# Combining in Vitro and in Vivo Pharmacokinetic Data for Prediction of Hepatic Drug Clearance in Humans by Artificial Neural Networks and Multivariate Statistical Techniques

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Several statistical regression models and artificial neural networks were used to predict the hepatic drug clearance in humans from in vitro (hepatocyte) and in vivo pharmacokinetic data and to identify the most predictive models for this purpose. Cross-validation was performed to assess prediction accuracy. It turned out that human hepatocyte data was the best predictor, followed by rat hepatocyte data. Dog hepatocyte data and dog and rat in vivo data appear to be uncorrelated with human in vivo clearance and did not significantly contribute to the prediction models. Considering the present evaluation, the most cost-effective and most accurate approach to achieve satisfactory predictions in human is a combination of in vitro clearances on human and rat hepatocytes. Such information is of considerable value to speed up drug discovery. This study also showed some of the limitations of the approach related to the size of the database used in the present evaluation.

## Introduction

Early knowledge of the human pharmacokinetics of potential drug candidates is of major importance for the selection process. Knowing whether a compound will be subject to a high oral first-pass effect in humans is key information since liver first-pass limits the systemic oral bioavailability. As the liver is the most important organ for drug metabolism, predictions of the hepatic metabolic clearance are of primary importance. Various approaches including in vitro−in vivo correlation and allometric scaling combining in vivo and in vitro data were proposed and successfully applied to predict the in vivo clearance in humans and the corresponding maximum achievable bioavailability.[1−3] Also, significant efforts are being made toward developing physiological models for the prediction of pharmacokinetics in humans.[4−6] In this study, multiple linear regression models (MLR), partial least squares regression (PLS), and artificial neural networks (ANN) were evaluated for their ability to predict the in vivo hepatic clearance in humans. Both PLS and ANN have been reported to be useful tools for a wide variety of pharmacokinetic issues.[7−11] In the early phases of drug discovery, usually only a limited amount of pharmacokinetic data is available for most of the drug candidates. Therefore, the potential of these methods to predict human drug clearance was investigated here. The principal objectives were (i) to evaluate the predictive value of combinations of in vivo data from different animal species with the corresponding in vitro (hepatocyte) data and (ii) to identify optimal combination(s) of predictor variables to classify compounds according to their clearance and maximally achievable bioavailability in humans.

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## Material and Methods

**Selection of Compounds.** In vivo pharmacokinetic and metabolic data of 22 compounds were either obtained from literature or generated in-house (Table 1). Parts of the data used in this evaluation were published previously.[1,2]

**In Vitro Data.** For all compounds, in vitro metabolic data were generated from three to four batches of hepatocytes isolated from rats, dogs, and humans. The metabolic stability of the substances in hepatocytes was quantified by their in vitro intrinsic clearance, determined from the disappearance of the parent compound in the incubation medium as previously described.[1,2]

**In Vivo Data.** Liver was shown or assumed to be the main site of metabolism for all compounds selected (Table 1), and in vivo pharmacokinetic data obtained after intravenous administration were available from rat and dog. In the case of mofarotene, oral data were utilized. The plasma clearances were converted to the corresponding blood clearances using the reported blood/plasma partition coefficients in the different species. When binding to erythrocytes was not known (e.g., caffeine, theophylline), the blood/plasma partition coefficient was assumed to be unity. Clearance values can be converted to the corresponding hepatic extraction ratios ($E_h$) using the following equation: $E_h = \mathrm{CL/LBF}$, where LBF corresponds to the liver blood flow in the various species (60, 40, and 20 mL/min/kg in rat, dog, and humans, respectively).[12]

**Multiple Linear Regression and Partial Least Squares Analysis.** Linear correlation analysis, principal component analysis (PCA), and multiple linear regression (MLR) were performed using the commercially available software package Statistica (v5.1, 1997, StatSoft Inc., Tulsa, OK). A detailed description of these methods can be found in the Statistica manual and elsewhere.[11] Partial least squares (PLS) analysis was performed using the software package Tsar (v3.2, 1998, Oxford Molecular Ltd., Oxford, England).

