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Research Paper

Physiologically Based Pharmacokinetic Modeling of Drug Disposition in Rat and Human: A Fuzzy Arithmetic Approach

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Purpose. Probabilistic methods are insufficient for dealing with the vagueness inherent in human judgment of minimal data available during early drug development. We sought to use fuzzy set theory as a basis for quantifying and propagating vague judgment in a physiologically based pharmacokinetic (PBPK) model for diazepam disposition.
Materials and Methods. First, using diazepam distribution data in rat tissues and fuzzy regression, we estimated fuzzy rat tissue-to-plasma partition coefficients (K_p's). We scaled the coefficients prior to human PBPK modeling. Next, we constructed the fuzzy set of hepatic intrinsic clearance (CL_{int}) by integrating CL_{int} values measured *in vitro* from human hepatocytes. Finally, we used these parameters, and other physiological and biochemical information, to predict human diazepam disposition. We compared the simulated plasma kinetics with published concentration-time profiles.
Results. We successfully identified rat K_p's by fuzzy regression. The predicted rat tissue concentration-time contours enveloped the animal tissue distribution data. For the human PBPK model, the mean *in vivo* plasma concentrations were contained in the simulated concentration-time envelopes.
Conclusions. We present a novel computational approach for handling information paucity in PBPK models using fuzzy arithmetic. Our methodology can model the vagueness associated with human perception and interpretation of minimal drug discovery data.

KEY WORDS: diazepam; fuzzy arithmetic; fuzzy regression; fuzzy sets; physiologically based pharmacokinetic model.

INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models can predict the pharmacokinetics of promising analogues in drug development (1,2). Their implementation, however, is hampered by the incompleteness and scarcity of data suitable for estimating the numerous model parameters. With a limited data base upon which to make predictive assessments, a degree of human judgment is needed. This is accompanied by the imprecision and vagueness that characterizes human reasoning and, consequently, a distinct form of uncertainty can be introduced, rooted not in randomness, but in vagueness. It is in such circumstances that a quantitative technique that incorporates the concept of vagueness, or fuzziness, may provide a useful perspective.

In uncertainty analysis of PBPK models to date, the vagueness inherent in the data modeler's perception and judgment of scant information is usually treated using probabilistic methods, such as Bayesian probability theory (3,4). The Bayesian method requires the data modeler to provide the probability that a parameter value has a particular likelihood of occurrence, i.e., a prior parameter distribution. As such, this procedure is anchored by available prior knowledge about what values the parameter can assume. This approach, however, can have serious limitations. First of all, if little or no information about the parameter distribution is known, a broad, uninformative prior distribution may be assigned. However, a vague prior may result in the problematic computation of the posterior distribution by, e.g., Markov Chain Monte Carlo estimation. More importantly, when human judgment is involved, it may be unnatural to force the data modeler to choose whether the parameter assumes a certain range of values or not, and state the relevant probabilities within the range. For instance, if the data modeler believes that "the drug concentration is 'about' 5 ng/ml", it may not make sense for him or her to attach a probability measure to this fuzzy observation. All in all, entering subjective judgments into PBPK model analysis as probabilities—conditional or otherwise—may be complicated or restrictive and therefore, a more heuristic approach may offer an appealing mathematical tool for handling such vague descriptors.

Fuzzy set theory was introduced more than 40 years ago as the foundation for a formalized logic that reflects the vagueness inherent in human reasoning (5). The fuzzy set theoretic approach has a distinct conceptual foundation from the Bayesian approach. Rather than demand that the data

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modeler quantifies the prior probabilities of parameter values, perhaps based on limited measurements related to frequency of occurrence, the fuzzy approach bypasses the quantification of probabilities and models uncertainty itself [(5–7) include extensive treatments of fuzzy set theory *versus* probability theory/Bayesian statistics from both technical and philosophical perspectives]. This is done through the concept of fuzzy sets, where the degree to which a parameter value belongs to a fuzzy set, i.e., its membership grade, inherently incorporates the uncertainty associated with the value (8). For humans, it is generally much easier to estimate grades of membership or degrees of possibility (e.g., what is the degree to which the concentration of drug A is high?; what is the degree to which drug B is effective?) rather than probabilities (9). Therefore, it can be argued that the fuzzy theoretic method is a natural one to represent and implement the vagueness of a data modeler's judgment. This makes fuzzy approaches particularly suitable for data analysis in drug discovery and early development, where human reasoning needs to be frequently employed to interpret incomplete and scarce drug candidates' data. Hence, these methods are increasingly adopted in pharmaceutical risk assessments and pharmacotherapy studies (9–12).

Using a rat PBPK model, Gueorguieva *et al.* (10) employed fuzzy sets to describe uncertain tissue-to-plasma partition coefficients (K_p's) and intrinsic hepatic clearance ($CL_{\text{int, } in\ vivo}$) in order to analyze the propagation of parametric vagueness in diazepam pharmacokinetics following intravenous administration. In that study, the authors derived the fuzzy sets of rat K_p's from their corresponding probability density functions (PDF's) using the probability-possibility transformation technique (13). These PDF's were in turn estimated by naïve pooled data analysis using ordinary least-squares (OLS) regression. However, the PDF-to-fuzzy set transformation approach may suffer from several drawbacks. First, statistical regression analysis may not yield valid estimates when the sample size is small. Furthermore, the availability of four or fewer measured diazepam concentrations per time t per tissue in the data set of (10) may make it more intuitive for the data modeler to state that "the diazepam concentration at $t = x$ min is 'more or less' y ng/ml" (where y is an 'average' measure of the available concentrations), instead of "the diazepam concentration at $t = x$ min follows a normal distribution with mean equal to y ng/ml and standard deviation equal to z ng/ml" (it can be expected that the estimate of the standard deviation in particular would be heavily affected by the small sample size). Probability theory presently does not offer specific techniques for dealing with fuzzy quantifiers like 'more or less' and 'about'. Second, the conversion of a PDF into its fuzzy set counterpart is dependent on the heuristic selection of a confidence level – which corresponds to the data modeler's preferred probability level of value(s) that will be transformed into the 'most likely' one(s) (i.e., with a membership grade of one) in the resultant fuzzy set (13). Moreover, the PDF may not be successfully transformed into a fuzzy set if its variance is comparatively large (10). Lastly, the practice of converting a PDF to a fuzzy set has been discouraged, since in doing this objective probabilistic data—if available—is replaced by fuzzy values, resulting in the loss of very valuable information (14). To address these concerns, in this paper, we employed a fuzzy set-based technique called fuzzy least-squares (FLS) regression to directly estimate the rat K_p's as fuzzy sets.

The objective of this study was to suggest an alternative way of handling minimal information in (10) by using fuzzy set theoretic approaches to quantify uncertainty due to vagueness attached to human perception and judgment. In doing so, we refrained from analyzing the data using both probabilistic and fuzzy set approaches, which quantify data randomness and data vagueness, respectively, and therefore have different domains of applicability. We illustrated our modeling framework by quantifying the pharmacokinetics of diazepam in rat and in human, since its *in vivo* kinetics in various animal species and human have been reasonably well studied, providing a body of useful information with regard to key absorption, distribution, metabolism and elimination processes (15–17).

