

# Interpretation of Non-Clinical Data for Prediction of Human Pharmacokinetic Parameters: In Vitro-In Vivo Extrapolation and Allometric Scaling

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**Abstract:** Extrapolation of pharmacokinetic (PK) parameters from in vitro or in vivo animal to human is one of the main tasks in the drug development process. Translational approaches provide evidence for go or no-go decision-making during drug discovery and the development process, and the prediction of human PKs prior to the first-in-human clinical trials. In vitro-in vivo extrapolation and allometric scaling are the choice of method for projection to human situations. Although these methods are useful tools for the estimation of PK parameters, it is a challenge to apply these methods since underlying biochemical, mathematical, physiological, and background knowledge of PKs are required. In addition, it is difficult to select an appropriate methodology depending on the data available. Therefore, this review covers the principles of PK parameters pertaining to the clearance, volume of distribution, elimination half-life, absorption rate constant, and prediction method from the original idea to recently developed models in order to introduce optimal models for the prediction of PK parameters.

**Keywords:** pharmacokinetics; in vitro-in vivo extrapolation; allometric scaling; animal scale-up; translational approach; non-clinical study

## 1. Introduction

One of the main reasons associated with the termination of drug development is inappropriate pharmacokinetic (PK) properties in humans [1]. Drugability is mainly dependent on the drug's metabolism and pharmacokinetic (DMPK) properties, which are the main hurdles in pharmaceutical R&D. Approximately 40% of drug failures are attributed to DMPK issues [2]. The main cause of failure in clinical trials is poor efficacy [3]. Although issues related to PK and bioavailability have improved since the 2000s [4], nearly half of all the therapeutic candidates in drug development are lost due to poor absorption, distribution, metabolism, excretion (ADME), toxicology, and pharmacology (safety) [5].

Therefore, the prediction of PK properties in humans before the first-in-human clinical trials is one of the main purposes of non-clinical studies in the drug discovery–development process. The two ways of predicting PK in humans include in vitro-in vivo extrapolation (IVIVE) and allometric scaling (AS).

The physiologically-based IVIVE model is based on physiological, biochemical, and biopharmaceutical factors such as organ size, blood flow rate, enzyme kinetics, drug permeability,

partitioning factor into the organ, and various in vitro clearance data. These data are incorporated into the IVIVE model to provide valuable insight into drug properties and evidence to guide decision-making in the drug discovery-development process. Despite its advantages, construction of the IVIVE model requires knowledge of PKs and an understanding of complex mathematical equations. Moreover, this approach is expensive and time-consuming [6]. Although to project in vitro to in vivo data is difficult due to the complexity of the interdependent biological processes and their dynamic nature [7], it is more physiologically relevant than AS, considering that IVIVE incorporates physiological factors and includes the possibility to expand with the mechanistic model [8,9]. In IVIVE, although methods to predict the various forms of clearance are available (e.g., biliary [10], renal [11–13], glucuronidation [11], and hydrolysis [14] clearance), we focus on the prediction of hepatic clearance which is the primary elimination pathway. AS is an empirical approach to predict human PK parameters. The origin idea and application of AS in PKs have been discussed in detail by numerous works [15–21]. Although AS is empirical and has limitations for drugs with high protein bound, extensive active renal secretion, and other transport processes or have species-specific binding or distribution, that may poorly predict human PK parameters [22], it is simple and less complicated, while providing a valuable insight as well.

Although basic principles and methodologies of the two methods vary, they have a common goal which is human PK prediction. Data required for IVIVE and AS were obtained from non-clinical studies prior to the entry of clinical trials [23–25]. Animal PK data are routinely obtained in non-clinical drug development processes [26]. These two methods are practically used to estimate the first-in-human dose in clinical trials [25].

Until now, numerous IVIVE and/or AS methods have been developed and comparative analyses have been conducted. However, a general overview of the fundamental principle of PK parameters for the application of IVIVE and AS is lacking, and the available methods are scattered. Therefore, this review will provide a comprehensive overview of the underlying principles of PK parameters with mathematical equations.

## 2. Theoretical Background for the Prediction of Clearance

### 2.1. Physiological Clearance Concept

Clearance (CL) is considered the most important PK parameter as it is related to drug elimination and bioavailability [27]. Further, the main purpose of IVIVE is to predict human CL using in vitro data and physiologically relevant mathematical equations. Therefore, an understanding of the basic principles of the CL concept is the first step prior to applying IVIVE. Due to the significance of the parameter itself, and physiological relevance, prediction of CL is one of the key steps in drug discovery and development.

There are three methods for the calculation of CL in PKs [28].

1. Non-compartmental analysis (NCA): This method employs data-dependent and model-independent calculations without the need to define a specific compartment model. The elimination constant is derived from the linear-regression of the elimination phase of a drug. The CL in NCA is calculated using Equation (1), in which the dose is the amount of drug introduced to systemic circulation and  $AUC_{0-\infty}$  is area under the concentration-time curve from zero to infinity. In this equation, the volume of distribution (V) does not need to be defined. In case of administration involving the absorption pathway, the dose is adjusted based on bioavailability (F).

$$CL = \frac{\text{Dose} \cdot F}{AUC_{\infty}} \quad (1)$$

2. Compartmental analysis: CL is calculated using the elimination rate constant (e.g., expressed as  $k_{el}$ , or  $k_{10}$ ) and V. This method assumes a defined compartment model. In this method, CL is calculated based on the following equation:

$$CL = k \cdot V \quad (2)$$

3. Physiological model: This model describes CL by incorporating physiological, anatomical, and/or biochemical aspects. The knowledge of the physiological PK model is crucial to understand IVIVE since it has improved by the efforts to explain the PK phenomenon as more physiologically relevant. Therefore, comprehensive physiological CL concepts are described in the following subsections.

#### 2.1.1. Organ Clearance

The concept of organ clearance is based on the loss of a parent drug across an organ of elimination [29–32]. A well-perfused clearing organ exhibits the ability to clear xenobiotics. If a drug is cleared in the clearing organ, then  $C_{out}$  is less than  $C_{in}$  ( $C_{out} < C_{in}$ ), in which  $C_{in}$  and  $C_{out}$  indicate drug concentration in artery and venous, respectively.