**Artificial Neural Networks.** Three-layered feed-forward networks were used to find a mathematical model of the relationship between hepatocyte data and/or animal in vivo clearance and human in vivo clearance values. The general architecture of these systems follows conventional fully connected networks as described in the literature.[13] An in-depth treatment of these systems and a comparison with more conventional statistical techniques can be found elsewhere.[14,15]

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**Table 1.** Compounds Selected for Model Development and Testing, Sorted by Ascending Human Liver Clearance

| | in vitro clearance [$\mu$L/min/$10^6$ cells] | | | in vivo clearance[a] [mL/min/kg] | | |
|---|---|---|---|---|---|---|
| compound | rat | dog | human | rat | dog | human |
| diazepam | 21.0 | 30.7 | 0.7 | 44.0 | 30.7 | 0.4 |
| antipyrine | 0.4 | 1.0 | 0.1 | 7.0 | 4.9 | 0.5 |
| theophylline | 0.2 | 0.2 | 0.1 | 2.2 | 1.5 | 0.6 |
| lorazepam | 3.8 | 18.1 | 0.3 | 27.2 | 26.1 | 1.1 |
| oxazepam | 1.3 | 1.2 | 0.4 | 29.1 | 33.0 | 1.1 |
| caffeine | 0.6 | 0.2 | 0.1 | 13.0 | 2.6 | 2.0 |
| tolcapone | 2.6 | 1.4 | 1.2 | 15.0 | 3.3 | 2.7 |
| bosentan | 1.3 | 0.2 | 0.2 | 55.0 | 1.9 | 3.7 |
| mibefradil[b] | 5.4 | 6.2 | 0.9 | 94.0 | 36.0 | 7.0 |
| nicardipine | 27.0 | 16.0 | 7.3 | 115.0 | 48.0 | 7.0 |
| midazolam[c] | 16.7 | na[d] | 4.5 | 130.0 | na | 11.0 |
| mofarotene | 2.3 | 1.0 | 2.0 | 16.0 | 5.8 | 11.0 |
| felodipine | 29.0 | 14.2 | 7.5 | 83.3 | 19.5 | 11.0 |
| diltiazem | 43.6 | 2.7 | 1.9 | 87.2 | 46.1 | 11.5 |
| Ro 24-6173[b] | 39.0 | 13.0 | 2.9 | 110.0 | 35.0 | 12.0 |
| propranolol | 51.0 | 19.0 | 4.2 | 92.0 | 34.0 | 13.0 |
| nitrendipine | 24.0 | 8.1 | 12.0 | 16.5 | 21.7 | 18.7 |
| remikiren[b,c] | 16.6 | na | 19.5 | 119.0 | 3.0 | 19.6 |
| nilvadipine | 29.7 | 10.9 | 13.3 | 94.0 | 15.3 | 20.0 |
| naloxone | 54.6 | 32.1 | 16.7 | 48.8 | 42.5 | 25.0 |
| Ro 48-6791[b] | 50.4 | 41.2 | 13.7 | 95.0 | 18.0 | 26.5 |
| Ro 48-8684[b] | 45.4 | 44.1 | 9.0 | 83.0[b] | 36.0 | 27.7 |

[a] Animal and human in vivo clearance values obtained from refs 1 and 2. [b] Data on file, F. Hoffmann-La Roche Ltd. [c] Only used for model testing. [d] na: not available.

Applications of neural networks to drug design have been reviewed recently.[16,17] The networks used here consisted of up to five input neurons, one or more sigmoidal or linear hidden neuron(s), and a single linear output neuron. The overall type of function represented by the networks containing hidden neurons was

$$y = f(\mathbf{x}) = \sum_k (v_k\ T(\sum_i w_{i,k} x_i + \vartheta_k)) + \Theta$$

where $y$ is the output of the network, $\mathbf{x}$ is the input vector, $\mathbf{w}$ is the weight vector connecting the input and the hidden layer, $\mathbf{v}$ are the output weights, $\boldsymbol{\vartheta}$ are the hidden neurons' bias values, and $\Theta$ is the output bias. For sigmoidal hidden neurons $T(\text{in}) = 1/(1 + \exp(-\text{in}))$; for linear hidden neurons $T(\text{in}) = \text{in}$. The number of input and hidden neurons was systematically changed in different runs to identify optimal network architectures. One to five input values (predictors) and one to five hidden neurons were used. An evolutionary algorithm was employed for network training.[18,19] The mean-square-error (mse) served as the quality function for network training

$$\text{mse} = \frac{1}{N}\sum_{i=1}^{N}(y^i - t^i)^2 \rightharpoonup \text{Min.}$$

where $N$ is the number of training patterns, $y$ is the predicted clearance in humans, and $t$ is the experimentally determined clearance in humans. All neural networks were developed in-house at Roche as a series of C modules.[20]