MATERIALS AND METHODS

Mathematical Background

Fuzzy Sets and Fuzzy Arithmetic

A fuzzy set is defined on an interval of possible values, and a membership function is used to define weights between 0 and 1 for all values within the interval (support). The membership function can be considered as a possibility distribution for values within the interval limits. Interval values with higher membership grades are 'more likely' than those with lower membership grades. The simplest of all fuzzy sets are those with a triangular membership function (when the exact distribution is not known, it may be impractical or unwarranted to assign a more complex-shaped function); this is most often used, and will also be employed in this study. A generic way of representing a fuzzy set is by expressing it as a series of intervals at different grades of membership called α-cuts. It follows that any mathematical operation involving such fuzzy sets can be implemented using the α-cut strategy. Using the intervals at a specific α level ($0 < \alpha \leq 1$), an interval analysis is performed. The result is an output interval that includes all possible combinations of input interval values, and the membership grade of any combination is the maximum membership grade of any of the individual input. Finally, the fuzzy output is assembled from the resulting output α-cuts. For a detailed exposition of fuzzy sets and fuzzy arithmetic, the reader is referred to (8,10,18).

FLS Regression

Fuzzy regression operates on fuzzy sets. A fuzzy regression model is characterized by coefficients that are fuzzy sets and, as such, the output values predicted by the model are also fuzzy sets. In our PBPK modeling framework, each regression equation of the well-stirred single tissue-based model (19) was identified using nonfuzzy input (t) and fuzzy output (tissue concentration $\tilde{C}$) variables, and the regression coefficient, e.g., tissue-to-plasma partition coefficient $\tilde{K}_p$, was estimated as a fuzzy set. In this paper, a letter with '~' denotes a fuzzy set. Consequently, values of the predicted dependent variable $\tilde{\hat{C}}$ were also fuzzy sets. A

regression model under our PBPK modeling framework was represented as:

$$\tilde{\tilde{C}}_k(t_k) = f\left(t_k, \tilde{K}_p\right), k = 1,2,\ldots n, \quad (1)$$

where f denoted the well-stirred single tissue-based model and n was the number of measurement time points (indexed by k). To conduct FLS regression, we implemented a generalized version of a published algorithm (20) that used least squares of errors between the observations and the estimations in the possibilistic space as a fitting criterion for parameter estimation (Seng *et al*., in preparation). In order to develop the model in Eq. 1, we adopted the following two-stage approach. First, we constructed a 'cost' function Γ based on a difference measure $D_k\left(\tilde{C}_k, \tilde{\tilde{C}}_k\right)$ between $\tilde{C}_k$ and $\tilde{\tilde{C}}_k$ for the kth observation (21). Formally,

$$D_k\left(\tilde{C}_k, \tilde{\tilde{C}}_k\right) = \sqrt{\left\{\int_{\alpha=0}^{\alpha=1} w^2(\alpha) \bullet d_k\left[\left(\tilde{C}_k\right)_\alpha, \left(\tilde{\tilde{C}}_k\right)_\alpha\right] d\alpha\right\}}, \quad (2)$$

where $\alpha \in [0, 1]$, $w^2(\alpha)$ is a monotone increasing function in [0, 1] and $d_k\left[\left(\tilde{C}_k\right)_\alpha, \left(\tilde{\tilde{C}}_k\right)_\alpha\right] = \left[(C_k)_\alpha^L - \left(\tilde{C}_k\right)_\alpha^L\right]^2 + \left[(C_k)_\alpha^U - \left(\tilde{C}_k\right)_\alpha^U\right]^2$, with $\left(\tilde{C}_k\right)_\alpha = \left[(C_k)_\alpha^L, (C_k)_\alpha^U\right]$ and $\left(\tilde{\tilde{C}}_k\right)_\alpha = \left[\left(\tilde{C}_k\right)_\alpha^L, \left(\tilde{C}_k\right)_\alpha^U\right]$. Here, $\left[(C_k)_\alpha^L, (C_k)_\alpha^U\right]$ and $\left[\left(\tilde{C}_k\right)_\alpha^L, \tilde{C}_k\right)_\alpha^U\right]$ contain the lower L and upper U bounds of the α-cuts of $\tilde{C}_k$ and $\tilde{\tilde{C}}_k$ respectively. $w^2(\alpha)$ can be interpreted as the weight of $d_k\left[\left(\tilde{C}_k\right)_\alpha, \left(\tilde{\tilde{C}}_k\right)_\alpha\right]$, ensuring that the larger the membership of the α-cut, the more significant it would be in determining the difference between $\tilde{C}_k$ and $\tilde{\tilde{C}}_k$. In this study, we chose $w^2(\alpha)$ to be equal to the α level. D_k in Eq. 2 thus quantifies the Euclidean difference between $\tilde{C}_k$ and $\tilde{\tilde{C}}_k$ as a weighted mean of the squared distances between their corresponding α-cuts. Next, we computed Γ as an arithmetic mean of the squared D_k's from all observations, i.e., $\Gamma = \frac{1}{n}\sum_{k=1}^{n}\left\{D_k\left(\tilde{C}_k, \tilde{\tilde{C}}_k\right)\right\}^2$. In step two, we obtained the optimal estimates for rat $\tilde{K}_p$, in the sense of best fit to the observed data, by solving a constrained nonlinear programming problem with an objective function minimizing Γ.

Data

Data that were used to illustrate the utility of our fuzzy PBPK modeling framework in predicting diazepam pharmacokinetics in human came from several sources.

Diazepam Disposition Data in Rats

We obtained the *in vivo* tissue distribution data for estimating rat $\tilde{K}_p$'s from a study of intravenous dosing of diazepam in rats (22). In that study, diazepam concentrations in the rat liver (LI), kidneys (KI), brain (BR), intestine (SPL), stomach (ST), muscle from the hind limb (MU), adipose tissue (AD), hair-free skin (SK), testes (TE), heart (HT) and lungs (LU) were measured at 7, 10, 20, 35, 95 and 245 min, following a 1 mg intravenous infusion of the drug over 5 min. Four or less measurements were obtained per t instance. Additionally, four to ten arterial diazepam concentrations were also measured per t instance at 2, 5, 7, 10, 20, 35, 95 and 245 min.

Diazepam Disposition Data in Humans

To verify the prediction from our human diazepam PBPK model, we obtained an independent set of human *in vivo* distribution data from the literature (23). This consisted of seven instances of plasma concentration-time data (where an instance corresponded to a single plasma concentration-time profile sampled at 5, 8, 11, 15, 20, 30, 45 and 60 min) for diazepam dosed intravenously in human subjects (weight, 84 ± 17 kg). The diazepam dose was 5 mg, intravenously administered over 1 min.

