DMD Fast Forward. Published on October 12, 2005 as doi:10.1124/dmd.105.004838
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DMD #4838

APPLICATION OF A GENERIC PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL TO THE ESTIMATION OF XENOBIOTIC LEVELS IN HUMAN PLASMA

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Running title: GENERIC PBPK MODEL APPLIED TO HUMAN PLASMA LEVEL ESTIMATION

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Number of text pages: 33

Number of tables: 3

Number of figures: 5

Number of references: 32

Number of words in the Abstract: 248

Number of words in the Introduction: 375

Number of words in the Discussion: 1500

Non-standard abbreviations: ADME, absorption, distribution, metabolism and elimination; $AUC_{t1\text{-}tlast\text{-}DN}$, dose-normalized *AUC* from the first to the last recorded time points; CL_{int}, hepatic intrinsic metabolic clearance; fu_p, fraction unbound in plasma; fu_t, fraction unbound in the interstitial fluid; IQ, interquartile; MLFE, mean log fold error; PBPK, physiologically-based pharmacokinetic; PK, pharmacokinetic(s); QSPR, quantitative structure-property relationship; wMLFE, weighted MLFE.

ABSTRACT:

Estimation of xenobiotic kinetics in man frequently relies upon extrapolation from experimental data generated in animals. In an accompanying paper, we have presented a unique, generic, physiologically-based pharmacokinetic model, and described its application to the prediction of rat plasma pharmacokinetics from *in vitro* data alone. Here we demonstrate the application of the same model, parameterized for human physiology, to the estimation of plasma pharmacokinetics in man, and report a comparative evaluation against some recently published predictive methods that involve scaling from *in vivo* animal data. The model was parameterized through an optimization process, employing a training set of *in vivo* data taken from the literature, and validated using a separate test set of published *in vivo* data. On average, the vertical divergence of the predicted plasma concentrations from the observed data, on a semi-log concentration-time plot, was 0.47 log units. For the training set, more than 80% of the predicted values of a standardized measure of *AUC* were within threefold of the observed values; over 70% of the test set predictions were within the same margin. Furthermore, in terms of predicting human clearance for the test set, the model was found to match or exceed the performance of three published interspecies scaling methods, all of which showed a distinct bias towards over-prediction. We conclude that the generic PBPK model, as a means of integrating readily-determined *in vitro* and/or *in silico* data, is potentially a powerful, cost-effective tool for predicting human xenobiotic kinetics in drug discovery and risk assessment.

Physiologically-based pharmacokinetic (PBPK) models are mathematical descriptions of the flow of blood throughout the body, developed for the simulation of xenobiotic absorption, distribution and elimination. Such models have been employed by scientists from a number of different disciplines who are interested in the simulation and prediction of exposure (Grass and Sinko, 2002; Leahy, 2003).

The application of a generic form of a PBPK model to the prediction of xenobiotic plasma levels in rat following an intravenous dose has been reported in an accompanying publication (Brightman et al., 2005). Here we describe the work that we have done to parameterize the same PBPK model for man, and to assess the reliability of the model in estimating plasma levels of xenobiotics, where these values are known from experimentation. In addition, we draw comparisons with alternative methods for predicting human pharmacokinetic properties that involve extrapolation from experimental data generated in animals.

Just as there are numerous published compound-specific PBPK models for the rat that utilize data derived from *in vivo* studies (Sugita et al., 1982; Igari et al., 1983; Tsuji et al., 1983; Bernareggi and Rowland, 1991; Kawai et al., 1994; Blakey et al., 1997), there are many examples of comparable PBPK models for man that rely upon scaling from *in vivo* animal data in order to simulate the human pharmacokinetics of a particular compound, and frequently incorporate observed clearance data from human subjects (Igari et al., 1983; Sawada et al., 1985; Tsuji et al., 1985; Bernareggi and Rowland, 1991; Kawai et al., 1994; Kawai et al., 1998). The PBPK model for man presented herein appears to represent the only truly generic model to be published to date, since it has been parameterized for human physiology, independently of any specific compound, and the manner in which *in vivo* distribution and elimination kinetics are predicted is

the same for all xenobiotics. Furthermore, the only compound-dependent inputs that the model requires are readily determined *in vitro*, or even *in silico*.

In this paper, we have concentrated on predicting the *in vivo* pharmacokinetics of compounds for which plasma levels have been determined following an intravenous dose. Work that we have done to extend the model to predict both human and rat plasma levels following an oral dose will be reported separately.

Methods

Model Inputs. A generic PBPK model, which enables the prediction of the pharmacokinetic behaviour of any given compound dosed intravenously in a specified human population, without recourse to data derived through *in vivo* studies, is presented herein. The compound-dependent inputs required by the model are the same as those listed previously (Brightman et al., 2005)**.**

Model Description. The PBPK model is based upon that published by Bernareggi and Rowland (Bernareggi and Rowland, 1991), as shown in their Fig. 1, but with substantial modification of the tissue distribution and elimination components, and comprises a series of compartments representing 14 major organs and tissues in the body, interconnected by further compartments representing arterial and venous blood pools, according to the principles developed by Bischoff and others (Bischoff, 1975).

The additional features of the model, including adaptations to facilitate modelling of 'diffusion-limited' distribution of an intravenously administered compound into the various tissues and organs, as well as the various processes involved in renal excretion, are described in detail in the accompanying paper, discussing its application to the prediction of rat *in vivo* pharmacokinetics (Brightman et al., 2005).