**Test of Prediction Accuracy.** Complete leave-one-out procedures and five-times cross-validation (20% random cancellation groups) were performed to assess the generalization ability of the models. Prediction quality was measured by the squared linear regression coefficient of the predicted vs observed clearance values (coefficient of determination, training data: $r^2$, test data: $q^2$) and by classification accuracy (fraction of correct class predicted). The compounds used for network training and testing were divided into three distinct classes according to their clearance in man, $CL_{man}$: (i) "low clearance" ($CL_{man} < 6$ mL/min/kg), (ii) "medium clearance" (6 mL/min/kg $\le CL_{man} <$ 14 mL/min/kg), and (iii) "high clearance" ($CL_{man} \ge 14$ mL/min/kg).

Twenty of the 22 collected data were used for model development. Remikiren and midazolam were added to the test data after completion of the prediction models. From the $N = 20$ different cross-validation runs per prediction model, the average percentage of correct predictions was calculated to estimate reclassification (prediction of training data). Test data prediction accuracy was determined for the test compounds in the cross-validation runs (the "left-outs"). These values provided the basis for evaluation of prediction accuracy and selection of useful ANN architectures and input parameters (predictor variables).

**Table 2.** Pearson Correlation Coefficients of the Model Input Parameters

| | rat in vitro | rat in vivo | dog in vitro | dog in vivo | human in vitro | human in vivo |
|---|---|---|---|---|---|---|
| rat in vitro | 1 | 0.68 | 0.76 | 0.63 | 0.72 | 0.81 |
| rat in vivo | | 1 | 0.48 | 0.64 | 0.38 | 0.48 |
| dog in vitro | | | 1 | 0.45 | 0.69 | 0.78 |
| dog in vivo | | | | 1 | 0.29 | 0.34 |
| human in vitro | | | | | 1 | 0.88 |
| human in vivo | | | | | | 1 |

## Results

Three different methods were applied to build a prediction system for estimation of human in vivo drug clearance: multiple linear regression (MLR), partial least squares (PLS), and artificial neural networks (supervised feed-forward systems, ANN). Scaled data were used for model building (standardized by mean/std dev).

**Multiple Linear Regression and Principal Component Analysis.** MLR of all data points using the original five predictors produced a squared regression coefficient of $r^2 = 0.82$ and $q^2 = 0.74$. Backward variable elimination indicated that animal in vivo and dog in vitro data did not significantly contribute. In our final model, only human and rat hepatocyte data are included ($r^2 = 0.84$, $q^2 = 0.79$). Judging from this analysis and the linear pairwise correlation of the predictor variables (Table 2), human in vitro data is the dominant predictor (partial correlation = 0.88), followed by rat in vitro data (partial correlation = 0.81).

The correlation of the first three varimax-rotated principal components (PC) with individual predictor variables was determined ("loadings"). PC1 (eigenvalue = 3.3, 66% of the total variance) correlates most with humans in vitro data (loading = 0.92); PC2 (eigenvalue = 0.9, 18% of the total variance) with dog *in vivo* (loading = 0.92); and PC3 (eigenvalue = 0.4, 7% of the total variance) with rat in vivo (loading = 0.9). MLR using PC1, PC2, and PC3 as predictors and backward elimination of variables showed that PC3 did not significantly contribute (partial correlation = −0.09; eigenvalue = 0.4) and could be removed from the model. PC1 and PC2 contain sufficient information to build a useful prediction system. Judging from the factor loadings, human in vitro data seem to be indispensable for reliable estimation of human in vivo clearance. The maximal training residuals decrease in the following order: mofarotene > Ro-48−8684 > nitrendipine > mibefradil. Complete leave-one-out analysis based on PC1 and PC2 as predictor variables again yielded $r^2 = 0.85$ and $q^2 = 0.79$. As expected, complete leave-one-out analysis based on the original human and rat hepatocyte data led to a very similar result ($q^2 = 0.79$) (Figure 1a). Three major conclusions were drawn from this analysis:

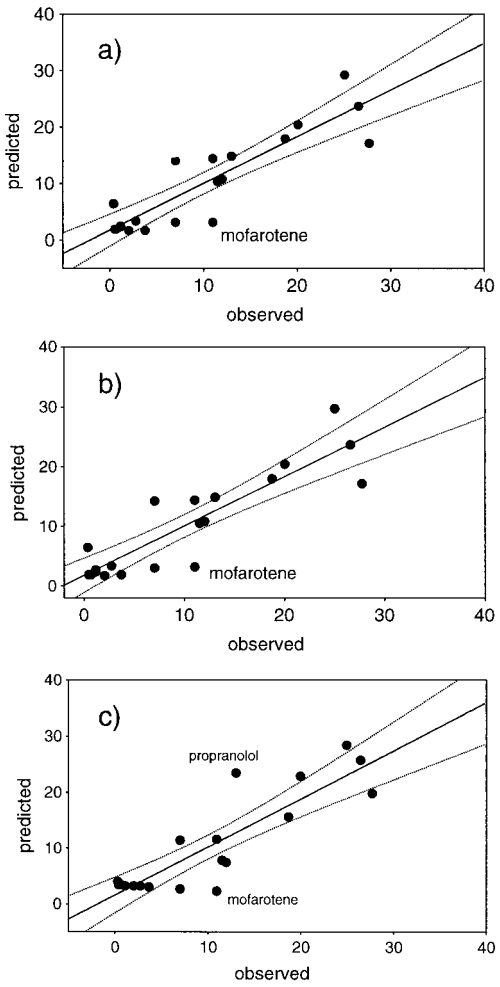


**Figure 1.** Predicted vs observed clearance values (mL/min/kg): (a) multiple linear regression model ($q^2 = 0.79$), (b) neural network model with two linear hidden neurons ($q^2 = 0.79$), (c) neural network model with two sigmoidal hidden neurons ($q^2 = 0.77$). Linear regression lines (solid) and 95% confidence intervals (dashed) are shown. Predictions stem from complete leave-one-out procedure (test data predictions); human and rat hepatocyte data were used as predictor variables.

(i) Human hepatocyte data contains the most significant information for prediction of human drug clearance.

(ii) The prediction can be improved if rat hepatocyte data is added to the model.

(iii) Rat and dog in vivo clearance data add little useful information to the estimation of human drug clearance.

A more detailed analysis and interpretation of the MLR results appear to be dangerous in this case as the input variables possess a correlation of greater than 0.7 (Table 2).

**Partial Least Squares Regression.** PLS analysis is a regression technique using principal component-like quantities ("latent variables") derived from the explanatory variables (here, in vitro and in vivo clearance data). It has much in common with MLR and seems to be particularly suited to dealing with large numbers of descriptor columns. For our full data set (five explanatory variables), the best PLS model (two components) gave $r^2 = 0.86$ and $q^2 = 0.77$. Taking only hepatocyte data for PLS analysis increased the prediction accuracy, yielding a model (three input variables, one component) with $r^2 = 0.83$ and $q^2 = 0.79$. Adding either rat or dog in vivo data did not increase prediction accuracy of our PLS models. The PLS regression (one component) generated for rat and humans in vitro data as the explanatory variables had $r^2 = 0.83$ and $q^2 = 0.79$, where man_vivo = 1.401 + 0.214 rat_vitro + 0.866 man_vitro. These results clearly substantiate the conclusions drawn from the MLR analysis.

**Neural Network Models.** Two types of artificial neural networks (ANN) were applied to predict human drug clearance from animal and hepatocyte data: (i) a three-layered network with two linear hidden units and (ii) three-layered networks with one to five nonlinear (sigmoidal) hidden units. In the latter case all possible combinations of input variables and number of hidden neurons were tested. The linear network was trained to have a comparison with the MLR model, whereas the nonlinear networks were developed to see if a nonlinear system could improve the predictions.