Structure of PBPK Models in Rat and Human

The concept and methodology of PBPK modeling are presented elsewhere (19). In this study, the structures of the rat and human diazepam PBPK models were identical to that used by Gueorguieva *et al.* (10). Briefly, the PBPK model comprised compartments representing the aforementioned tissues, interconnected by two compartments representing the arterial (ART) and mixed venous (VEN) pools. To preserve mass balance, a rest of the body (RE) compartment was also incorporated. For both the rat and human PBPK models, diazepam was administered by constant-rate infusion into VEN. The LI compartment received diazepam directly from the hepatic artery as well as from SPL and ST via the hepatic portal vein. The LU compartment closed the circulation loop and received blood at a flow rate equal to the cardiac output. Elimination was accounted for by metabolism in LI (15,24,25). Additionally, we assumed each tissue to be characterized by perfusion-rate limited distribution, i.e., it was modeled using a single, well-stirred compartment. The reader is referred to (10) for the compartmental mass balance equations of the 14 compartments.

Fuzzy PBPK Modeling Strategy

The procedure for our fuzzy PBPK modeling framework is depicted in Fig. 1. In the context of diazepam disposition following intravenous administration, it consisted broadly of two major steps, namely application of fuzzy set-based estimation tools to first derive parameters characterizing the key distribution and metabolism processes, and then their combination in a PBPK model to predict, in a second step, the *in vivo* distribution. It may be worth mentioning here that, unlike in (10), we estimated the rat $\tilde{K}_p$'s by FLS regression.

Fuzzy Set-Based Estimation (I): Rat $\tilde{K}_p$'s and $\tilde{K}_{pu}$'s

Using FLS regression, we first estimated rat $\tilde{C}_k$'s for tissues that received diazepam only from the arterial blood (i.e., MU, AD, TE, SK, HT, BR, KI, ST and SPL). To this end, we fitted the well-stirred parametric model representation to the $\tilde{C}_k$ data for each of these tissues separately, using an *a priori* fitted arterial concentration profile as the forcing

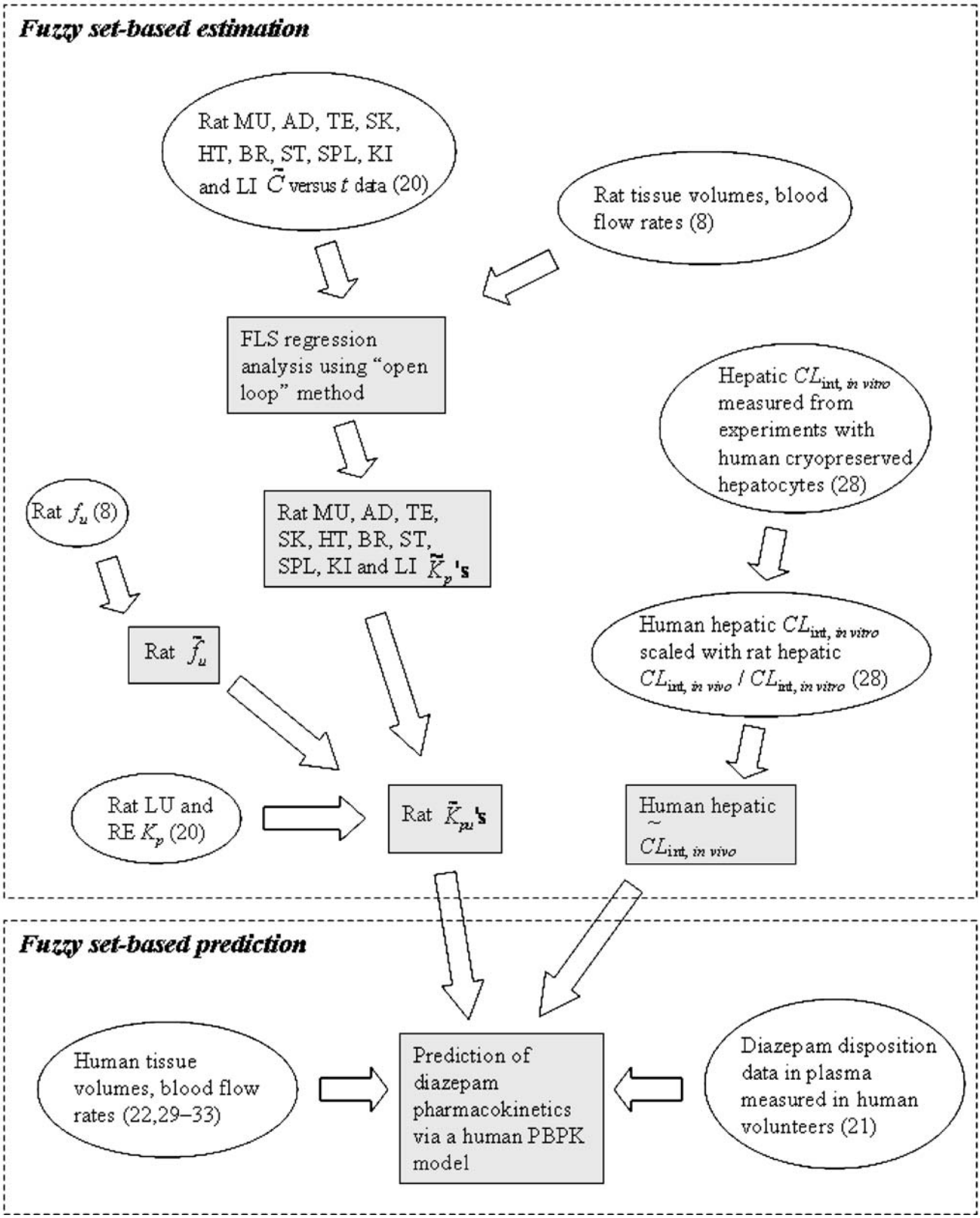

Fig. 1. Procedure of PBPK modeling framework for fuzzy set-based estimation and prediction of diazepam pharmacokinetics in human. Nonfuzzy data and/or parameters obtained from the literature are encapsulated by ellipses. Gray rectangles signify that estimation and/or prediction using fuzzy arithmetic are performed.

function, a procedure commonly termed the 'open loop' estimation approach (17). The forcing function was the best fitting multi-compartmental pharmacokinetic model of the rat plasma diazepam distribution data; model fitting was performed using the Nonlinear Mixed Effects Modeling (NONMEM) software (version V; NONMEM Project Group, University of California, San Francisco, San Francisco, CA). Before implementing FLS regression, we also fuzzified the concentration data for each t with an asymmetrical triangular membership function [following procedures in (21)], since the tissue concentration values per t were derived from no more than four rats and displayed large variability. More concretely, for the kth observation, the bounds of the support of $\widetilde{C}_k$ were assigned the minimum and maximum concentration values, while the 'most likely' value (i.e., the concentration at which $\alpha = 1$) of $\widetilde{C}_k$ was set equal to the median of the measured concentrations. We selected the median as a measure of the fuzzy concentration's 'most likely' value so that it would not be 'distorted' by outliers in the small sample of measurements. We assumed that t was recorded with little imprecision and it therefore remained nonfuzzy. Since our data comprised asymmetrical triangular fuzzy sets and each regression model contained only one regression coefficient, the estimated rat MU, AD, TE, SK, HT, BR, KI, ST and SPL $\widetilde{K}_p$'s had an asymmetrical triangular membership function shape. For the purpose of estimating the rat $\widetilde{K}_p$'s, we assumed the tissue volumes and blood flow rates to be that for a 250 g rat (10; Table I). We assigned constants to these parameters because they were typically well-characterized in the literature and, hence, were less imprecise or uncertain compared to rat K_p's. Furthermore, doing so would facilitate comparison of the fuzzy diazepam concentrations in rat tissues predicted in this study and in (10), since the authors in (10) also assigned constants to the rat tissue volumes and blood flow rates.