Model Parameters. The physiological parameters used in the model were obtained from the literature and are given in the Appendix (which is available online as supplemental data); these were scaled according to the actual body weights of the subjects used in the clinical studies being simulated. Tissue and organ volumes were largely derived from a single comprehensive compilation of physiological data for use in pharmacokinetic models (Brown et al., 1997), supplemented by data from other sources for skin (Mapleson, 1963) and testes (Spector, 1956)

volumes, and represent the extravascular (combined interstitial fluid and intracellular space sub-compartments) volumes only. Blood flow rates were taken almost exclusively from Bernareggi and Rowland (Bernareggi and Rowland, 1991), with the exception of the blood flow to the testes (Williams and Leggett, 1989). The glomerular filtration rate and urine flow rate were from Davies and Morris (Davies and Morris, 1993) and Tang-Liu *et al*. (Tang-Liu et al., 1983), respectively, whilst the renal tubular lumen volume was obtained from a textbook of physiology (Pitts, 1974). A haematocrit of 0.441 (Altman and Dittmer, 1971) was assumed.

Parameterization of the distribution and elimination components of the generic PBPK model for human required the development of a number of correlation models. Two such models, for the prediction of parameters corresponding to the effective *in vivo* lipophilicity and plasma protein binding, were derived through a process of optimization of the performance of the PBPK model, as described in the companion paper (Brightman et al., 2005). A comprehensive training set of *in vivo* data was used for this purpose, and is described in greater detail below.

The following parameters were derived as detailed previously (Brightman et al., 2005): permeability-surface area products for organ and tissue distribution; intracellular space/interstitial fluid (unbound) partition coefficients; blood/plasma concentration ratio (R); parameters governing renal excretion; hepatic microsomal intrinsic clearance (CL_{int}) and fractions unbound in plasma (fu_p) and interstitial fluid (fu_t).

Stochastic simulations were performed in order to incorporate known variability in the subject body weights and imprecision in the values of the physicochemical parameters, as described previously (Brightman et al., 2005).

Training Dataset. The set of *in vivo* data employed in training the model comprised 180 instances of data (where an instance corresponds to a single plasma concentration-time profile) for 69 different compounds, and was derived from numerous published clinical studies of intravenous dosing in human subjects. The compounds in this diverse training set were drawn from many therapeutic areas. The required model inputs for the training set compounds were obtained as described previously (Brightman et al., 2005). As before, no attempt was made to eliminate compounds from the training set on the basis of any features of the *in vivo* pharmacokinetic behaviour.

Test Dataset. In order to objectively evaluate the performance of the model, an independent set of *in vivo* test data was constructed. This consisted of 39 instances of plasma concentration-time data for 18 compounds dosed intravenously in human subjects. These data were derived from the literature.

The test set compounds were varied in terms of physicochemical properties, and represented diverse therapeutic areas. The requisite model inputs for these compounds were derived as described previously (Brightman et al., 2005).

Calculation of the Plasma Concentration Weighted Mean Log Fold Error (wMLFE). For each pair of *in vivo* and simulated plasma concentration-time profiles, the log fold prediction error was determined at each simulated time point for which there were corresponding *in vivo* data, and the mean of these errors over all time points was calculated, to give an overall mean prediction error for each instance of simulated data. The wMLFE represents the weighted mean of these individual means. The weights used in the calculation arise from there being multiple instances and/or sources of *in vivo* data for several compounds, and hence the contribution of

each individual log fold prediction error to the overall mean is weighted accordingly; i.e., so that each compound contributes equally, whatever the number of instances of *in vivo* data for that compound.

Results

Model Validation. For any given set of input data, output from the PBPK model is in the form of a predicted plasma concentration-time profile. When stochastic simulations are performed for a single set of input data, each iteration generates a predicted profile, and hence the total output consists of a population of profiles that reflect the inherent uncertainty in the input data. Examples of typical simulation results, plotted on the same axes as the corresponding *in vivo* data, are given in Fig. 1 for selected training set compounds and in Fig. 2 for a similar selection of test set compounds.

The simulated profiles in Figs. 1A and B and Figs. 2A and B illustrate accurate estimation of plasma concentrations over time, for selected training set and test set compounds, respectively. There is little variation within the population of profiles generated for either of the training set compounds dexamethasone and verapamil (Figs. 1A and B), and the fit of the model output to the single set of observed data is precise and almost exact in both cases. Conversely, Figs. 2A and B demonstrate accurate, but less precise simulation of the *in vivo* plasma data for the test compounds, biperiden and acecainide; although the median predicted profiles for these compounds depart very slightly from the observed profiles, the ranges of predicted profiles encompass the *in vivo* data. The variation within the model output for biperiden, for example, was generated from both known variability in the subject weights, and a combination of variability and uncertainty arising from multiple estimates of *in vivo* fu_p and CL_{int}.

Some other simulation results are shown in Figs. 1C and D and Figs. 2C and D. Somewhat inaccurate estimation of the *in vivo* tissue distribution of the training set compound phenytoin, resulting in a tendency to under-predict plasma levels, is indicated by Fig 1C. However, there is

clearly a degree of variability in the observed data for this compound, which is reflected in the model output. In contrast, the elimination half-life of alprazolam has been underestimated (Fig. 1D). The median predicted profiles for the test set compounds dofetilide and budesonide deviate from the observed profiles (Figs. 2C and D), but the *in vivo* data are captured by the ranges predicted; hence, the model results can still be considered acceptable.

For the remainder of the results presented here, the median of the population of predicted profiles generated from each set of input data was used as an individual estimate of the plasma concentration time course. In order to assess the overall performance of the model in terms of successfully predicting *in vivo* plasma levels, the plasma concentration wMLFE was determined for both the training and test sets; this statistic corresponds to the mean vertical deviation (in log units) of a simulated data point from a corresponding observed data point on a semi-log plot of plasma concentration versus time. A plasma concentration wMLFE of 0.47 was determined for both the training set and the test set. By way of illustration, the median predicted profiles shown in Fig. 1C and Fig. 2C both have an associated MLFE of approximately 0.47.