The rate of the input and output of a drug can be expressed by multiplying drug concentration with flow,  $Q$ . Regarding mass balance, the rate of elimination is defined by the difference between input and output as described below.

$$\text{Rate of elimination} = C_{in} \cdot Q - C_{out} \cdot Q = Q \cdot (C_{in} - C_{out}) \quad (3)$$

Organ extraction ratio (ER) is the ratio of the elimination rate to the input rate. Thus, ER can be understood as the efficiency with which the organ clears the drug under a specific blood flow,  $Q$ . ER is calculated using the following equation:

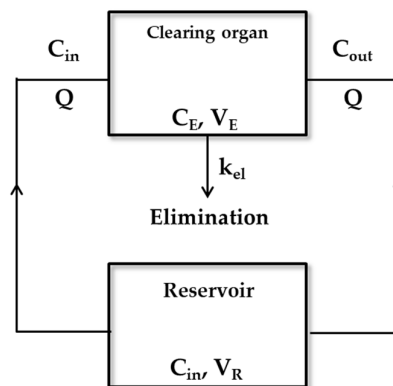
$$ER = \frac{Q(C_{in} - C_{out})}{Q \cdot C_{in}} = \frac{C_{in} - C_{out}}{C_{in}} = 1 - \frac{C_{out}}{C_{in}} \quad (4)$$

Organ clearance is the volume of blood cleared of a drug by an organ per unit of time. It is expressed by the following equation [33]:

$$CL = \frac{\text{The rate of elimination}}{C_{in}} = \frac{Q(C_{in} - C_{out})}{C_{in}} = Q \cdot ER \quad (5)$$

The ER is a dimension-less parameter. As shown in Equation (5), it is obvious that the organ clearance is limited by the blood flow in the specific organ. Since ER is greater than or equal to 0 but less than or equal to 1 ( $0 \leq ER \leq 1$ ), CL is greater than or equal to 0 but less than or equal to  $Q$  ( $0 \leq CL \leq Q$ ).

In Figure 1, the perfusion model explains the relationship between  $Q$  and ER [34]. This model follows the well-stirred model that will be mentioned later. It assumes that the drug distribution in intra-cellular and extra-cellular fluids can instantaneously reach equilibrium, where the blood flow rate is rapid enough not to limit the distribution of a drug. If a drug is bolus administered into the reservoir, the mass balance equations are described by the following Equations (6) to (9).



**Figure 1.** The perfusion model including one reservoir and one clearing organ. In this model,  $Q$  refers to the rate of perfusate or blood flow.  $C_{in}$  is the drug concentration in the artery entering the reservoir and clearing organ.  $C_{out}$  denotes the drug concentration in veins leaving the clearing organ and entering the reservoir, which is a non-clearing organ.

$V_E$  and  $V_R$  indicate the volume of clearing organ and reservoir, respectively. The elimination process is followed by first-order kinetics and its elimination constant is represented by  $k_{el}$ .  $C_E$  is the drug concentration in the clearing organ.

Differential equations for the reservoir and clearing organs are as follows:

$$-V_R \frac{dC_{in}}{dt} = Q(C_{in} - C_{out}) \quad (6)$$

$$V_E \frac{dC_E}{dt} = Q(C_{in} - C_{out}) - k_{el} \cdot V_E \cdot C_E \quad (7)$$

where  $C_E$  is drug concentration in the clearing organ. However, in a practical setting, the analysis of the actual drug concentration in the organ is impossible. Therefore,  $C_E$  is substituted by  $C_{out}$  which can be measured in a practice setting using the partition coefficient between  $C_E$  and  $C_{out}$  as shown in the equations below:

$$K_P = \frac{C_E}{C_{out}} \quad (8)$$

$$K_P \cdot V_E \cdot \frac{dC_{out}}{dt} = Q(C_{in} - C_{out}) - k_{el} \cdot K_P \cdot V_E \cdot C_{out} \quad (9)$$

Solving Equations (7) and (9) for  $C_{in}$  and  $C_{out}$  and substituting these solutions into Equation (1), the final solution yields Equation (10) below. The detailed solving method has been represented in Rowland et al. [34]:

$$CL_{org} = \frac{Q \cdot k_{el} \cdot K_P \cdot V_E}{Q + k_{el} \cdot K_P \cdot V_E} \quad (10)$$

in which  $CL_{org}$  denotes the organ clearance.

In Equation (10),  $k_{el}K_PV_E$  is defined as intrinsic clearance ( $CL_{int}$ ); in other words, an intrinsic capability of a liver to remove a drug from the blood without any flow limitations. The unit of  $k_{el}K_PV_E$  is identical to  $CL$ , and it is expressed by the following equation:

$$CL_{int} = k_{el} \cdot K_P \cdot V_E \quad (11)$$

which from Equation (10) implies

$$CL_{org} = \frac{Q \cdot CL_{int}}{Q + CL_{int}} \quad (12)$$

Equation (12) indicates that  $CL_{org}$  is a function of  $Q$  and  $CL_{int}$ . There are two circumstances depending on the relative size of the two variables.

1. The first situation is when the clearance capacity (i.e.,  $CL_{int}$ ) exceeds the  $Q$  ( $CL_{int} \gg Q$ ). In this situation, Equation (12) collapses and transforms to Equation (13).

$$CL_{org} \cong Q, \quad \text{if } CL_{int} \gg Q \quad (13)$$

2. The second situation is when  $C_{out}$  is a small fraction of  $C_{in}$  (i.e., when  $K_P$  is high, or ER is low). [34]. In this case, Equation (12) collapses in the following equation:

$$CL_{org} \cong CL_{int}, \quad \text{if } CL_{int} \ll Q \quad (14)$$

The basic assumption of the  $CL$  concept is that only an unbound free drug is accessible to the enzyme and is subjected to metabolism or biliary excretion. Therefore, the actual intrinsic clearance should be based on the unbound fraction in plasma ( $f_p$ ) or blood ( $f_b$ ). In practical settings, the calculation of protein binding and the analysis of drug concentration are usually performed with plasma. Interconversion between the free fractions in blood and in plasma is shown below:

$$f_b = \frac{f_p \cdot C_p}{C_B} \quad (15)$$

$$C_B = C_{RBC} + C_P(1 - H_{ct}) \quad (16)$$

where  $C_B$  and  $C_P$  refer to the total drug concentration in blood and in plasma, respectively.  $H_{ct}$  is the hematocrit with a value of 0.44 in humans [35] and  $C_{RBC}$  refers to the drug concentration in red blood cells.

Therefore,  $CL_{org}$  is expressed by the equation below by incorporating  $f_p$ :

$$CL_{org} = \frac{Q \cdot f_p \cdot CL_{int}}{Q + f_p \cdot CL_{int}} \quad (17)$$

### 2.1.2. Consideration of Enzyme Kinetics

In Equation (12), if the clearing organ is the liver, the correlation between hepatic clearance ( $CL_H$ ) and enzyme kinetics is expressed by the equation below.

The metabolic rate ( $V_{met}$ ) in the liver is described by the Michaelis–Menten equation:

$$V_{met} = \frac{V_{max} \cdot C}{K_m + C} \quad (18)$$

where  $V_{max}$  is the maximal rate of the reaction,  $C$  is the concentration of the substrate, and  $K_m$  is the Michaelis constant. If both sides of Equation (18) are divided by  $C$ , then  $V_{met}/C$  is the hepatic intrinsic clearance ( $CL_{int,H}$ ) as shown in the following equation:

$$CL_{int,H} = \frac{V_{met}}{C} = \frac{V_{max}}{K_m + C} \quad (19)$$

Since liver enzymes are rarely saturated in clinical practice, generally the value of  $K_m$  is much greater than  $C$ . Thus, Equation (19) can be simplified into the following equation:

$$CL_{int,H} = \frac{V_{met}}{C} = \frac{V_{max}}{K_m} \quad (20)$$

Intrinsic clearance is also expressed by the summation of enzyme activities of all parallel metabolic pathways as shown in the following equation:

$$CL_{int,H} = \sum_{i=1}^n \frac{V_{max,i}}{K_{m,i}} \quad (21)$$

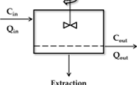
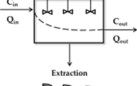
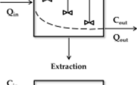
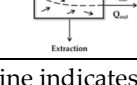
In an *in vitro* setting, the  $V_{max}$  and  $K_m$  are calculated. Then hepatic clearance is estimated by embedding the  $CL_{int,H}$  into Equation (12).