Table I. Physiological Parameter Values for PBPK Modeling in a 250 g Rat and a 70 kg Human

Compartment	Volume (ml)		Blood Flow Rate (ml/min)	
	Rat[a]	Human[b]	Rat[a]	Human[b]
LU	1.2	504	80	4,951.8
LI	11	1,799	3.55	301
ST	1.1	140	1.9	39.2
SPL	15	966	20.25	1,099
KI	2	294	16.61	1,099
MU	125	30,786	16.25	749
AD	10	13,601	2.55	259
SK	43.8	7,126	7.1	301
HT	1	322	4.2	149.8
BR	1.2	1,351	0.78	700
TE	2.5	28	1.9	2.8
RE	15.8	7,854	4.91	252
ART	6.8	2,037	80	4,951.8
VEN	13.6	3,192	80	4,951.8
Whole body	250	70,000	80	4,951.8
Mean unbound fraction of diazepam in plasma f_u	0.14[c]	0.032[c]		
Mean blood-to-plasma ratio of diazepam R	1.037[c]	1.037[d]		

[a] Blood flow rates and tissue volumes for the rat were referenced from (10).
[b] Blood flow rates and tissue volumes for the human were referenced from (24,31–35).
[c] Referenced from (15).
[d] Mean for man was assumed to be equivalent to that for rat (15).

Prior to identifying rat LI $\widetilde{K}_p$ by the 'open loop' method, we derived rat hepatic *in vivo* intrinsic clearance ($CL_{\text{int, in vivo}}$) via the well-stirred liver model (26) and using values of rat plasma unbound fraction of diazepam (f_u), blood-to-plasma concentration ratio of diazepam (R), liver blood flow rate (11) and hepatic blood CL, with the latter derived from dividing the administered diazepam dose by the area under the previously fitted plasma concentration-time curve. Here, using the finding that extrahepatic metabolism of diazepam in rat is negligible (15,24,25), we assumed that the values for the total blood CL and the hepatic blood CL were identical. Additionally, we defuzzified rat ST and SPL $\widetilde{K}_p$'s into their nonfuzzy counterparts prior to FLS regression of the LI parametric model. This was achieved using the centroid method (27):

$$K_p^* = \frac{\int K_p \bullet \mu(K_p) dK_p}{\int \mu(K_p) dK_p} \tag{3}$$

where K_p^* denotes the defuzzified value of $\widetilde{K}_p$. Since we utilized the 'open loop' approach for estimating rat $\widetilde{K}_p$'s in this paper, we could not estimate rat tissue-to-plasma concentration ratios for LU and RE as fuzzy sets. Instead, we obtained nonfuzzy rat LU and RE K_p's from (22), which also computed K_p's by the 'open loop' method with the same set of rat *in vivo* tissue distribution data.

To obtain appropriate tissue-to-plasma concentration ratios for the human diazepam PBPK model, we used the assumption that the ratio of tissue concentration to unbound plasma concentration of diazepam (K_{pu}) obtained in rat could be utilized to predict its disposition kinetics in human (15,28). That assumption was predicated on the finding that the steady-state volumes of unbound diazepam in rat and human were equivalent, which indicated that any difference in total diazepam pharmacokinetics was primarily due to the difference in serum protein binding of diazepam between rat and human (24). The approach of scaling rat K_p to K_{pu} to quantify diazepam pharmacokinetics in human had been previously shown to elicit accurate prediction of its disposition kinetics in a PBPK model (15). Accordingly, we divided all rat K_p's by rat f_u to yield K_{pu}'s. Furthermore, to capture the potential vagueness inherent in this scaling, we represented rat f_u as a symmetrical triangular fuzzy set with half-support equal to $0.05 \times f_u$, i.e., $\widetilde{f}_u = \langle f_u - 0.05\times\ f_u, f_u, f_u + 0.05 \times f \rangle$ according to the triplet notation of Hanss (29). As a consequence, all rat K_{pu}'s were represented as fuzzy sets in this paper.

Fuzzy Set-Based Estimation (II): Human Hepatic $\widetilde{CL}_{\text{int, in vivo}}$

We constructed the human hepatic $\widetilde{CL}_{\text{int, in vivo}}$ based on $CL_{\text{int, in vivo}}$ values reported by Naritomi *et al.* (30). In that study, primary cultures of cryopreserved human hepatocytes were used to estimate the hepatic intrinsic CL measured *in vitro* (expressed in ml/min/cell) based on diazepam disappearance. $CL_{\text{int, in vivo}}$, expressed in ml/min/kg, was then calculated from $CL_{\text{int, in vitro}}$ by direct scaling up using the

factor $CL_{\text{int, in vivo}}/CL_{\text{int, in vitro}}$ measured in rat. $CL_{\text{int, in vivo}}$ in rat was obtained from *in vivo* pharmacokinetic data using the well-stirred liver model, whereas $CL_{\text{int, in vitro}}$ in rat was measured from freshly isolated rat hepatocytes. However, as acknowledged by the authors, the human $CL_{\text{int, in vivo}}$ values were characterized by imprecision since (1) limited (six) and nonuniform (differing in age, race and sex) human cryopreserved hepatocytes were used; (2) difficulties arose during the measurement of low values of human and rat $CL_{\text{int, in vitro}}$; and (3) marked differences among the rat $CL_{\text{int, in vivo}}/CL_{\text{int, in vitro}}$ values existed. In order to capture the uncertainty associated with the human $CL_{\text{int, in vivo}}$ values (209.4, 227.9, 338.8, 585.2, 893.2 and 1570.8 ml/min as scaled for a 70 kg human), we used an asymmetrical triangular fuzzy set to embody them. In particular, we assigned 209.4 and 1570.8 ml/min as the minimum and maximum values, respectively, of the support of $\widetilde{CL}_{\text{int,invivo}}$. We assigned the 'most likely' value of $\widetilde{K}_p$ as the median of the six $CL_{\text{int, in vivo}}$ values (i.e., 462 ml/min).