The frequency distributions of the actual mean fold errors in plasma concentration prediction for both sets of compounds are shown in Fig. 3. Interestingly, the results appear rather better for the test set than for the training set, in that plasma concentration predictions are on average within a factor of two above or below the observed data points for a far greater percentage of the test set compounds. However, given the relatively small size of the test set, this discrepancy is unlikely to be significant. In fact, very similar proportions of the two sets (59% of the training set and 61% of the test set) are on average within threefold of the observed data, and the same percentage of each set mean has a mean prediction error of more than fivefold.

Although the primary outputs from the PBPK model are the predicted *in vivo* plasma concentration-time profiles that are generated, these can be utilized for estimation of standard pharmacokinetic (PK) parameters of interest, including area under the concentration-time curve (*AUC*), allowing direct comparisons to be made with analogous *in vivo* data. Since different methods of extrapolating *AUC* from zero time to the first time point and from the last time point to infinity can vary in the estimates they yield, the simulation results were compared to observed data in terms of a standardized parameter, the dose-normalized *AUC* from the first to the last recorded time points ($AUC_{t1\text{-}tlast\text{-}DN}$).

The capability of the PBPK model to accurately predict $AUC_{t1\text{-}tlast\text{-}DN}$ in man has been evaluated in terms of the median values and interquartile (IQ) ranges of the predicted/observed ratios, for both the training and test sets, as given in Table 1. These summary data indicate that prediction of $AUC_{t1\text{-}tlast\text{-}DN}$ is generally successful, although the predictions for the test set are again apparently more accurate than those for the training set. Thus, the median predicted/observed ratio is close to 1.0 for either set of compounds, and half of the test set predictions are within a factor of approximately 1.5 above or below the observed values, but the range is slightly greater for the same proportion of training set predictions.

The frequency distributions of the predicted/observed ratios of $AUC_{t1\text{-}tlast\text{-}DN}$ for both the training and test sets are more clearly demonstrated by the histograms shown in Fig. 4. The majority (55%) of the predictions for the training set compounds are within twofold of the observed values, and more than 80% are within threefold. Similarly, over 60% of the test set predictions are within a factor of two of the observed values, and greater than 70% are within a factor of three. The predicted/observed ratios for this small test set are somewhat skewed towards low values.

DMD Fast Forward. Published on October 12, 2005 as DOI: 10.1124/dmd.105.004838
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Comparison of the PBPK Model with Interspecies Pharmacokinetic Extrapolation Methods. In order to further evaluate the potential of the generic PBPK model as a means for estimating the human pharmacokinetics of novel compounds within a drug discovery program, a performance-related comparison was made between the model parameterized for human and three recently published interspecies scaling methods for the prediction of clearance in man from rat *in vivo* data alone, derived for possible use as early pharmacokinetic screening tools (Caldwell et al., 2004; Ward and Smith, 2004). Both of the methods published by Caldwell *et al*. utilize simple allometric scaling (Caldwell et al., 2004), whilst the technique of Ward and Smith considers clearance as a fixed proportion of liver blood flow (Ward and Smith, 2004).

Predictions of human clearance for the different sets of compounds used in training the PBPK model, by Caldwell *et al.* and by Ward and Smith, were evaluated in terms of the average fold error, as defined by Caldwell *et al*. (Caldwell et al., 2004), and by the proportion of predictions within two-, three- and fourfold of the observed values. The statistics presented for the two Caldwell *et al.* methods have been reproduced directly from the original source. In order to calculate comparable statistics for the Ward and Smith method, it was necessary to regenerate the original results of these authors from rat clearance data presented graphically in the source publication. The majority, but not all (97 out of 103), of the data points could be extracted from the source. However, the median prediction fold error calculated for this subset was the same as that reported in the publication for the full training set, and hence for the purposes of the analysis described here, the fold error distribution we derived was assumed to be acceptably close to the true distribution.

For many of the compounds in the PBPK model training set there were multiple instances of *in vivo* data, derived from one or more published sources. The statistics shown for the PBPK model

have therefore been calculated on the basis of the weighted mean fold prediction error for each compound, and hence are directly comparable with those for the published interspecies scaling methods, which were also determined on a per-compound basis. The weighting system employed was the same as that used in the calculation of the plasma concentration wMLFE. There was considerable overlap in the compounds comprising the training sets for the four different methods.

The results of this analysis for the individual methods and their respective training sets, as shown in Table 2, demonstrate that the PBPK model apparently compares favourably with the Ward and Smith method, with the former having a lower average fold error and predicting a slightly greater proportion of compounds within a factor of three of the observed values. However, they also suggest that the current version of the PBPK model might be somewhat less accurate than the methods of Caldwell *et al*. in the quantitative prediction of human clearance.

In order to further investigate the relative capabilities of the four methods, a similar statistical analysis of their predictions of clearance in man for the PBPK model test set compounds was carried out, and is summarized in Table 3. This test set was completely independent, so that the compounds selected for inclusion were not present in any of the training sets, but also represented those for which suitable rat and human *in vivo* intravenous dosing data were readily available. Predictions of human clearance made by the Caldwell *et al.* and Ward and Smith methods were calculated from published values of rat *in vivo* clearance for the test set compounds and evaluated against the corresponding published human clearances. Again, the statistics for the PBPK model were derived from the weighted mean fold prediction error for each compound.