### 2.1.3. Hepatic Clearance Model

Liver is one of the key organs for drug clearance via metabolism and/or excretion through the bile acid. For most drugs, the elimination process in PKs involves hepatic metabolism. Alteration of liver blood flow, synthesis of albumin, and/or enzyme activity could occur by liver impairment, concomitant drug use, environmental factors, and so on [36,37]. Therefore, predicting drug behavior in the liver facilitates the analysis of hepatic drug elimination in virtual scenarios [38].

In the field of PKs, there are four representative hepatic clearance models (Table 1).

**Table 1.** Four hepatic clearance models.

Model	Scheme <sup>1</sup>	CL <sub>H</sub> <sup>2</sup>	ER <sub>H</sub>
Well-stirred		$\frac{Q_H \cdot CL_{int,H} \cdot f_p}{Q_H + CL_{int,H} \cdot f_p}$	$\frac{CL_{int,H} \cdot f_p}{Q_H + CL_{int,H} \cdot f_p}$
Parallel tube		$Q_H \cdot \left\{ 1 - e^{-\left( \frac{f_p \cdot CL_{int,H}}{Q_H} \right)} \right\}$	$1 - e^{-\left( \frac{f_p \cdot CL_{int,H}}{Q_H} \right)}$
Distributed		$Q_H \cdot \left\{ 1 - e^{-\left( \frac{f_p \cdot CL_{int,H}}{Q_H} + \frac{1}{2} \varepsilon^2 \left( \frac{f_p \cdot CL_{int,H}}{Q_H} \right)^2 \right)} \right\}$	$1 - e^{-\left( \frac{f_p \cdot CL_{int,H}}{Q_H} + \frac{1}{2} \varepsilon^2 \left( \frac{f_p \cdot CL_{int,H}}{Q_H} \right)^2 \right)}$
Dispersion		$Q_H \cdot \left\{ 1 - \frac{4a}{(1+a)^2 \cdot e^{\left[ \frac{a-1}{2DN} \right]} - (1-a)^2 \cdot e^{-\left[ \frac{a+1}{2DN} \right]}} \right\}$	$1 - \frac{4a}{(1+a)^2 \cdot e^{\left[ \frac{a-1}{2DN} \right]} - (1-a)^2 \cdot e^{-\left[ \frac{a+1}{2DN} \right]}}$

<sup>1</sup> Dotted line indicates the concentration–distance profile within liver.

<sup>2</sup> Where  $Q_H$  is hepatic liver flow expressed as a unit of mL/min/kg.

### Well-Stirred Model

The well-stirred model is a widely applied model, in which the liver is viewed as a single, well-mixed compartment with a fixed drug concentration. This model is expressed in simple equations.

CL<sub>H</sub> is described by Equation (5):

$$CL_H = Q_H \cdot ER_H \quad (22)$$

where  $Q_H$  is the hepatic blood flow (20.7 mL/min/kg in humans), and ER<sub>H</sub> is the hepatic extraction ratio. Since ER is dependent on  $Q_H$ , CL<sub>H</sub> is not directly proportional to  $Q_H$ . Typically ER decreases with increasing  $Q_H$  [32]. Additionally, hepatic availability ( $F_H$ ) is calculated by the following equation using ER<sub>H</sub>:

$$F_H = 1 - ER_H = \frac{Q_H}{Q_H + f_p \cdot CL_{int,H}} \quad (23)$$

For the drugs with high ER<sub>H</sub>, equations of CL<sub>H</sub>, ER<sub>H</sub>, and  $F_H$  are simplified as the following equations:

$$CL_H \cong Q_H \quad (24)$$

$$ER_H \cong \frac{CL_{int,H} \cdot f_p}{CL_{int,H} \cdot f_p} \cong 1 \quad (25)$$

$$F_H \cong \frac{Q_H}{f_p \cdot CL_{int,H}} \quad (26)$$

For the drugs with low ER<sub>H</sub>, the equations of CL<sub>H</sub>, ER<sub>H</sub>, and  $F_H$  are to be simplified to the following equations:

$$CL_H \cong CL_{int,H} \quad (27)$$

$$ER_H \cong \frac{CL_{int,H} \cdot f_p}{Q_H} \quad (28)$$

$$F_H \cong \frac{Q_H}{Q_H} \cong 1 \quad (29)$$

### Parallel-Tube Model

The parallel-tube model describes the liver as a set of tubes representing a sinusoid where the elimination occurs in hepatocytes. Drug concentration within the liver (i.e., sinusoids and hepatocytes) exponentially decreases in the direction of the hepatic vein [39].

In this model,  $F_H$  is expressed by the following equation:

$$F_H = 1 - ER_H = e^{-\left(\frac{CL_{int,H}}{Q_H}\right)} \quad (30)$$

When  $ER_H$  and  $Q_H$  are known, the  $CL_{int,H}$  is estimated by this model. Taking the natural logarithm of the Equation (30):

$$\ln(1 - ER_H) = -\frac{CL_{int,H}}{Q_H} \quad (31)$$

$$CL_{int,H} = -Q_H \times \ln(1 - ER_H) = -Q_H \times \ln F_H \quad (32)$$

Both well-stirred and parallel-tube models assume that drug permeability is not a rate-limiting step in drug elimination [40]. However, recently, an extended clearance model has been developed in which permeability is one of the important factors affecting the  $CL_H$  [41,42].

In many cases, the well-stirred model is the choice of method for the estimation of  $CL_{org}$ . However, in certain situations, the estimation of  $CL_H$  differs between the two models. Pang and Rowland have shown these differences [43–45]. In their studies, using lidocaine with an  $ER$  of 0.99 or higher, a liver perfusion experiment was conducted in mice. Its metabolite profile is well described by the well-stirred model. The major differences between these two models are  $F_H$  based on changes of  $Q_H$  and oral bioavailability ( $F_{po}$ ). When a drug with high  $ER_H$  (e.g., lidocaine) is administered via per oral (PO) route, its  $F_{po}$  is expressed by the following equation:

$$F_{PO} = F_H = 1 - ER_H = e^{-\left(\frac{CL_{int,H}}{Q_H}\right)} \quad (33)$$

Based on the well-stirred and parallel-tube model, the Equation (33) could be transformed into Equations (34) and (35), respectively:

$$F_{PO} \cong \frac{Q_H}{f_p \cdot CL_{int,H}} \quad (34)$$

$$F_{PO} \cong e^{-\left(\frac{f_p \cdot CL_{int,H}}{Q_H}\right)} \quad (35)$$

As shown in these equations,  $F_{PO}$  is associated with  $Q_H$ . In a well-stirred model,  $F_{PO}$  shows a linear relationship with  $Q_H$ . However, in the parallel-tube model,  $F_{PO}$  changes exponentially with  $Q_H$ . By comparing the observed values with predicted values using these two models, the investigator can select the model that better explains the organ clearance. However, under practical experimental settings, it is hard to determine the model with a good fit prior to an investigation. Therefore, unless there is obvious evidence, most investigators use the well-stirred model based on the principle that models should be as simple as possible, but not simpler [40,46].