Fuzzy Set-Based Prediction of Diazepam Disposition (I): Rat

First, we described the interval membership of rat $\widetilde{K}_p$'s and rat hepatic $\widetilde{CL}_{\text{int, in vivo}}$ using 11 α-cuts each ($\alpha = 0$, 0.1,...,1). We obtained the fuzzy rat hepatic $\widetilde{CL}_{\text{int, in vivo}}$ from (10). The rat hepatic K_p^* had an asymmetrical trapezoidal membership function, with the support and 'most likely' interval equal to [200, 1,200] and [400, 800] ml/min respectively. We simulated the PBPK model for a 250 g rat receiving 1 mg of diazepam (intravenously administered over 5 min) over $t = 0$–245 min (in intervals of 1 min) using the α-cut strategy outlined in 'Materials and Methods'. For this purpose, as we mentioned, we used nonfuzzy 250 g-rat tissues volumes and blood flow rates, as well as R and f_u of diazepam from (10 (Table I). We compared the predicted fuzzy-valued diazepam concentrations against the tissue distribution data in (22).

Fuzzy Set-Based Prediction of Diazepam Disposition (II): Human

First, we expressed rat $\widetilde{K}_{pu}$'s and human hepatic $\widetilde{CL}_{\text{int, in vivo}}$ as 11 α-cuts each ($\alpha = 0$, 0.1,...,1). Again using the α-cut strategy, we simulated the PBPK model for a 70 kg human receiving 5 mg of diazepam (intravenously administered over 1 min) over $t = 1$–60 min (in intervals of 1 min). We obtained tissue blood flow rates from Bernareggi and Rowland (31), and Williams and Leggett (32). We referenced tissue volumes from a variety of reports (33–35). In addition, we obtained R and f_u of diazepam for human from (24). Table I lists these nonfuzzy physiological and biochemical parameters. We evaluated the accuracy of our predictions by qualitatively comparing them with plasma concentration values reported by Lindhardt *et al.* (23).

RESULTS

Fuzzy Set-Based Estimation

Our results showed that a two-compartment model provided the best fit to the plasma data for diazepam in rat. We estimated the values of the population parameters using the first-order conditional estimation (with interaction effects) method in NONMEM (Table II). We modeled the between-subject and residual unknown variability according to an exponential model and a proportional model respectively. The relative standard errors of the fixed-effect mean parameters were between 5.8 and 18.1%, which suggests that these parameters were estimated with good precision.

The rat $\widetilde{K}_p$'s identified by FLS regression are presented in Fig. 2. Our results indicated that the convergence properties of the underlying nonlinear programming problem in FLS regression were good: in most cases we obtained optimized parameters with less than ten iterations. For comparison purposes, we showed the corresponding membership functions obtained via the probability-possibility transformation technique (10). To further facilitate comparison, we also displayed the defuzzified, nonfuzzy rat K_p values from this study and from (10). In Fig. 3, we compared the membership functions of $\widetilde{C}$'s and $\tilde{\bar{C}}$'s of selected rat tissue compartments after their respective single tissue-based models were identified by FLS regression. Visually, we observed that more than half of the 'most likely' values of $\tilde{\bar{C}}$'s were generally contained in the supports of the corresponding $\widetilde{C}$'s. These results suggest that the fuzzy regression models were successful in establishing the respective functional relationships between t and $\widetilde{C}$ in the rat tissues. In Table III, we list the α-cuts of the rat $\widetilde{K}_{pu}$'s at $\alpha = 0.1$, 0.5 and 1.

Fuzzy Set-Based Prediction of Diazepam Disposition in Rat

In Fig. 4, we plot the envelopes for $\tilde{\bar{C}}$'s in several rat tissue compartments across the simulation time history at α-cuts = 0.1 and 1. To this end, we profiled the minimum (i.e., lower bound of α-cut) and maximum (i.e., upper bound of α-cut) concentration values over $t = 0$–245 min for an α level. For comparison purposes, we also computed the fuzzy concentrations at these α-cuts using the rat $\widetilde{K}_p$'s obtained from (10). Overall, considering the differences in the membership functions between the rat $\widetilde{K}_p$'s from our study and from (10), we observed that simulated profiles were morphologically similar. Additionally, we noted that our predicted output envelopes of $\alpha \geq 0.1$ membership typically

Table II. Population Parameters of the Biexponential Pharmacokinetic Model, $C = Ae^{-at} + Be^{-bt}$

Parameter	Parameter Estimates		Between-subject Variability	
	Value[a]	S.E.[b]	BSV[c]	S.E.[b]
A (ng/ml)	3,080	11.4	49.4	40.4
B (ng/ml)	175	18.1	57	35.7
a (1/min)	0.15	8.1	NE	NE
b (1/min)	0.0128	5.8	NE	NE
σ^2	0.0563	29		

σ^2, variance of the proportional residual unknown error model. *NE* Not estimated.
[a] Fixed-effect parameters.
[b] Standard error expressed as percent coefficient of variation.
[c] *BSV*, between-subject variability expressed as percent coefficient of variation.

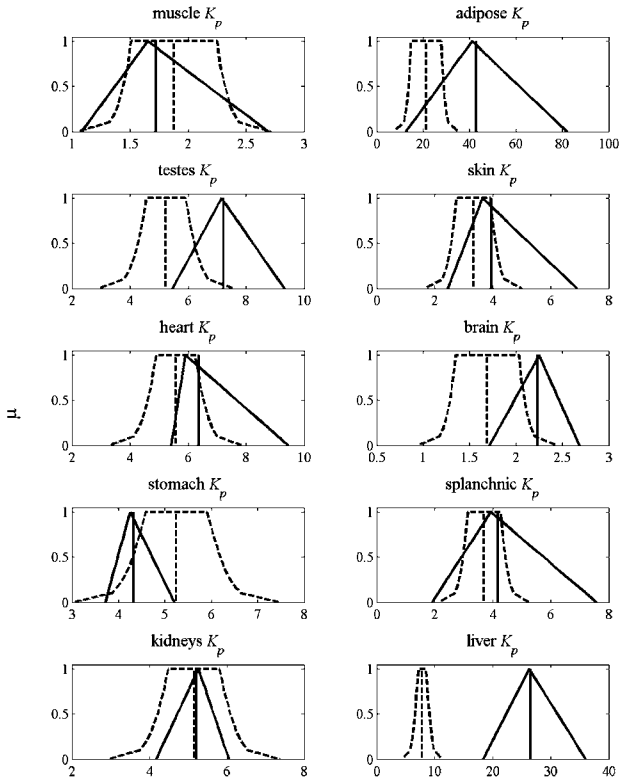

Fig. 2. Comparison of rat $\widetilde{K}_p$'s identified from FLS regression analysis (*continuous line*) and $\widetilde{K}_p$'s obtained using the probability-possibility transformation method (*dash line*) (8). Each defuzzified rat K_p value from FLS regression analysis (continuous line) and the probability-possibility transformation method (dash line) is depicted as a singleton, i.e., the nonfuzzy value has a membership of 1.

enveloped all the raw diazepam concentration data, further supporting the reliability of FLS regression for parameter estimation as well as verifying the fuzzy prediction step.

