance. *Drug Metab Dispos* 34: 1829-1836.
68. Hallifax D (1998) Hepatocellular binding of drugs and its implications in determination of metabolic clearance (Unpublished Doctoral Thesis). University of Manchester, Manchester, UK.
69. Ishida R, Obara S, Masubuchi Y, Narimatsu S, Fujita S, and Suzuki T (1992) Induction of propranolol metabolism by the azo dye sudan III in rats. *Biochem Pharmacol* 43: 2489-2492.
70. Fremstad D, Jacobsen S, and Lunde KM (1977) Influence of serum protein binding on the pharmacokinetics of quinidine in normal and anuric rats. *Acta Pharmacol Toxicol (Copenh)* 41: 161-76.
71. Harashima H, Sawada Y, Sugiyama Y, Iga T, and Hanano M (1985) Analysis of nonlinear tissue distribution of quinidine in rats by physiologically based pharmacokinetics. *J Pharmacokinet Biop* 13: 425-440.
72. Katashima M, Fujimoto R, Ishibashi K, Tokuma Y, and Noda K (1993) Pharmacokinetics of quinotolast sodium in rats, guinea pigs and dogs. *Drug Metab Pharmacokinet* 8: 505-514.
73. Choi JS, Choi I, and Choi DH (2013) Effects of nifedipine on the pharmacokinetics of repaglinide in rats: Possible role of CYP3A4 and P-glycoprotein inhibition by nifedipine. *Pharmacol Rep* 65: 1422-1430.
74. Li C, Choi DH, and Choi JS (2012) Effects of efonidipine on the pharmacokinetics and pharmacodynamics of repaglinide: possible role of CYP3A4 and P-glycoprotein inhibition by efonidipine. *J Pharmacokinet Pharmacodyn* 39: 99-108.
75. Gertz M, Harrison A, Houston JB, and Galetin A (2010) Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos* 38: 1147-1158.

76. Li AP, (2010) Evaluation of drug metabolism, drug-drug interactions, and in vitro hepatotoxicity with cryopreserved human hepatocytes, in *Hepatocytes in Methods in Molecular Biology* (P. Maurel eds) pp. 281-294, Humana Press,
77. Kitamura S, Maeda K, Wang Y, and Sugiyama Y (2008) Involvement of multiple transporters in the hepatobiliary transport of rosuvastatin. *Drug Metab Dispos* 36: 2014-2023.
78. Fukuda H, Ohashi R, Tsuda-Tsukimoto M, and Tamai I (2008) Effect of plasma protein binding on in vitro-in vivo correlation of biliary excretion of drugs evaluated by sandwich-cultured rat hepatocytes. *Drug Metab Dispos* 36: 1275-1282.
79. Watanabe T, Maeda K, Kondo T, Nakayama H, Horita S, Kusuhara H, and Sugiyama Y (2009) Prediction of the hepatic and renal clearance of transporter substrates in rats using in vitro uptake experiments. *Drug Metab Dispos* 37: 1471-1479.
80. Shibata Y, Takahashi H, Chiba M, and Ishii Y (2002) Prediction of hepatic clearance and availability by cryopreserved human hepatocytes: An application of serum incubation method. *Drug Metab Dispos* 30: 892-896.
81. Choi MR, Kwon MH, Cho YY, Choi HD, Kim YC, and Kang HE (2014) Pharmacokinetics of tolbutamide and its metabolite 4-hydroxy tolbutamide in poloxamer 407-induced hyperlipidemic rats. *Biopharm Drug Dispos* 35: 264-274.
82. Yamao T, Nakagami H, Furuhashi K, Onodera T, Kurosaki Y, Nakayama T, and Kimura T (1994) Pharmacokinetics of tolbutamide following intravenous and oral administrations in rats with obstructive-jaundice. *Biol Pharm Bull* 17: 691-695.
83. Eagling VA, Tjia JF, and Back DJ (1998) Differential selectivity of cytochrome P450 inhibitors against probe substrates in human and rat liver microsomes. *Br J Clin Pharmacol* 45: 107-114.
84. Veronese ME, Mcmanus ME, Laupattarakasem P, Miners JO, and Birkett DJ (1990) Tolbutamide hydroxylation by human, rabbit and rat-liver microsomes and by purified forms of cytochrome P-450. *Drug Metab Dispos* 18: 356-361.
85. Izumi T, Enomoto S, Hosiyama K, Sasahara K, Shibukawa A, Nakagawa T, and Sugiyama Y (1996) Prediction of the human pharmacokinetics of troglitazone, a new and extensively metabolized antidiabetic agent, after oral administration, with an animal scale-up approach. *J Pharmacol Exp Ther* 277: 1630-1641.
86. Lee YS, Yoon JN, Yoon IS, Lee MG, and Kang HE (2012) Pharmacokinetics of verapamil and its metabolite norverapamil in rats with hyperlipidaemia induced by poloxamer 407. *Xenobiotica* 42: 766-774.
87. Choi DH, Chang KS, Hong SP, Choi JS, and Han HK (2008) Effect of atorvastatin on the intravenous and oral pharmacokinetics of verapamil in rats. *Biopharm Drug Dispos* 29: 45-50.
88. Yacobi A and Levy G (1977) Comparative pharmacokinetics of coumarin anticoagulants 29: Elimination kinetics and anticoagulant activity of (S)-(-)-warfarin in rats before and after chronic administration. *J Pharm Sci* 66: 1275-1277.
89. Baars LGM, Schepers MT, Hermans JJR, Dahlmans HJJ, and Thijssen HHW (1990) Enantioselective structure-pharmacokinetic relationships of ring substituted warfarin analogs in the rat. *J Pharm Pharmacol* 42: 861-866.
90. Chan E, Hegde A, and Chen X (2009) Effect of rutin on warfarin anticoagulation and pharmacokinetics of warfarin enantiomers in rats. *J Pharm Pharmacol* 61: 451-458.
91. Yacobi A and Levy G (1974) Pharmacokinetics of warfarin enantiomers in rats. *J Pharmacokinet Biop* 2: 239-255.
92. Huang L, Berry L, Ganga S, Janosky B, Chen A, Roberts J, Colletti AE, and Lin M-HJ (2010) Relationship between passive permeability, efflux, and predictability of clearance from in vitro metabolic intrinsic clearance. *Drug Metab Dispos* 38: 223-231.