#### Distributed Model and Dispersion Model

It is obvious that the liver is neither a well-stirred compartment nor a series of identical tubes [47]. There have been efforts to explain hepatic clearance as more physiologically relevant by using a dispersion model [48,49] or a distributed model [50,51]. The distributed model describes the liver as a series of parallel tubes with different geometrical properties. In this model,  $\varepsilon^2$  is an estimated parameter used to express variance for each sinusoid in the whole liver [52]. In the distributed model, the mixing of blood in the sinusoids is incorporated into flow rates and path length. The degree of mixing is defined by the dispersion number  $D_N$  which is estimated in this model. When  $D_N \rightarrow \infty$  or  $D_N \rightarrow$  zero, the dispersion model is collapsed in the well-stirred model and the parallel

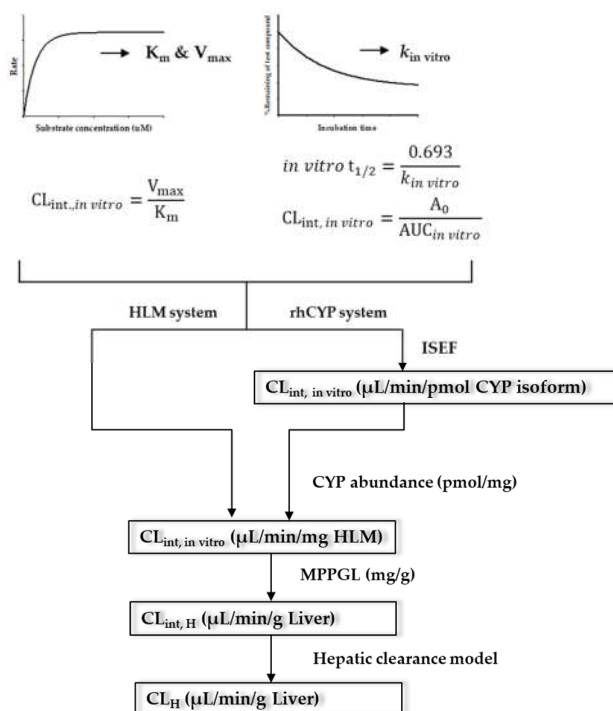
tube model, respectively. The variable 'a' in the dispersion model is equal to  $(1+4R_N D_N)^{1/2}$ , where the efficiency number of  $R_N$  is equal to  $f_p \cdot CL_{int,H}/Q_H$ .

Other models presented by scholars include the series-compartment model [53] and transit-time model [54–57]. However, the IVIVE mainly uses the four models described above.

### 3. Prediction of Human Clearance Using IVIVE Method

#### 3.1. IVIVE

The purpose of IVIVE is to perform quantitative extrapolation of in vitro data to predict human parameters. A reliable extrapolation method to predict hepatic metabolic clearance utilizes in vitro kinetic data and mathematical equations [58]. The general approach of IVIVE using human liver microsomes (HLM) or recombinant human cytochrome P450 (CYP) system (rhCYP) is presented in Figure 2. Using these systems, metabolite production or substrate depletion are used to calculate the in vitro metabolic kinetic parameters (i.e.,  $K_m$ ,  $V_{max}$ , and  $k_{in\ vitro}$ ). The IVIVE method has been improved since its introduction by Rane et al. [59]. Scale-up of in vitro data to in vivo is performed by analyzing the correlation between in vitro and in vivo data or applying physiological correction factors. Many investigators have tried to improve the accuracy of prediction (Table 2).



**Figure 2.** The scheme of the overall in vitro-in vivo extrapolation (IVIVE) process using human liver microsomes or recombinant human cytochrome P450 (CYP) system. MPPGL refers to the microsomal protein per gram of liver.

##### 3.1.1. Empirical IVIVE Model

Scaling factors have been used to predict in vivo clearance from in vitro data. Correction factors are key components in this method. Various physiological or empirical values have been suggested in this approach. Appropriate scaling factors have been developed to improve the predictability of the IVIVE model. A direct physiological scaling factor was incorporated to predict  $CL_H$  using in vitro hepatocytes and rat microsomes data by Houston [60]. In that study, the basic principal and process of IVIVE were presented. The physiological scaling factor was investigated. Results indicated that this simple scaling factor yielded adequate evidence supporting IVIVE.



Another empirical analysis was performed by Lavé et al. [61], who used human hepatocytes as an in vitro system to predict human  $ER_H$ . A scaling factor in Equation (41), shown in Table 2, was estimated using non-linear iterative least squares, which is not a fixed value. The predicted  $ER_{H,pred}$  and intrinsic in vitro clearance ( $CL_{int, in vitro}$ ) had a good relationship. In this method, no protein binding was considered, resulting in overestimation of  $ER_{H,pred}$  values of highly bound drugs. Nevertheless, the PK parameters of a few highly bound drugs, such as bosentan and lorazepam, were estimated with good agreement. The authors suggested that such discrepancy was attributed to the differences between the relative binding rate of the drug in the plasma and in hepatocytes, and/or its relative [61]. However, the overall predictability of human PK parameter was improved by applying a precise scaling factor, which plays a key role in the IVIVE method.

As shown in these results of Houston [46] and Lavé et al. [47], appropriate scaling factors are important in the IVIVE model to improve the predictability. Protein binding also has a critical impact on the prediction of in vivo PK parameters. The effect of binding properties on the prediction of CL has been investigated in other studies.

### 3.1.2. Correction Factor of IVIVE Model

#### Protein Binding Factor

Obach [62] has reported the prediction method of human intrinsic hepatic clearance ( $CL_{int, H, human}$ ) using the in vitro half-life ( $t_{1/2}$ ) to incorporate non-specific binding factors to microsomes ( $f_{u,mic}$ ) and/or the  $f_p$ . Twenty-nine drugs were classified according to their chemical property (i.e., basic, neutral, and acidic compounds). Generally, the basic compounds tend to have a large extent of binding. Results showed that human CL of neutral and basic compounds was adequately predicted with or without binding factors. However, in case of acid compounds, excluding binding factors, human CL values were predicted with a high degree of error.