Fuzzy Set-Based Prediction of Diazepam Disposition in Human

We plot the envelopes for $\tilde{\tilde{C}}$'s in the human LU, LI, HT, BR, AD and ART compartments from $t = 1$ min to $t = 60$ min at α-cuts = 0.1, 0.5 and 1 in Fig. 5. We also display the measured mean (±SD) concentrations in human plasma (23). We noted that the mean *in vivo* data for all measured t instances were typically contained in the simulated concentration-time envelopes.

DISCUSSION

In this study, we have developed a computational framework using fuzzy arithmetic and FLS regression to systematically account for vague human judgment for uncertainty analysis in PBPK models. While we do not propose replacing probabilistic PBPK models for uncertainty analysis, when they are applicable, we illustrate, through our results, the utility and potential contribution of applying fuzzy set descriptions of dramatically sparse data—not uncommon during early drug discovery—in PBPK models. In particular, we suggest an alternative approach to the way such data were used to estimate rat $\widetilde{K}_p$'s in (10) by conducting FLS regression analysis. In doing so, we permit a fuzzy set theoretic treatment of the imprecise concepts associated with human perception and comprehension of minimal data, spanning model identification through prospective simulation.

Fuzzy Set-Based Estimation

A key difference between the work presented in this study and in (10) was in our implementation of FLS regression analysis to estimate the rat $\widetilde{K}_p$'s. For the sake of completeness, we compared $\widetilde{K}_p$'s obtained from FLS regression and their counterparts determined via the probability-possibility transformation technique (10). In the absence of 'standard' rat tissue $\widetilde{K}_p$'s (due to the myriad ways with which the tissue distribution data could be analyzed, e.g., data assumed to follow a Gaussian distribution; data represented as fuzzy sets; and so on)—by which one could conduct an objective evaluation of the performances of the probability–possibility transformation technique and FLS regression—we have resorted to a qualitative comparison of the rat $\widetilde{K}_p$'s, following the procedures of (21,36,37). Our results showed that the supports of many fuzzy parameters estimated by FLS regression exhibited variability of comparable magnitude with respect to those transformed from the respective PDF's, differing by 23% on average. Furthermore, the defuzzified rat K_p values of the cross-referenced membership functions compared favorably (differences were less than 25%), leading to substantial overlaps between the membership functions, with the exception of the ST and LI $\widetilde{K}_p$'s. However, we recognize that this discrepancy could be minimized, e.g., by performing FLS regression analysis with the initial guesses for the ST $\widetilde{K}_p$'s and LI $\widetilde{K}_p$'s 'most likely' values set equal to, respectively, the centers of the 'most likely' intervals of the ST $\widetilde{K}_p$ and LI $\widetilde{K}_p$ identified in (10). All these considerations suggest that the estimation results of FLS regression were numerically close to that obtained in (10).

These findings notwithstanding, the fuzzy set theoretic approach appears to be an improvement over the probability-possibility transformation technique (10) for handling vague or subjective descriptors. As mentioned previously in "Introduction", the latter method requires the data modeler to first estimate the rat K_p's probability distributions by statistical regression. However, the scarcity, and by extension, vagueness of rat tissue diazepam concentration data might render statistical regression methods inapplicable, since these approaches assume that the uncertainty in the underlying data must be explained by statistical randomness. Furthermore, the transformation of a PDF to its fuzzy set counterpart is inherently subjective—and thus, we argue, it defeats the purpose of statistical regression analysis for quantifying data randomness in the first place—and may also be unsuccessful if the PDF is characterized by a high variance value (10). In contrast, fuzzy regression analysis is designed for constructing the relationship between explanatory and response variables with fuzzy, not random, data, which arise from human perception and judgment. Under FLS regression, the lower and upper bounds of the estimated rat $\widetilde{K}_p$'s support definitively characterize the vagueness in the underlying data. The same, however, cannot be said of the support