The ANN with two linear hidden neurons led to $r^2 = 0.86$ (97% correct class) when using all data for training. Exhaustive leave-one-out analysis yielded $q^2 = 0.79$ (75% correct class) (Figure 1b), which is identical to the performance of our best statistical MLR model (Figure 1a). This result demonstrates that linear regression models seem to be sufficient for clearance prediction.

ANN with sigmoidal hidden neurons were successfully trained on the prediction of human clearance from in vitro (hepatocyte) and in vivo animal data. All possible combinations of input data were used, and five different network architectures (one to five hidden neurons) were optimized per combination of input parameters. Complete cross-validation (leave-one-out) was performed with each architecture. The evolutionary training algorithm led to reproducible results and converged within short periods of time (500 training cycles, mse deviation in multiple runs <0.1%; data not shown). The network architecture leading to the highest classification accuracy (percent correct classification of test data) was selected as the final prediction system.

A network architecture with three sigmoidal hidden neurons and three input variables (man in vitro, rat in vitro, dog in vivo) led to the most accurate prediction in the complete leave-one-out test ($q^2 = 0.77$, 95% correct class) and $r^2 = 0.88$ with all data for network training. In contrast, 10 times cross-validation with 20% randomly selected test data led to an averaged $q^2 = 0.64$ of the fit to the cancellation groups. A possible explanation is that either the leave-one-out test is not suited to reliably measure generalization ability or 20% reduction of the training data removes essential information. There is generally a high risk of having an underdetermined system when only a small data set is available ("overfitting" effect). A similar observation was made for the combination of rat and human hepatocyte data as network input and a network containing two sigmoidal hidden neurons.

Using only rat and human hepatocyte data for nonlinear network training, $q^2 = 0.77$ was obtained (Figure 1c). This value is identical to the model including dog in vivo data (see above). Obviously the ANN including dog data was able to ignore the noise added by this additional predictor variable.

**Table 3.** Accuracy of the Best Models for Clearance Prediction

| method | $r^2$ [a] | $q^2$ [b] |
|---|---|---|
| MLR (all variables) | 0.84 | 0.74 |
| MLR (rat + human in vitro) | 0.84 | 0.79 |
| PCA + MLR | 0.85 | 0.79 |
| PLS | 0.83 | 0.79 |
| ANN linear | 0.86 | 0.79 |
| ANN sigmoidal | 0.88 | 0.77 |

[a] Coefficient of determination (training data). [b] Coefficient of determination (cross-validation data, complete leave-one-out).

The following conclusions were drawn from the neural network experiments:

(i) Prediction accuracy of neural networks containing linear neurons was very similar to the results obtained by more conventional statistical regression techniques ($q^2 = 0.79$).

(ii) The relative importance of human and rat hepatocyte data was confirmed for prediction of drug clearance in humans. Animal in vivo data did not significantly contribute to the predictions.

(iii) Test data prediction of simple neural networks containing sigmoidal hidden neurons was acceptable when a complete leave-one-out validation study was performed ($q^2 = 0.77$), but it was not better than the other models.

(iv) Test data prediction accuracy dropped significantly when 20% of the random cancellation groups were used for multiple cross-validation; this observation is probably due to the small data set available.

## Discussion and Conclusion

For the given application, the advanced nonlinear approximation capability of ANN was not required for construction of a useful quantitative prediction model. The conventional linear regression systems even slightly outperformed the nonlinear neural networks. One possible explanation is that due to the small number of data points the more flexible nonlinear neural network model carried out erroneous interpolation, whereas the linear regression model captured the trends more accurately. A summary of the performance of the different prediction models is given in Table 3.

The comparably worse prediction result of the networks containing sigmoidal hidden neurons results mainly from the inaccurate prediction of propranolol clearance (Figure 1c). Omitting propranolol from the test set yields a drastically improved cross-validated $q^2 = 0.83$ (compared to $q^2 = 0.77$ including propranolol). One possible explanation for the surprisingly poor propranolol clearance prediction is the relative position of propranolol in the data distribution (Figure 2a). Propranolol marks the borderline between substances with medium and high clearance in humans. If this reference point is excluded from the training data, the steepest part of the prediction surface (network predictions) is shifted toward lower input values (rat and man hepatocyte clearance) (Figure 2b). This observation clearly shows that more experimentally verified data are needed to fine-tune the prediction models. Despite the above-mentioned insufficiency, the networks containing sigmoidal hidden neurons more adequately reflect the relation between hepatocyte data and human in vivo drug clearance by including an upper limit for the predicted clearance value in humans.