In practice, in the absence of prior PKs and/or ADME knowledge of a compound of interest, one cannot easily decide whether or not to consider protein binding when predicting human PK parameters. Therefore, the projection of human CL considering both binding factors (i.e., in vitro microsomes binding and the fraction unbound in plasma) is a strategy to decrease significant risks of over/under estimation of human CL while expanding the predictability.

The effect of microsomal protein binding on the prediction of  $CL_{int}$  was also investigated by Austin et al. [63]. In their work, rat liver microsomes were used as an in vitro system. Their results showed that the  $CL_{int}$  was dependent on microsomal concentration. However, this relationship can be ignored when  $f_{u,mic}$  is considered. The authors also found that  $f_{u,mic}$  was correlated with lipophilicity. Based on these results, the authors formulated an equation for the calculation of  $f_{u,mic}$  based on the physicochemical properties of drugs. Equation (36) can be used to calculate  $f_{u,mic}$  as follows:

$$f_{u,mic} = \frac{1}{C \times 10^{0.56(\log P/D)-1.41} + 1} \quad (36)$$

where C denotes the microsomal protein concentration (mg/mL) and log P/D refers to log P of a basic compound ( $pK_a > 7.4$ ) or logD<sub>7.4</sub> of acidic compound ( $pK_a < 7.4$ ), where logD<sub>7.4</sub> stands for the partition coefficient between octanol-0.02 M phosphate buffer (pH 7.4 at 20 °C). The logP is equal to the logD<sub>7.4</sub> for compounds designated as neutral and the logP is also calculated using the following equation:

$$\log P = \log D_{7.4} + \log(1 + 10^{7.4A+BpK_a}) \quad (37)$$

where A = 1 and B = −1 for an acidic compound, and A = −1 and B = 1 for a basic compound [64].

Howgate et al. [65] revealed that most of the  $f_{u,mic}$  values are high enough to be ignored in the prediction of clearance. However, the few compounds with high microsomal binding should be considered to accurately predict the in vivo clearance. Therefore, when basic knowledge of the compound of interest is lacking in the early stage of drug discovery and development process, incorporating  $f_{u,mic}$  is a preferable way to predict in vivo situations.

## Animal Scaling Factor

Naritomi et al. [66] recommended the IVIVE method of the animal scaling factor, which is defined as  $CL_{int, in vivo}$  divided by  $CL_{int, in vitro}$  to improve the human  $CL_{int, in vivo}$ . This scaling factor is similar across species, since it depends on the compound itself. When the animal scaling factor in a rat or a dog was not considered, the average fold error increased (from an average two-fold to four-fold error). These results indicate that the scaling factor of each drug is conserved across an inter-species system. However, an animal scaling factor is difficult to use in the absence of adequate information for various species.

**Table 2.** Mathematical equations of the IVIVE approach for prediction of clearance from in vitro data.

Equation	Comment *	Ref.
$CL_{int, in vitro} = \frac{V_{max}}{K_m} = \frac{\text{rate of metabolism}}{C_E}$ (38)	Basic principle of IVIVE was suggested Provide the 4 stages for the IVIVE	[60]
$CL_{H, in vivo} = \frac{Q_H \cdot f_b \cdot CL_{int, in vitro}}{Q_H + f_b \cdot CL_{int, in vitro}}$ or $CL_{int} = \frac{CL_H}{f_b(1 - E)}$ (39)		
$CL_{int, in vitro} = \frac{\text{Initial amount in the incubation}}{AUC_{in vitro}}$ (40)	Empirically the scaling factor (SF) was estimated as the value of 8.9	[61]
$ER_{H, pred} = \frac{SF \cdot CL_{int, in vitro}}{Q_H + (SF \cdot CL_{int, in vitro})}$ (41)	Predicted $ER_H$ and observed $ER_H$ are $ER_{H, pred}$ and $ER_{H, obs}$ , respectively	
$ER_{H, obs} = \frac{CL}{Q_H}$ (42)	Provide criteria for the classification of the drugs into: low extraction, $ER_H < 0.3$ ; intermediate, $0.3 < ER_H < 0.7$ ; high extraction, $ER_H > 0.7$	
$CL_{int, H, human} = \frac{0.693}{in vitro t_{1/2}} \cdot \frac{mL incubation}{mg microsomes} \cdot \frac{45 mg microsomes}{g liver} \cdot \frac{20 mg liver}{kg body weight}$ (43)	Investigation of the effect of the protein binding into the plasma and microsomes	[62]
$f_{u, mic} = \frac{\text{drug/ISTD peak height ratio in buffer sample}}{2 \cdot \text{drug/ISTD peak height ratio in microsome sample}}$ (44)	The ISTD refers to the internal standard	
$CL_{int, H, pred.} = CL_{int, in vitro} \cdot \text{animal scaling factor}$ (45)		
$\text{Animal Scaling factor} = \frac{CL_{int, H, in vivo}}{CL_{int, in vitro}}$ (46)	Animal scaling factor was incorporated into IVIVE	[66]
$f_{u, mic} = \frac{\text{unchanged compound concentration in buffer}}{\text{unchanged compound concentration in microsome}}$ (47)		
$CL_{int, in vivo, pred} = CL_{int, in vitro} \cdot MPR = \frac{V_{max}}{K_M} \cdot MPR$ (48)	Microsomal protein recovery (MPR) ratio was incorporated	[67]