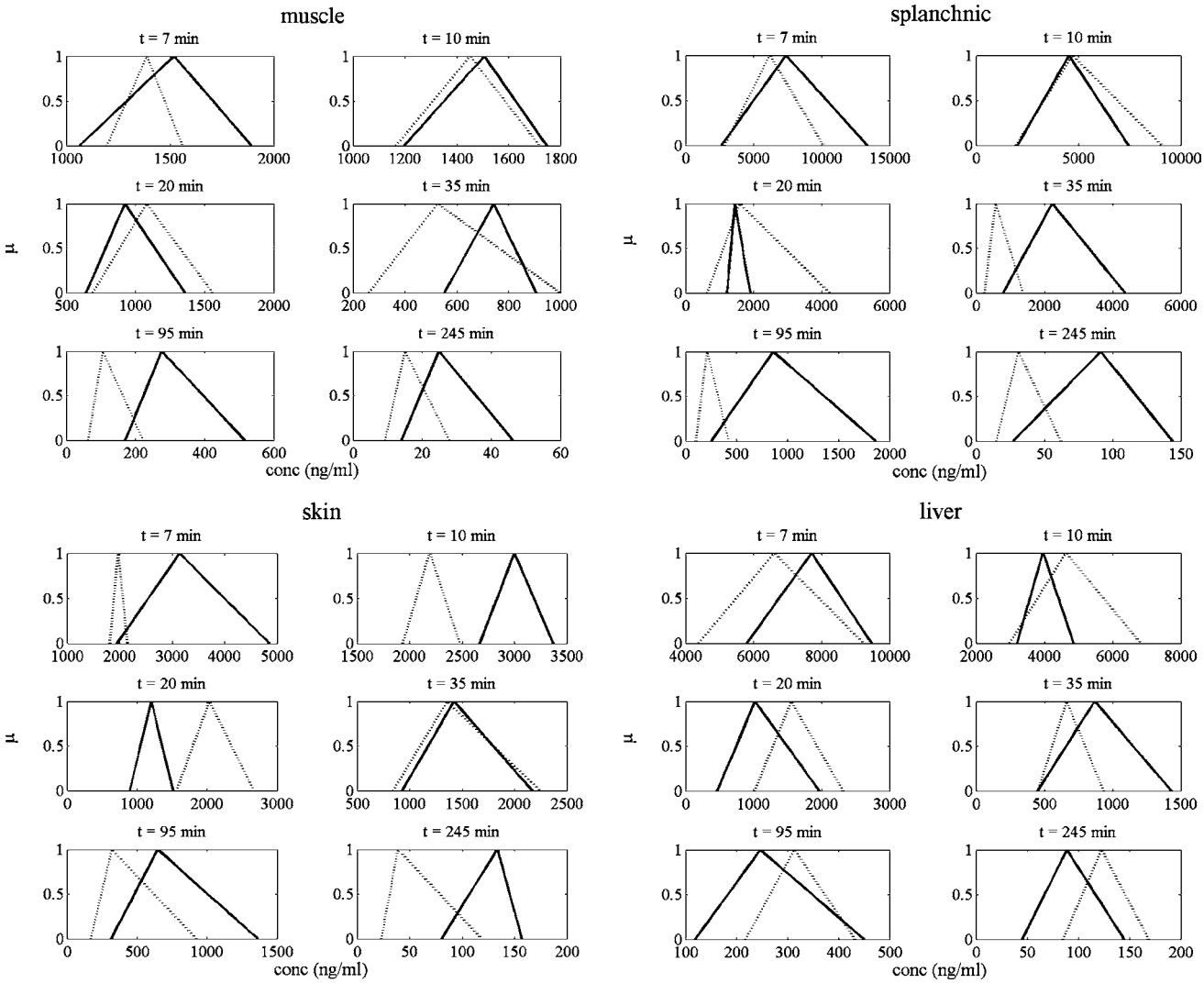

Fig. 3. Comparison between the membership functions of diazepam $\widetilde{C}$'s (*continuous line*) and their corresponding $\widetilde{\widetilde{C}}$'s (*dot line*) based on the fuzzy regression model and estimated rat $\widetilde{K}_p$'s in the MU, SPL, SK and LI compartments. '*Conc*' denotes concentration.

of rat $\widetilde{K}_p$ transformed from its PDF counterpart, since the probability distribution characterizes statistical variability, not vagueness, in the data. In conclusion, fuzzy regression serves as an intuitive tool for robust estimations in situations where the availability of minimal data is characterized by vagueness, a finding consistent with the literature (38–40).

Table III. α-cuts of Rat $\widetilde{K}_{pu}$'s at $\alpha = 0.1$, 0.5 and 1

Parameter	α-cut at $\alpha = 0.1$	α-cut at $\alpha = 0.5$	α-cut at $\alpha = 1$
LU K_{pu}	[50.1, 55.3]	[51.3, 53.9]	[52.6, 52.6]
LI K_{pu}	[159.1, 222.3]	[173.3, 204.9]	[187.5, 187.5]
ST K_{pu}	[29.9, 31.6]	[30.1, 30.9]	[30.4, 30.4]
SPL K_{pu}	[26.1, 31.7]	[27.1, 29.9]	[28.1, 28.1]
KI K_{pu}	[29.8, 43.2]	[33.7, 40.4]	[37.6, 37.6]
MU K_{pu}	[7.7, 19.2]	[9.8, 15.5]	[11.8, 11.8]
AD K_{pu}	[90.1, 584.8]	[192, 439.3]	[293.9, 293.9]
SK K_{pu}	[17.5, 49.3]	[21.8, 37.7]	[26.1, 26.1]
HT K_{pu}	[38.7, 67.5]	[40.4, 54.8]	[42.2, 42.2]
BR K_{pu}	[12.3, 19.2]	[14.2, 17.6]	[16.1, 16.1]
TE K_{pu}	[49.4, 53.3]	[50.3, 52.2]	[51.1, 51.1]
RE K_{pu}	[102.5, 113.3]	[105, 110.4]	[107.6, 107.6]

The two numbers in each interval denote the minimum and maximum K_{pu}'s per α-cut.

A hallmark of the fuzzy set theoretic approach lies in its representation of the subjective preferences of the data modeler via the use of mathematical constructs such as membership functions. Although this permits fuzzy sets to be more flexible than probability assignments in handling human thinking and judgment, it is only logical to expect that different data modelers will characterize data vagueness differently, with different membership function shapes, and that these variations will likely affect the output fuzzy sets. In this study, we have fuzzified the diazepam concentrations and the human *in vitro* metabolism data with a triangular membership function because: (1) fuzzy sets with a triangular membership function are most often used in the literature; (2) it seemed natural to assign the median of the diazepam concentration values as its 'most likely' value and the minimum and maximum values as its maximum range of uncertainty; and (3) we have intentionally kept all membership functions simple, so that the computational procedure of our modeling framework could be made clear. Quite possibly, a different group of data modelers analyzing the

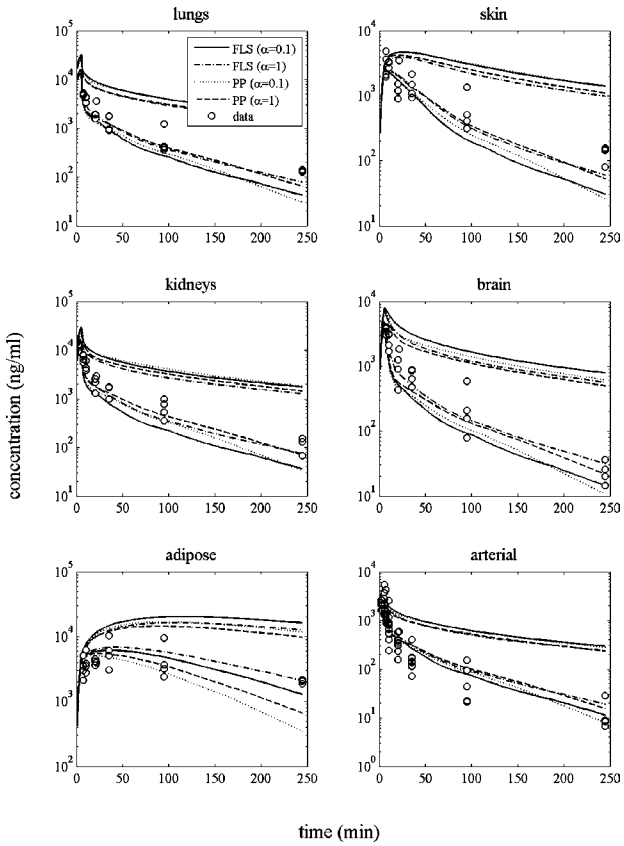

Fig. 4. Fuzzy diazepam C (ng/ml) *versus* t (min) in rat plasma and tissue compartments, as predicted using rat $\widetilde{K}_p$'s estimated by FLS regression and by the probability–possibility (P–P) transformation technique in (10). The predicted minimum and maximum profiles are shown at α-cuts of 0.1 and 1. The measured nonfuzzy concentrations (22) are shown in open circles for comparison purposes.

same data set might have considered an interval of concentration values to be equally 'most likely' at every t instance, and characterized the diazepam concentrations as trapezoidal fuzzy sets. Hence, it would be informative to assess the impact of different input membership function preferences on rat $\widetilde{K}_p$'s, and by extension, the predicted fuzzy concentration in human tissues in a study involving sensitivity analysis. In this regard, we expect that changing the shape of all input membership functions from triangular to, for example, trapezoidal, will alter the shape of the membership functions of rat $\widetilde{K}_p$'s and of the diazepam concentrations in human. However, assuming that the supports of the revised input membership functions are still bounded by the minimum and maximum measurements, we anticipate the spreads of these predicted fuzzy sets to be comparable to those obtained from the present study.