The predictive accuracy of each method, and particularly of the Ward and Smith method, is generally poorer for the test set compounds than for those comprising the respective training sets, but still at what might be considered an acceptable level for the early stages of drug discovery (Caldwell et al., 2004; Ward and Smith, 2004). However, the deterioration in overall performance is proportionately less for the PBPK model than for the other methods. Consequently, the expected accuracy of the PBPK model seems to be at least comparable to that of Caldwell *et al.*'s methods in terms of predicting human clearance for a set of novel compounds. For both methods, the same number of predicted values are within a factor of two of the observed values, and an equal or greater proportion of the PBPK model predictions are within a factor of three or four above or below the observed values, although the average fold error for the PBPK model is rather higher. On the evidence shown, the PBPK model would appear however, to considerably outperform the method of Ward and Smith in predicting human clearance for novel compounds. Moreover, the PBPK modelling approach has the obvious advantage over all three interspecies extrapolation methods that no *in vivo* data are required in order to predict human pharmacokinetics, with a better or similar degree of accuracy.

Consideration of the median values of the predicted/observed ratios for the test set (Table 3) also reveals an interesting trend in the values predicted by the interspecies scaling methods, which all appear to have a tendency to over-predict human clearance, to varying degrees. This is confirmed by the histograms shown in Fig. 5, illustrating the frequency distribution of the predicted/ observed ratios for each method. Although most of the values predicted by either of the Caldwell *et al*. methods are within a factor of two of the observed values, an almost equivalent number are between two and ten times the observed values (Fig. 5A and B). The majority (56%) of the values predicted by the Ward and Smith method are also between two and ten times greater than

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the observed values (Fig. 5C). In contrast, the errors in the PBPK model predictions are normally distributed, with the highest single proportion of predictions, and the majority overall, being within twofold of the observed values (Fig. 5D).

It is notable that all four methods were found to substantially over-predict clearance in man of the test set compound digitoxin, which is also a significant outlier for the prediction of $AUC_{\text{t1-tlast-DN}}$ by the PBPK model: the predicted/observed ratios for clearance are 14.5, 9.5, 15.0 and 22.1 for the two Caldwell *et al*. methods, Ward and Smith's method and the PBPK model, respectively. However, the human clearance of quinine, also in the test set, is similarly poorly predicted by the three interspecies scaling methods (predicted/observed ratios of 9.5, 10.5 and 16.6 for Caldwell *et al*.'s and Ward and Smith's methods, respectively), but more successfully predicted by the PBPK model, with a predicted/observed ratio of 4.3.

Finally, the same analysis was applied to a further method published by Ward and Smith for predicting human clearance, requiring scaling from clearance in monkey (Ward and Smith, 2004). The average fold error for the training set data of these authors was found to be 2.04, with 64%, 78% and 89% of the predictions being less than two-, three- and fourfold in error, respectively. The overall level of performance therefore does not greatly exceed that demonstrated by the Caldwell *et al*. methods when applied to the corresponding training set. Hence, it would have been interesting to establish whether the same deterioration in predictive capability might be shown by this alternative method of Ward and Smith, when applied to the PBPK model test set, as observed for their analogous method that depends on scaling from rat clearance. However, appropriate monkey *in vivo* data were unfortunately not available for this particular set of compounds. Similarly, Obach et al. have reported several methods of predicting clearance in man (as well as volume of distribution and half-life) from preclinical

pharmacokinetic data and/or *in vitro* data (Obach et al., 1997), but the compounds used in their analysis were not identified, and hence we were unable to directly compare the performances of the PBPK model and these particular methods.

Discussion

In this and the preceding paper (Brightman et al., 2005), we have demonstrated that a generic PBPK model can produce reliable predictions of mammalian PK following intravenous dosing, for a wide range of organic compounds, from a small number of readily-determined, compound-dependent inputs. We expect the overall physiological description to be common to all mammalian species, and that the fundamental model could be successfully adapted to any species by changing the physiological parameters.

Since a PBPK model is physiologically realistic, with explicit equations for flows into the major tissues and organs of the body, the compound-dependent parameters required to simulate PK are real properties that can be measured. Consequently, such models can be used to define a minimum set of absorption, distribution, metabolism and elimination (ADME) properties that must be obtained by *in vitro* or *in silico* screening in order to predict *in vivo* PK for any compound. In the same way that clinical trials are the point at which all the experimental data generated during drug discovery and preclinical development are integrated to produce a clear picture of the clinical potential of a new drug, so PBPK models can create the framework for integrating ADME, toxicity and efficacy data throughout discovery and even preclinical development. Our aim is to develop models that are driven by inputs that can be determined within imposed cost and time constraints, and can reliably inform compound selection, by being able to predict human PK with sufficient confidence for a particular phase of the discovery/development process.

The PBPK model that we have described in this paper generates predictions of plasma concentrations and clearance in man that appear to be sufficiently reliable to inform compound

selection during early drug discovery. We have not, so far, been able to validate the human version of the model against drug-discovery compounds to the same extent as the rat version. Nonetheless, there is no reason to believe that the relative robustness shown by the latter model in its ability to predict PK for compounds that are distant from the training set is not also a property of the human model. We have, however, demonstrated the equal or even superior ability of the current version of the human model to predict clearance when compared to interspecies extrapolation methods. Further development of the model, for example, through incorporation of additional physiological or biochemical processes currently not simulated, extension of the training set and/or the use of additional inputs, will serve to further enhance the predictive capability of the model above methods that are dependent on *in vivo* animal experimentation.