$\frac{\text{MPR (mg protein/g liver)}}{\text{Liver homogenate CYP content (nmol/g liver)}} = \frac{\text{Microsomal CYP content (nmol/mg protein)}}{\text{Microsomal CYP content (nmol/mg protein)}} \quad (49)$	in IVIVE R <sub>B/P</sub> refers to blood to plasma ratio
$\text{CL}_{\text{int.in vivo,obs}} = \frac{\text{CL}}{f_p \cdot R_{B/P}} \quad (50)$	
$\frac{\text{P450 content correcting factor}}{\text{P450 isozyme content/g liver}} = \frac{\text{P450 isozyme content/mg protein}}{\text{P450 isozyme content/mg protein}} \quad (51)$	CYP abundance was incorporated in IVIVE [68]
$\text{RAF} = \frac{V_{\text{max}}(\text{HML})}{V_{\text{max}}(\text{rhCYP})} \quad (52)$	Relative activity factor (RAF) introduced for scaling rhCYP data to HLM
$\text{RAF} = \frac{\text{CL}_{\text{int}}(\text{HML})}{\text{CL}_{\text{int}}(\text{rhCYP})} \quad (53)$	[69,70]
$\text{CL}_{\text{int}} = \left[ \sum_{j=1}^n \left( \sum_{i=1}^n \frac{V_{\text{max}}(\text{rhCYP})_i \times \text{RAF}_{ij}(V_{\text{max}})}{K_m(\text{rhCYP})_i} \right) \right] \times \text{MPPGL} \times \text{Liver weight} \quad (54)$	Modified RAF taking into account of K <sub>m</sub>
$\text{ISEF} = \frac{V_{\text{max}_{ji}}(\text{HML})}{V_{\text{max}_i}(\text{rhCYP})_i \times \text{CYP}_j \text{abundance (HLM)}} \quad (55)$	Inter-system extrapolation factor (ISEF) is introduced for scaling rhCYP data to HLM
$\text{CL}_{\text{int}} = \left[ \sum_{j=1}^n \left( \sum_{i=1}^n \frac{V_{\text{max}_{ji}}(\text{rhCYP})_i \times \text{CYP}_j \text{abundance}}{K_m(\text{rhCYP})_i} \right) \right] \times \text{MPPGL} \times \text{Liver weight} \quad (56)$	[69]
$\text{CL}_H = \frac{R_{B/P} \cdot Q_H \cdot \text{CL}_{\text{int,liver,human}} \cdot f_p \cdot F_I}{R_{B/P} \cdot Q_H + \text{CL}_{\text{int,liver,human}} \cdot f_p \cdot F_I} \quad (57)$	The ionization factor is incorporated into the IVIVE
$F_I = \frac{f_p^n}{f_{IW}^n} = \frac{1 - f_p^i}{1 - f_{IW}^i} \quad (58)$	F <sub>I</sub> is an ionization factor Subscript letter IW denotes intracellular water
$f_{\text{acid}}^i = \frac{[A^-]}{[AH]_0} = \frac{1}{1 + 10^{pK_a - \text{pH}}} \quad (59)$	Upper letter i and n indicate compounds of ionized and neutral forms, respectively
$f_{\text{base}}^i = \frac{[BH^+]}{[B]_0} = \frac{1}{1 + 10^{\text{pH} - pK_a}} \quad (60)$	
$\text{CL}_H = \frac{Q_H \cdot \text{CL}_{\text{int,liver,human}} \cdot f_{u,\text{liver}}/f_{u,\text{mic}}}{Q_H + \text{CL}_{\text{int,liver,human}} \cdot f_{u,\text{liver}}/f_{u,\text{mic}}} \quad (61)$	The unbound fraction into the liver (f <sub>u,liver</sub> ) is incorporated into the IVIVE
$f_{u,\text{liver}} = \frac{\text{PLR} \cdot f_{u,\text{p,app}}}{1 + (\text{PLR} - 1) \cdot f_{u,\text{p,app}}} \quad (62)$	Plasma to whole liver concentration ratio (PLR) = 13.3

$CL_{\text{int,liver,in vitro}} = \frac{PS_{\text{uptake,total}} \cdot CL_{\text{met}} + PS_{\text{bile}}}{CL_{\text{met}} + PS_{\text{efflux,total}} + PS_{\text{bile}}} \quad (63)$	<p>Physiologically-based IVIVE model</p> <p>Total apparent uptake clearance (<math>PS_{\text{uptake,total}}</math>) consists of saturable and/or non-saturable processes</p> <p><math>CL_{\text{met}}</math> and <math>PS_{\text{bile}}</math> refer to metabolic and biliary clearance, respectively</p>	[73]
$CL_H = \frac{Q_H \cdot CL_{\text{int,liver,in vitro}} \cdot f_p}{Q_H + CL_{\text{int,liver,in vitro}} \cdot f_p} \quad (64)$	<p>Apparent sinusoidal total efflux clearance from the intracellular side of hepatocytes back into blood (<math>PS_{\text{efflux, total}}</math>) consists of saturable and/or non-saturable processes</p>	
$fn_H = fn_{\text{sec}} + fn_{\text{met}} \quad (65)$	<p>Provide the method for the prediction of total clearance and relative elimination contributions</p>	[8]
$fn_H = 1 - e^{-0.01741 PS_{\text{inf}}} \quad (66)$	<p>The <math>fn_H</math>, <math>fn_{\text{sec}}</math>, and <math>fn_{\text{met}}</math> refers to a fractional contribution of hepatic, biliary, and metabolic elimination to overall clearance</p>	
$fn_{\text{met}} = 1 - e^{-0.01521 PS_{\text{inf,pas}}} \quad (67)$	<p><math>PS_{\text{inf, act}}</math> and <math>PS_{\text{inf, pas}}</math> refer to the sinusoidal active and passive influx clearance, respectively</p>	
$CL_{\text{renal}} = CL_{\text{total}} - CL_H \quad (68)$	<p>Sinusoidal efflux from hepatocytes back into blood (<math>PS_{\text{eff}}</math>) is assumed to occur via passive diffusion, therefore <math>PS_{\text{eff}} = PS_{\text{inf,pas}}</math></p>	
$CL_{\text{total}} = \frac{CL_H}{fn_H} \quad (69)$	<p><math>CL_{\text{int,sec}}</math> and <math>CL_{\text{int,met}}</math> refer to intrinsic secretory and metabolic clearance, respectively</p>	
$CL_{\text{int,in vitro}} = \frac{(PS_{\text{inf,act}} + PS_{\text{inf,pas}}) \cdot (CL_{\text{int,sec}} + CL_{\text{int,met}})}{PS_{\text{eff,total}} + CL_{\text{int,sec}} + CL_{\text{int,met}}} \quad (70)$	<p><math>PS_{\text{inf}}</math> equals to the sum of <math>PS_{\text{inf,act}}</math> and <math>PS_{\text{inf,pas}}</math> which are determined by suspension of pooled human hepatocytes (unit: mL/min/kg)</p>	
$CL_H = \frac{Q_H \cdot CL_{\text{int,in vitro}} \cdot f_p}{Q_H + CL_{\text{int,in vitro}} \cdot f_p} \quad (71)$		

\* Each comment corresponds to all the equations within each major section of the table defined by horizontal lines.

### 3.1.3. Inter-Individual Variability (IIV) in the IVIVE Method

The prediction of human  $CL_H$  by IVIVE is generally limited by IIV, most likely due to drug metabolizing enzymes [74]. Several studies have reported the substantial differences in CYP expression and significant differences in the activity of different CYP isoforms in HLM [68,75,76]. The

potential variation in the abundance of protein expression in relevant organs can be incorporated into IVIVE [77].

The microsomal protein per gram of liver (MPPGL) value can be used as a scaling factor to calculate  $CL_{\text{int, in vivo}}$  from  $CL_{\text{int, in vitro}}$ . Generally, a value of 45 mg/g liver [60] originally obtained from rat data, or 52.5 mg/g based on hepatocyte data reported in the literature via back calculation, is commonly used as MPPGL. Since the pharmacogenetic data of laboratory animal models are less than those of humans because of their genetics and environment, the variation in MPPGL of humans may be greater than that of rats [78].