The computational complexity of our FLS regression algorithm depends on the number of iterations required to obtain the optimal fuzzy coefficients as well as the computational complexity of a single iteration, which is dependent on the numerical complexity of the underlying model (e.g., number of equations, number of model parameters). Due to its computational and analytical simplicity, we have chosen to use the 'open loop' estimation method to illustrate the framework of FLS regression analysis in this study, as opposed to the 'close loop' estimation approach by which all equations of the PBPK model are fitted simultaneously to the data. However, the 'open loop' estimation method does not account for the interdependence between the tissues in regard to the mass balance of diazepam in rat, therefore yielding potentially biased $\widetilde{K}_p$'s and concentration predictions (19,22). It may thus be desirable to fit all tissue distribution data simultaneously to estimate rat $\widetilde{K}_p$'s via the 'close loop' method. Since this estimation approach is numerically more complex than the 'open loop' method, enhanced computational resources as well as improved optimization tools may be needed to solve the mathematical programming problem we formulated using least-squares fitting.

Fuzzy Set-Based Prediction

Despite differences between the shapes of the membership functions of rat $\widetilde{K}_p$'s estimated via FLS regression and the probability-possibility transformation technique (10), the fuzzy diazepam concentrations in various rat tissues were numerically close, in the sense that the corresponding envelopes' profiles at various α-cuts were almost identical. Such similarity can be traced to these reasons: (1) the defuzzified K_p values were only slightly different; (2) on the average, the supports of rat $\widetilde{K}_p$'s did not differ appreciably; and (3) apart from the differences between rat $\widetilde{K}_p$'s, we and the authors of (10) used the same PBPK model structure for prediction. More importantly, we observed that the predicted

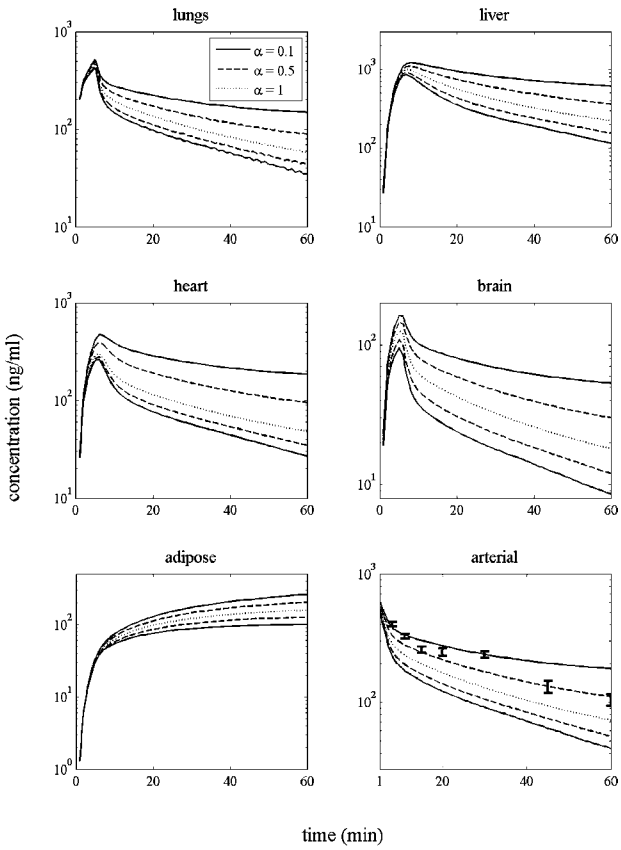

Fig. 5. Fuzzy-valued diazepam C (ng/ml) *versus* t (min) in human plasma and tissue compartments. The predicted minimum and maximum profiles are shown at α-cuts of 0.1, 0.5 and 1. The measured mean (±SD) concentrations (shown as *error bars*) (23) for the arterial compartment are also shown for comparison purposes.

contours of $\alpha \geq 0.1$ membership in our rat PBPK model typically enveloped the data, further indicating the reliability of FLS regression for parameter estimation. While not providing independent, rigorous validation of the human PBPK model's predictions following intravenous administration of diazepam, the comparison of the concentration-time envelopes with previously published mean *in vivo* data is rather encouraging.

It must be stressed, however, that prediction based on fuzzy set theory is more suitable for qualitative reasoning than quantitative estimation of uncertainty. As mentioned previously, this is because varying degrees of human subjectivity are inevitably incorporated when insufficient, imprecise or vague information is enshrined in the form of fuzzy sets and membership functions. Consequently, the predicted diazepam concentration α-cuts may differ in areas like the 'most likely' values and the maximum variability at the lowest level of certainty, i.e., at the α-cut equal to zero. Therefore, the superposition of the predicted fuzzy envelopes and the measured data in this study only served as evidences of the robustness of the computational framework, as well as the suitability of the model structure (and parameters), and not as definitive proof of model validation. Nonetheless, it appears that fuzzy PBPK model prediction based on fuzzy parameters can describe variability in model parameters on the basis of a limited number of values from experiments and/or human opinion. As such, it can (1) improve the typical value approach of simulating single curves or variables by including a measure of variability and uncertainty in the parameters; and (2) provide a formal framework for the representation of uncertainty in risk assessment when the limited data at hand cannot justify the assumptions underlying the use of probability distributions for statistical techniques, e.g., Monte Carlo simulation.

The major advantage of PBPK models is the opportunity to quantify concentrations and amounts in physiological compartments remote from the site of administration and sampling. The predictions of diazepam concentrations in such remote tissues (e.g. heart and kidneys) are conditional on the model structure and the (fuzzy) parameter values. Our results show that, following intravenous administration, most peripheral compartment concentrations reach their peaks at around 10 minutes after injection; the peaks are then followed by a steady decrease. Small numerical instabilities at large times and low concentrations (which can be especially appreciated in Fig. 5 in the lung compartment predictions) are mostly due to the large uncertainty in the $\widetilde{K}_p$'s estimated in the rat. The impossibility to independently validate the predicted remote site time courses against direct measurements is a clear limitation of these predictions, and in fact it can be argued that most PBPK modeling studies *in vivo* suffer from similar problems. However, we feel these results are promising, especially in light of published reports suggesting successful use of PBPK methods in scaling of drug disposition from rat to human (15,41) .

CONCLUSIONS

We present a novel and powerful computational framework to predict, via fuzzy set theoretic approaches, intravenous pharmacokinetic profiles in rat and in human based on limited rat *in vivo* tissue distribution and human *in vitro* metabolic data. A significant feature of our framework is the implementation of FLS regression analysis to estimate rat $\widetilde{K}_p$'s in the presence of uncertainty in data that arises as a consequence of the vagueness attached to human judgment. Further development of the fuzzy PBPK modeling framework, e.g., through estimation of $\widetilde{K}_p$'s via a 'closed loop' model, will serve to further enhance its modeling capability.

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