The maximum value that can be extracted from the use of this model within drug discovery largely depends upon the *modus operandi* of an individual company, drug-discovery programme or project. Significant factors that determine the relative merits of applying the model in any particular situation include: the number of compounds passing through the successive stages of the discovery process; the role of early ADME determination; access to low-cost ADME screens; the methods used for lead expansion and the usage of *in silico* techniques. Assays are available, for each of the required model inputs, that have sufficiently high throughput to enable human PK prediction using measured values within the timescale dictated by successive synthesis rounds of a typical project in lead optimization. Recent, well-documented changes in practice within the pharmaceutical industry have led to ADME data being generated more thoroughly and earlier in drug discovery than was previously the case. Hence, experimental measurement of some or all of the required inputs will be routinely available during lead optimization for many projects. Alternatively, *in silico* methods can be used to predict one or more inputs, in order to reduce cost,

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increase throughput and/or reduce the requirement for the physical compound, the availability of which may be restricted during early discovery. Consequently, we anticipate that the use of rapid *in vitro*/*in silico* screening of ADME and physicochemical properties, coupled to the prediction of human PK through PBPK modelling, will enable the optimization of human PK to be a significant determinant of the lead expansion and optimization phases, rendering rat and mouse PK studies increasingly unnecessary.

The logical extension is that all the required inputs could be predicted by *in silico* methods, enabling virtual human PK screening. The PK of large numbers of virtual compounds can be simulated by the model over a relatively short time scale (simulating a 24-hour time course takes a fraction of a second on a 1.3 GHz server with 1 GB of RAM running Red Hat Linux 7.2); calculation of the compound-dependent inputs, rather than execution of the PBPK model, is rate-limiting for some methods of input value prediction. In addition, due to the capability of the model to perform Monte-Carlo simulations, uncertainty in the values of the predicted inputs can be transformed into uncertainty in the predicted PK. This permits assessment of the associated risk when making assumptions based on any combination of predicted inputs and the uncertainty in those inputs. Thus, when using *in silico* prediction of its inputs, PBPK modelling has a potential role to play during lead identification, and even in prioritizing compounds to be passed through biological activity screens. The balance of how to use available computing resources most effectively, between the number of compounds to simulate and the number of Monte Carlo iterations to perform, depends on: the number of compounds (virtual or real) under consideration; the number to be taken forward; the quality of the predicted model inputs and the acceptable degree of uncertainty in PK when selecting compounds. It is important to note that the uncertainty in predicted PK that results from uncertainty in the inputs is highly dependent on the

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combination of input values for a compound. Thus, high uncertainty in CL_{int} transforms to relatively low uncertainty in predicted PK for compounds whose unbound CL_{int} (i.e., $f_{up} \cdot CL_{int}$) is greater than hepatic blood flow, whereas for compounds with low CL_{int}, uncertainty in predicted *AUC* is nearly proportional to uncertainty in CL_{int}.

During such a virtual screening process, care must be taken with the input prediction. Whilst commercial software is available for predicting many of the inputs to the PBPK model, the ability of such software to generalize with acceptable accuracy to drug discovery compounds is not guaranteed, as in most cases these compounds will lie outside the property space of the training data for the underlying models. Consequently the suitability of predictive software should be determined by comparison with experimental data for a subset of the compounds to be screened. In those cases where reliability is not sufficient, possible alternative courses of action include building quantitative structure-property relationship (QSPR) models to make corrections to the predicted output for particular chemistry (some commercial software permits this local training) or developing bespoke QSPR predictions. Fortunately, one of the most difficult properties to predict reliably, CL_{int}, is one of the simplest to determine experimentally. This provides the potential for inexpensively generating a significant experimental clearance database, for use in PK prediction and in the development of QPSR models for predicting clearance of further compounds.

As compounds progress towards preclinical development, the limitations of the current generic PBPK model, in both predictive reliability and the amount of information concerning the determinants of PK behaviour that project teams typically require, are likely to become apparent. The potential for further model development, however, means that such restrictions need only be provisional. In principle, any process that affects PK can be incorporated into the model. The

only limitations are the availability of relevant, usable data with which to modify the model and, if the changes require additional inputs, the availability of appropriate data to drive the model for novel compounds. As our understanding of the processes that govern PK increases, along with the availability of cost-effective *in vitro* techniques for their determination, so generic PBPK models can evolve to incorporate them. Within this scheme, the PBPK model can play an active role, informing *in vitro* and *in silico* method development regarding the accuracy and precision required for reliable *in vivo* prediction. We can, in time, expect to see developments in *in vitro* and *in silico* methods that enable successful PBPK-based prediction further along the discovery/development pipeline. Other potential developments of the PBPK model are less dependent on the provision of additional *in vitro* assays. These include improvements in the prediction of PK differences arising from sex, age and body-weight differences, permitting realistic inter-individual variability to be simulated.

As we have already discussed (Brightman et al., 2005), the capacity to reliably predict mammalian PK from *in vitro* or *in silico* inputs is also of great potential benefit in assessing the risk posed to populations from exposure to environmental chemicals, without recourse to animal experimentation, and the consequent uncertainties in interspecies extrapolation to man. Consequently, in the light of the results presented here, we conclude that the generic PBPK model can be a powerful, efficient and cost-effective tool for xenobiotic PK prediction and reduction of *in vivo* animal experimentation in industry.

Acknowledgements

The authors thank Dr. Darwin Cheney, for his helpful comments on the manuscript.

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Legends for Figures

FIG. 1. *Predicted and observed plasma concentration-time profiles for selected training set compounds: A, dexamethasone; B, verapamil; C, phenytoin; D, alprazolam.*

The *in vivo* data (filled symbols) are taken from the literature (Odar-Cederlof and Borga, 1974; Tsuei et al., 1979; Dominic et al., 1981; Smith et al., 1984). The simulated data are the median values (solid line) of a population of predicted profiles generated from 100 stochastic simulations; also indicated are the 10^{th} (dashed line) and 90^{th} (dotted line) percentiles of the population.