#### Microsomal Protein Content and CYP Abundance

Since a maximum limit for microsomal CL may exist [59], drugs with high CL tend to have under-predicted  $CL_{\text{int, in vivo}}$  if data are derived from microsomal protein [51]. Carliel et al. [67] have investigated diazepam as a model drug with high clearance. Its  $CL_{\text{int, in vivo}}$  is 160 mL/min/SRW, where SRW refers to standard rat weight of 250 g. Microsomal content was adjusted by treating phenobarbital and dexamethasone as CYP inducing agents. The scaling factor calculated from Equation (49) was used to estimate  $CL_{\text{int, in vivo}}$ . The results showed a good agreement with observed in vivo clearance. Although a  $CL_{\text{int, in vitro}}$  obtained from dexamethasone-treated microsomes provided an accurate estimate of 77% of the observed  $CL_{\text{int, in vivo}}$ , the limitation similar to that of Houston [60] persisted. The relationship between  $CL_{\text{int, in vitro}}$  and  $CL_{\text{int, in vivo}}$  was investigated empirically rather than mechanistically. Nonetheless, this study suggested that variation in CYP content affects the prediction of in vivo clearance. It provides evidence supporting the incorporation of CYP content as a covariate affecting the IIV in the IVIVE model.

Correction factors of both microsomal protein content and CYP abundances have been included in the IVIVE method using the  $f_{u,\text{mic}}$  factor by Howgate et al. [65]. Underestimation of the parameter is a general issue in the IVIVE method. Inclusion of the microsomal protein content and CYP abundances that affect the IIV did not show the trend of underestimation.

#### Microsomal Protein per Gram of Liver (MPPGL)

MPPGL is a key value for the scaling of  $CL_{\text{int, in vitro}}$  to  $CL_{\text{int, in vivo}}$  using liver microsomes data or the rhCYP system as shown in Figure 2. It is a value with varying degrees of IIV. However, investigators have been using fixed values either due to the lack of information or empirically.

Barter et al. [78] have reported MPPGL variability via meta-analysis and have investigated potential covariates affecting MPPGL [79]. In their studies, the authors reported an inverse correlation between age and MPPGL. The MPPGL values range from 40 mg/g and 31 mg/g for those in their 30s and 60s, respectively. The authors also provided the following equation to calculate age-related values of MPPGL from birth to adult:

$$\text{MPPGL (mg/g)} = 10^{(1.407 + 0.0158 \times \text{age} - 0.00038 \times \text{age}^2 + 0.0000024 \times \text{age}^3)} \quad (72)$$

The results provide key information to project PK parameters to humans, especially prior to clinical trials. Healthy subjects constitute the typical population for a clinical pharmacology study during the early phase of drug development, whereas real world patients are very disparate. Estimation of the PK parameters of special populations (e.g., pediatric or geriatric patients) is one of the challenging tasks in clinical trials. Of course, various factors that affect PKs in a special population have been studied. These results provide meaningful insight suggesting that non-clinical data may be considered for the design of clinical trials representing special populations.

#### Inter-System Extrapolation Factor (ISEF)

The use of a recombinant system represents an alternative in vitro method instead of human liver samples, for prediction of in vivo metabolic clearance. Iwatsubo et al. [68] have suggested the use of CYP450 isoform content in the recombinant method and proposed a P450 content correction factor. Since the levels of CYP450 reductase and cytochrome b5 differ from those of human livers

(lower, in most case) in a recombinant system, the authors have proposed an additional correction factor, expressed in Equation (51). The authors have concluded that the prediction of in vivo CL using recombinant system is possible if metabolic activity is corrected for the CYP isozyme content both in rhCYP and per gram liver in vivo.

ISEF, which is a dimensionless value based on the activity of CYP isoform and its contents, has been defined by Proctor et al. [70]. It is used to direct scale data from a rhCYP system to an HLM environment for evaluation of differences in intrinsic activity (per unit CYP) and IIV by incorporating CYP abundance as shown in Equations (52) to (56). Population approached IVIVE could be performed by combining the variance in physiological parameters (such as liver blood flow and liver weight) and the variance in scaling parameters (such as MPPGL and ISEF).

Nakajima et al. [69] have suggested a modified version of the relative activity factor (RAF) using  $CL_{\text{int, in vitro}}$  to correct a flaw in the original RAF, which was calculated with  $V_{\text{max}}$  alone while the  $K_m$  value was ignored. In their study, RAF represents the ratio of CL used to predict clearance of azelastine. It best reflects observed N-demethylation CL in HLM.

Chen et al. [80] have experimentally determined the ISEF of six CYP isoforms and investigated their utility in early phases of drug discovery and development. Venkatakrishnam et al. [81] have also investigated the role of CYP1A2, CYP2B6, CYP2C19, CYP3A, and CYP2D6 in a lymphoblastic cell line and suggested the incorporation of bridging factors between rhCYP and liver microsomes, such as RAF and abundance of CYP isoform in microsomes.

### 3.1.4. Additional Correction Factors

In the conventional IVIVE method, the prediction of in vivo CL using in vitro metabolic data has been performed with good agreement. However, this method could not be used for drugs with high binding rate to plasma and/or blood protein, low CL, or high interaction with transporters. To overcome these limitations, recently, investigators have tried to develop new IVIVE methods using physiologically-based and mechanistic approaches which are presented below in detail.

#### $F_i$

Only unbound and unionized forms of drugs have access to hepatocytes, which are the sites of metabolism. In the conventional IVIVE model, protein binding is a key factor contributing to the accuracy of CL prediction. Berezhkovskiy [71] has developed a modified equation to predict  $CL_H$  based on differences in intra- and extra-cellular pH of the unbound drug fraction using  $F_i$  as presented in Equations (57) to (60). These equations yielded higher values (up to 6.3-fold) of  $CL_H$  for a basic compound ( $F_i > 1$ ) for strong diprotic bases, but lower values (up to 6.3-fold) of  $CL_H$  for an acidic compound ( $F_i < 1$ ) for strong diprotic acids. The author suggests that the modified equation with  $F_i$  improved the issue of both under- and over-estimation commonly encountered in IVIVE. Therefore, for basic compounds, the modified equation could improve the prediction of  $CL_H$ . For acidic drugs, the conventional IVIVE equation tends to overestimate the  $CL_H$ . However, this modified equation also improves the prediction of  $CL_H$  for acidic compounds. Especially, the ionization factor significantly influences the calculation of  $CL_H$  for drugs with a low extraction ratio since  $CL_H$  is directly proportional to  $F_i$  in this case.

#### Effective Fraction Unbound in Plasma

Calculation of drug concentration in the site where metabolism occurs is important in IVIVE methodology since only free-form drugs penetrate the cellular membrane to reach the metabolic enzyme. Ionic interactions between extracellular binding proteins and the hepatocyte surface provide higher cellular exposure for the unbound drugs than without consideration of the interactions [82].

Poulin et al. [72,83,84] have developed a mechanistic IVIVE model based on two additional factors including pH differences between extracellular and intracellular water in liver, and protein-facilitated uptake induced by potential ionic interactions between protein-albumin bound drug complex and cell surface. This mechanistic IVIVE model overcomes the prediction of human CL

for drugs with low  $CL_{int}$  and high binding affinity for proteins commonly encountered when predicting human CL from in vitro data. Equations suggested by Poulin presented in Equations (61) to (62), incorporate the new correction factor of unbound fraction in the liver.