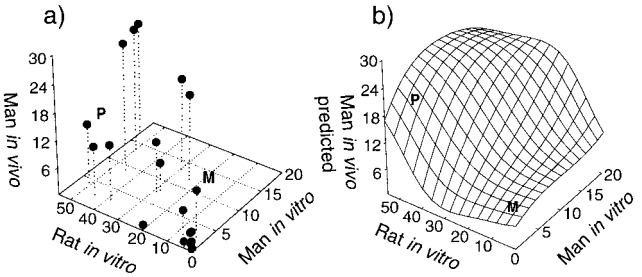


**Figure 2.** (a) Distribution of the training data given by the predictor variables (rat and man hepatocyte data). (b) Prediction surface generated by a neural network with two sigmoidal hidden neurons. Propranolol was omitted from the training data; its predicted (overestimated) clearance value in humans is indicated by "P". P: propranolol; M: mofarotene. Unit of drug clearance in man: (mL/min/kg); unit of hepatocyte clearance: $\mu$L/min/$10^6$ cells.

Mofarotene was a notorious outlier in all prediction models (Figure 1), i.e., its clearance value was underestimated (approximately 300% error). Interestingly, mofarotene was the only compound in the database for which oral clearance was used instead of the intravenous clearance (mofarotene was not administered intravenously to humans). This oral clearance of mofarotene most likely represents an overestimate of the intravenous clearance since the bioavailability of this compound in animals is approximately 50% (F. Hoffmann La Roche Ltd., data on file) (cf. Figure 2b).

Remikiren (human in vivo clearance: 19.6 mL/min/kg) and midazolam (human in vivo clearance: 11 mL/min/kg) were additional test cases, as these data were neither used for model development nor leave-one-out prediction (Table 1). Using both the best MLR and ANN models (Figure 1a,b) and the PLS regression, the following values for human liver clearance were predicted from rat and human hepatocyte data. Remikiren: 24.8 (MLR), 21.8 (PLS), and 16.3 (ANN) mL/min/kg. Midazolam: 9.1 (MLR), 8.7 (PLS), and 9.6 (ANN) mL/min/kg. The models correctly predicted the "high clearance" class of remikiren and the "medium clearance" class of midazolam. The quantitative predictions of PLS were the best for remikiren, whereas the ANN prediction was best for midazolam clearance.

The present study shows that useful predictions of drug clearance in humans can be obtained from in vitro (hepatocyte) data only. Inclusion of in vivo data from rat or dog did not significantly improve prediction accuracy. Such information can be of considerable practical value to speed up drug discovery programs by selecting the most appropriate in vitro and in vivo models to achieve satisfactory predictions of human pharmacokinetics. Considering the present evaluation, the most cost-effective and most accurate approach to achieve satisfactory predictions of drug clearance in humans is a combination of in vitro clearances on human and rat hepatocytes and a linear regression model.

This study also showed some limitations of the approach related to the size of the database. Although clear trends were identified, the results must be interpreted with some caution due to the limited amount of data available. This is especially valid for neural network training. The effect of variability and additional complexities such as nonlinear metabolic processes will

require a more thorough investigation. Other limitations deal with the empirical nature of the artificial neural network approach, which largely ignores physiological relevance. Despite these concerns, our prediction approach represents a useful alternative to conventional physiological techniques. The compounds included in our database cover a wide variety of metabolic reactions, including both phase I (cytochrome P450) and phase II (e.g., glucuronidation). On the basis of our data set, we were able to show that our approach is successful independent of the metabolic pathways involved. It would be of great interest to see whether prediction accuracy depends on the metabolic pathway. However, a drastically increased data set will be required to address this question. With the computational tools, which were developed here, we hope to enforce the discussion about prediction of human pharmacokinetic parameters and stimulate the search for better prediction models.

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## References

(1) Lave, T.; Dupin, S.; Schmitt, C.; Valles, B.; Ubeaud, G.; Chou, R. C.; Jaeck, D.; Coassolo, P. The use of human hepatocytes to select compounds based on their expected hepatic extraction ratios in humans. *Pharm. Res.* **1997**, *14*, 152−155.