FIG. 2. *Predicted and observed plasma concentration-time profiles for selected test set compounds: A, biperiden; B, acecainide; C, dofetilide; D, budesonide.*

The *in vivo* data (filled symbols) are taken from the literature (Ryrfeldt et al., 1982; Grimaldi et al., 1986; Coyle et al., 1991; Smith et al., 1992). The simulated data are the median values (solid line) of a population of predicted profiles generated from 100 stochastic simulations; also indicated are the 10^{th} (dashed line) and 90^{th} (dotted line) percentiles of the population.

FIG. 3. *Frequency distribution of the plasma concentration mean fold errors for the training set (A) and test set (B) compounds.*

The training set and test set comprise 69 and 18 different drugs, respectively.

FIG. 4. *Frequency distribution of the predicted/observed ratios of* $AUC_{t1\text{-}tlast\text{-}DN}$ *for the training set (A) and test set (B) compounds.*

The training set and test set comprise 69 and 18 different drugs, respectively.

FIG. 5. *Frequency distribution of the predicted/observed ratios of clearance for the 18 test set compounds: A, Method I of Caldwell et al.; B, Method II of Caldwell et al.; C, the method of Ward and Smith; D, PBPK Model.*

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Tables

TABLE 1

Summary of the $AUC_{t1\text{-}tlast\text{-}DN}$ predicted/observed ratio distributions for the training set and test set compounds.

Training Set		Test Set	
Median	**IQ Range**	**Median**	**IQ Range**
1.14	0.65-2.44	0.96	0.73-1.55

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TABLE 2

The prediction of human clearance by the PBPK model and the published interspecies extrapolation methods of Caldwell et al. (2004) and Ward and Smith (2004) for the respective training sets.

The value of n corresponds to the number of compounds in each training set.

	Caldwell *et al*. (n=176)		**Ward and Smith (n=97)**	**PBPK Model (n=69)**
	Method I	**Method II**		
Average Fold Error	2.16	2.22	2.64	2.51
Fold Error < 2	56%	52%	49%	46%
Fold Error < 3	77%	79%	70%	72%
Fold Error < 4	88%	86%	79%	75%

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TABLE 3

The prediction of human clearance by the PBPK model and the published interspecies scaling methods of Caldwell et al. (2004) and Ward and Smith (2004) for the PBPK model test set (n=18).

	Caldwell *et al*.		**Ward and Smith**	**PBPK Model**
	Method I	**Method II**		
Average Fold Error	2.61	2.53	3.44	2.78
Fold Error < 2	50%	50%	28%	50%
Fold Error < 3	56%	56%	44%	61%
Fold Error < 4	72%	67%	61%	72%
Median Predicted/Observed	1.74	1.60	2.54	1.06

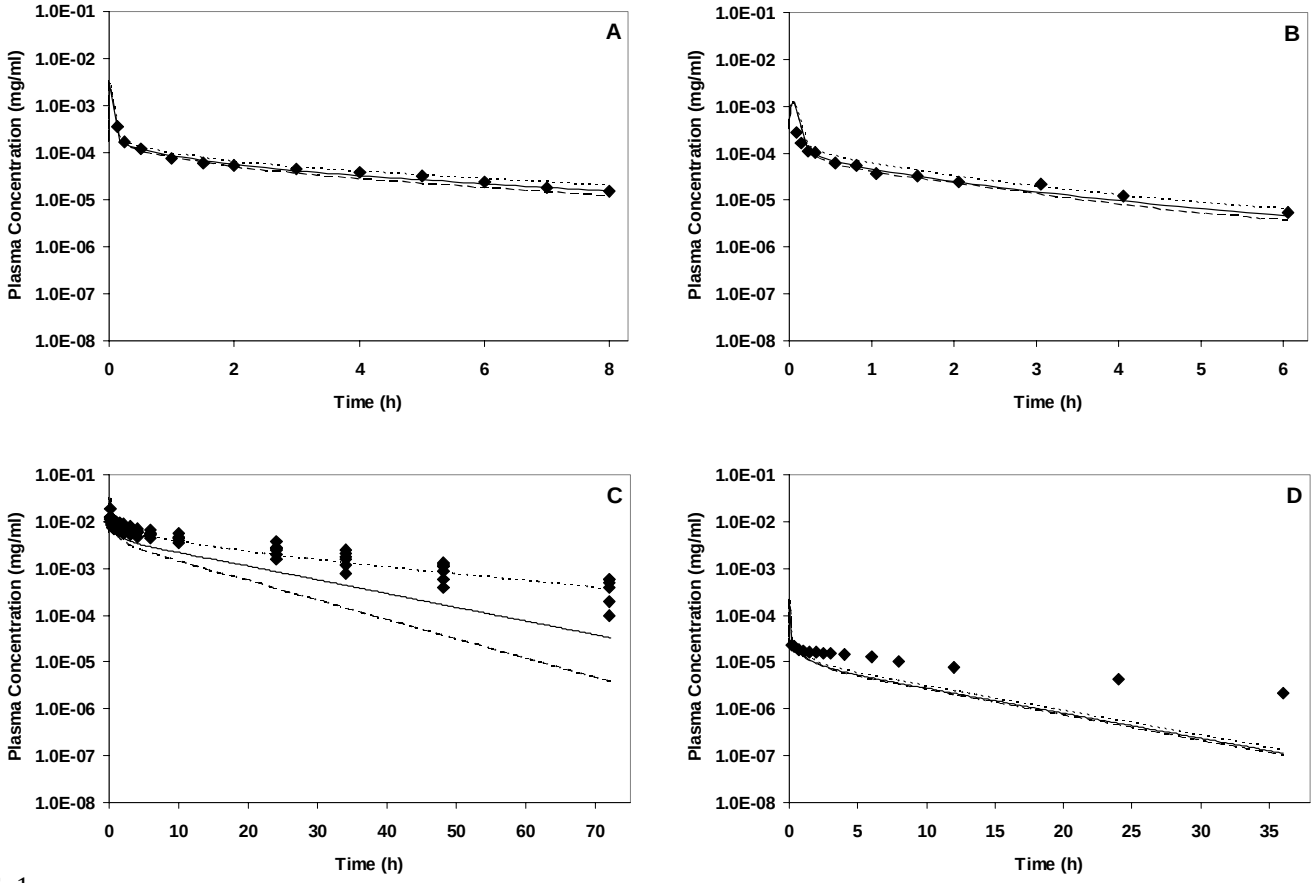

FIG. 1.

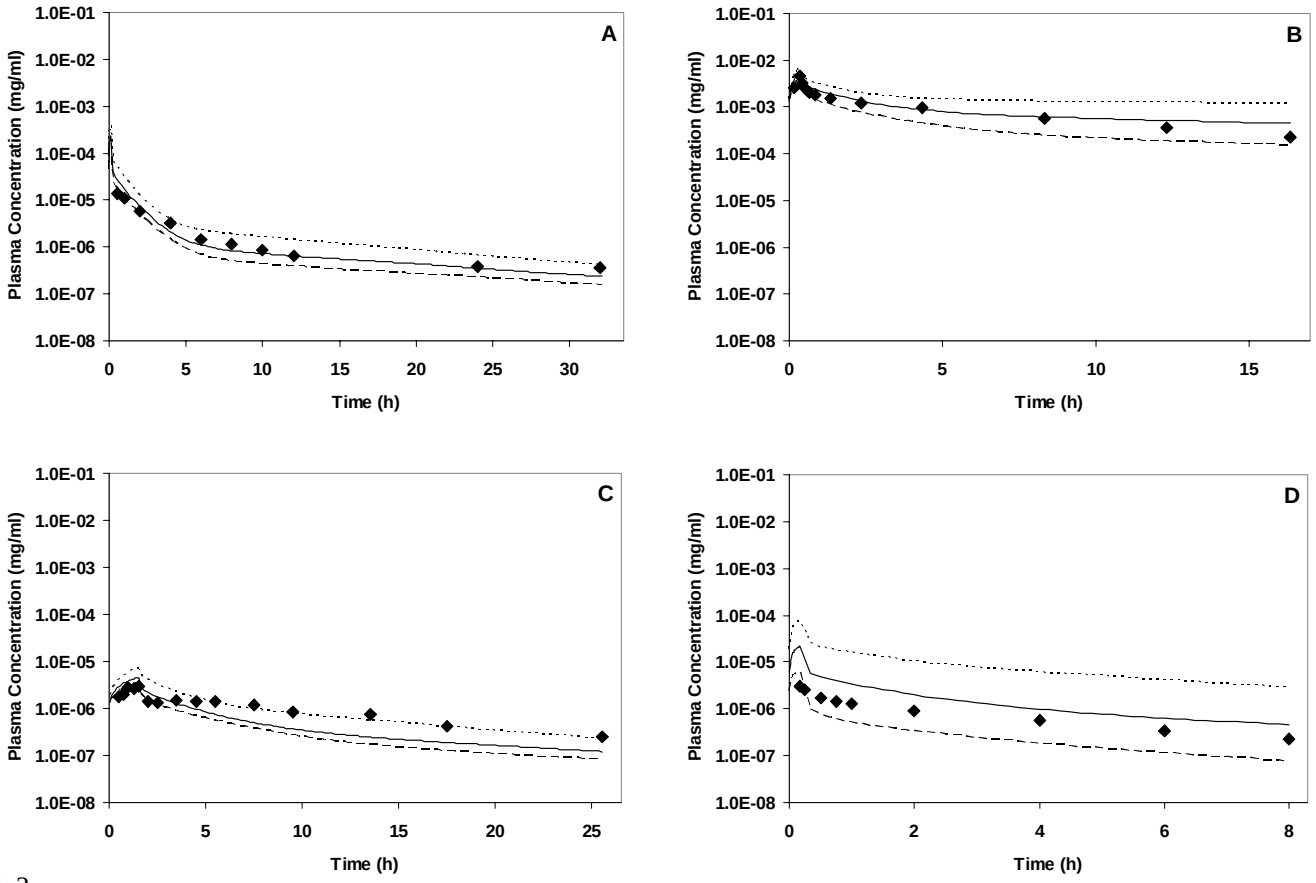

FIG. 2.