(2) Lave, T.; Dupin, S.; Schmitt, C.; Chou, R. C.; Jaeck, D.; Coassolo, P. Integration of in vitro data into allometric scaling to predict hepatic metabolic clearance in man: Application to 10 extensively metabolized drugs. *J. Pharm. Sci.* **1997**, *86*, 584−590.

(3) Lave, T.; Coassolo, P.; Reigner, B. G. Prediction of hepatic metabolic clearance based on interspecies scaling techniques and in vitro-in vivo correlations. *Clin. Pharmacokinet.* **1999**, *36*, 211−231.

(4) Charnick, S. B.; Kawai, R.; Nedelman, J. R.; Lemaire, M.; Niederberger, W.; Sato, H. Physiologically based pharmacokinetic modeling as a tool for drug development. *J. Pharmacokinet. Biopharm.* **1995**, *23*, 217−229.

(5) Carey, E., Jr.; Duff, H. J.; Roden, D. M.; Primm, R. K.; Wilkinson, G. R.; Wang, T.; Oates, J. A.; Woosley, R. L. Encainide and its metabolites. Comparative effects in man on ventricular arrhythmia and electrocardiographic intervals. *J. Clin. Invest.* **1984**, *73*, 539−47.

(6) Houston, J. B. Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. *Biochem. Pharmacol.* **1994**, *47*, 1469−1479.

(7) Hussain, A. S.; Johnson, R. D.; Vachharajani, N. N.; Ritschel, W. A. Feasibility of developing a neural network for prediction of human pharmacokinetic parameters from animal data. *Pharm. Res.* **1993**, *10*, 466−9.

(8) Gobburu, J. V.; Chen, E. P.; Ritschel, W. A.; Akileswaran, R.; Hussain, A. S. Artificial neural networks as a novel approach to integrated pharmacokinetic-pharmacodynamic analysis. *J. Pharm. Sci.* **1996**, *85*, 505−10.

(9) Ritschel, W. A.; Akileswaran, R.; Hussain, A. S.; Hussain, A. S.; Johnson, R. D.; Vachharajani, N. N.; Ritschel, W. A. Application of neural networks for the prediction of human pharmacokinetic parameters. *Methods Find. Exp. Clin. Pharmacol.* **1995**, *17*, 629−43.

(10) Erb, R. J. The back-propagation neural network − A Bayesian classifier − Introduction and applicability to pharmacokinetics. *Clin. Pharmacokinet.* **1995**, *29*, 69−79.

(11) Forina, M.; Casolino, M. C.; De la Pezula Martinez, C. Multivariate calibration: Applications to pharmaceutical analysis. *J. Pharm. Biomed. Anal.* **1998**, *18*, 21−33.

(12) Davies, B.; Morris, T. Physiological parameters in laboratory animals and humans. *Pharm. Res.* **1993**, *10*, 1093−1095.

(13) Hertz, J.; Krogh, A.; Palmer, R. G. *Introduction to the theory of neural computation*; Addison-Wesley: Redwood City, CA, 1991.

(14) Bishop, M. *Neural networks for pattern recognition*; Clarendon Press: Oxford, 1995.

(15) Ripley, B. D. *Pattern recognition and neural networks*; Cambridge University Press: Cambridge, 1996.

(16) Devillers, J. Strengths and weaknesses of the back-propagation neural network in QSAR and QSPR studies. In *Neural networks in QSAR and drug design*; Devillers, J., Ed.; Academic Press: London, 1996; pp 1−46.

(17) Schneider, G.; Wrede, P. Artificial neural networks for computer-based molecular design. *Prog. Biophys. Mol. Biol.* **1998**, *70*, 175−222.

(18) Schneider, G.; Wrede, P. Development of artificial neural filters for pattern recognition in protein sequences. *J. Mol. Evol.* **1993**, *36*, 586−595.

(19) Schneider, G.; Schuchhardt, J.; Wrede, P. Evolutionary optimization in multimodal search space. *Biol. Cybern.* **1996**, *74*, 203−207.

(20) Kernighan, B. W.; Ritchie, D. M. *The C programming language ANSI C*, 2nd ed.; Prentice Hall International: Englewood Cliffs, 1988.

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