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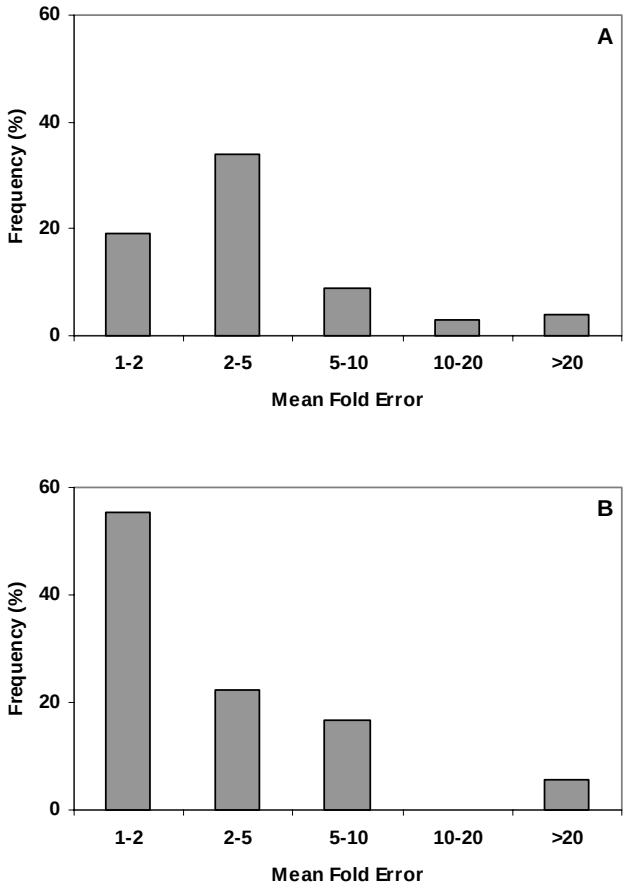

FIG. 3.

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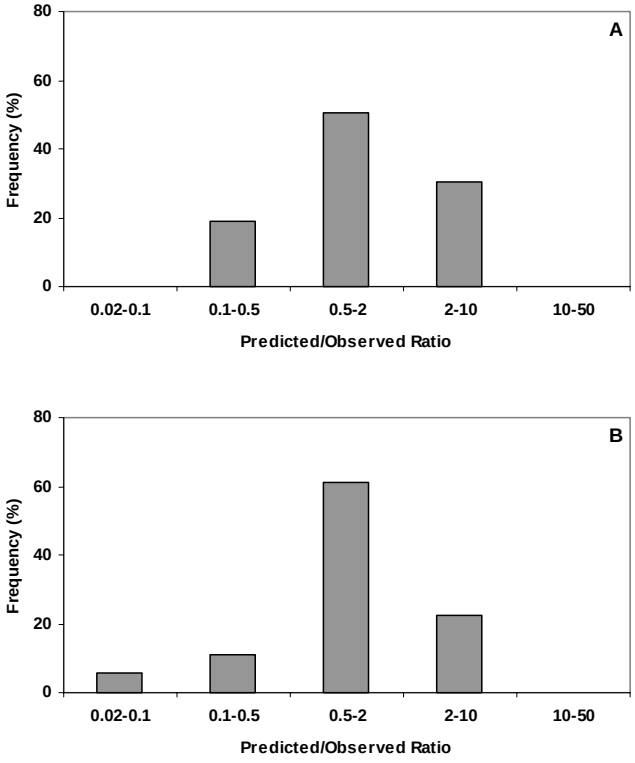

FIG. 4.

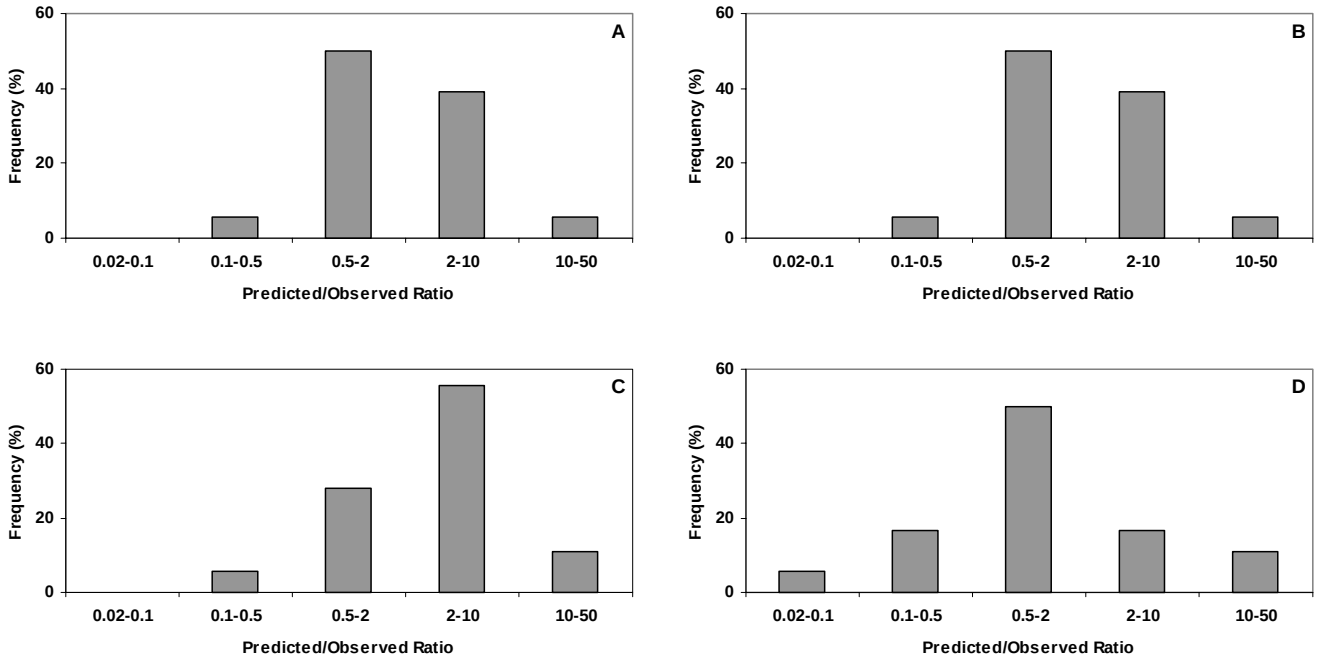

FIG. 5.