### 3.1.5. Physiologically-Based IVIVE Model

Despite several attempts to accurately predict drug concentrations in the liver where metabolism takes place, the comparative analysis from Hallifax and Houston [85] reported fewer differences in accuracy for the prediction in vivo CL, calculated by Berezhkovskiy and Poulin, using conventional methods. Furthermore, the authors have underscored the need to develop a model that reflects additional physiological factors and mechanistic elucidation to overcome the limitations of existing methods.

In the disposition process, transporters and enzymes play a key role by interacting with each other [86]. Conventionally, IVIVE methods are focused on a single pathway of drug metabolism. However, a drug introduced into the body is cleared via the ADME pathways, which involves numerous enzymes and transporters.

Wu et al. [87] have suggested a Biopharmaceutics Drug Disposition Classification System (BDDCS), which is modified by the Biopharmaceutics Classification System based on routes of drug elimination and the effect of efflux and absorptive transporters. Their study revealed that highly permeable compounds are highly metabolized whereas less permeable compounds tend to be eliminated via renal and/or biliary excretion in intact form.

A novel IVIVE method was developed to predict hepatic organ clearance via physiology-based modeling [42,73] as shown in Equations (63) to (64). This new method reflects additional physiologically relevant information (namely hepatic uptake, metabolism, biliary excretion, and sinusoidal efflux) compared with the conventional method. The proposed method was used to predict rat hepatic clearance of 13 compounds with various physicochemical and PK characteristics. In these studies, the hepatic clearance of valsartan (class 2 compound based on BDDCS) was underestimated with the highest fold-error of 3.95. The rate-limiting steps of class 2 compounds include metabolism and biliary excretion. Although this method incorporates both biliary excretion and metabolism in a typical single parameter prediction, underestimated cases such as valsartan prevailed probably due to its high plasma protein binding (97%) feature. However, since plasma protein binding is considered in the model, the error might have occurred due to unknown non-hepatic elimination.

Although these novel IVIVE methods provide precise prediction and detailed information of CL, additional in vitro data are required compared with the conventional single parameter prediction. Furthermore, in the early phases of drug discovery and development, it may be difficult to apply high-throughput screening, which is an advantage under in vitro experiment settings. However, this novel IVIVE method represents a very useful tool for the evaluation of optimized candidates prior to clinical trials.

An extended clearance model (ECM) based on hepatobiliary clearance has been reviewed by Camenisch et al. [41]. The same group proposed a new IVIVE method for the prediction of total clearance for accurate prediction of relative elimination contribution. Two mathematical Equations (66) and (67) depict the relationship between  $PS_{inf}$  and  $fn_H$ ; and  $PS_{inf,pas}$  and  $fn_{met}$ . In vitro data (i.e., hepatic uptake data) based on suspensions of human hepatocytes  $fn_H$  and  $fn_{met}$  can be calculated using the equations.

Along with estimated fractional parameters, total clearance can be presented as the sum of parallel connected organ clearances assuming the absence of extra-hepatic and/or renal clearance. This practical method facilitates the determination of the mechanism of elimination pathway using only in vitro data.

## 4. Application of AS for the Prediction of Human PK Parameters

### 4.1. Concept of AS

Allometry is the study of the relationship between size and physiological parameter. It is the study of the usual variation in measurable characteristics of anatomy and physiology as a function of overall body size [88]. The allometric equation is generally expressed as a power function based on the following equation [20]:

$$Y = aW^b \quad (73)$$

In Equation (73), the Y and X represent quantitatively measurable variables, a denotes constant of appropriate unit, and b is a power exponent. In the PKs, Y is a parameter of interest and B is a physiological parameter, and W is weight. In general, a is drug dependent and b is parameter-type dependent, which are approximately 0.75 for CL and chemical-specific factors associated with metabolism (e.g.,  $V_{max}$ ), 1 for volume of organs and blood flows, and 0.25 for physiological times [18, 88]. AS assumes that mammals share similar anatomical, biochemical, and physiological features [16,21].

#### 4.2. Prediction of Clearance by AS

There have been various attempts to predict human clearance. Mathematical equations are presented in Table 3. Since simple AS is one of the simplest methods for prediction of human PK parameter, it is widely used for scale-up of prediction from non-clinical PK data to humans. Although it is simple and useful by nature, simple AS has not been entirely successful for the prediction of human clearance. To overcome this limitation, various groups have reported new methods of AS.

**Table 3.** Methods for prediction of clearance (CL) using allometric scaling (AS).

Method	Equation	Comments *	Ref.
Simple AS	$CL = a(W)^b$	(74) Select a proper equation by the rule of exponent (ROE)	-
AS with MLP <sup>1</sup>	$CL \cdot MLP = a(W)^b$	(75) W and BW represent body and brain weight, respectively	-
AS with BW	$CL \cdot BW = a(W)^b$	(76)	[89]
Rule of exponent	If the exponent is 0.55 to 0.7, then use the simple AS, Equation (74) If the exponent is 0.71 to 1, then use the MLP, Equation (75) If the exponent is more than 1, then use the BW, Equation (76)		[90]
Two-term method	$CL = \theta(W)^a \cdot (BW)^b$	(77) $\theta$ is a constant, which is determined by multiple regression analysis	[91]
Multixponential	$CL_{human} = aW^b + \left[ \frac{\left(1 - \frac{3}{2}b\right)}{\left(1 - \frac{1}{2}b\right)} \right] aW^{0.9}$	(78) The unit of CL is mL/min	[92]
Normalized AS	$CL_{animal} \frac{CL_{int,human}}{CL_{int,animal}} = a(W)^b$	(79) $CL_{it}$ refers the unbound $CL_{it}$ in microsomes or hepatocytes in species and humans	[93]



One species AS	$CL_{human} = CL_{animal} \cdot \left(\frac{W_{human}}{W_{animal}}\right)^b$	(80)	The exponent b is a constant 0.75, which is physiologically relevant value (e.g., blood flow, filtration, etc)	[94,95]
One species AS	$CL_{pred} = 0.152 \cdot CL_{rat} \cdot \left(\frac{W_{human}}{W_{rat}}\right)$	(81)	Predict the CL of bound drug	[90]
	$CL_{pred} = 0.41 \cdot CL_{dog} \cdot \left(\frac{W_{human}}{W_{dog}}\right)$	(82)		
	$CL_{pred} = 0.407 \cdot CL_{monkey} \cdot \left(\frac{W_{human}}{W_{monkey}}\right)$	(83)		
Two species AS	$CL_{pred} = a_{rat-dog} \cdot W_{human}^{0.628}$	(84)	Predict the CL of bound drug	
	$CL_{pred} = a_{rat-monkey} \cdot W_{human}^{0.650}$	(85)		
Hepatic method	liver $CL_{pred} = CL_{animal} \cdot \left(\frac{Q_{H,human}}{Q_{H,animal}}\right)$	(86)		[96]
FCIM <sup>2</sup>	$CL = 33.35 \times \left(\frac{a}{Rf_u}\right)^{0.77}$	(87)	R <sub>fu</sub> is the f <sub>u</sub> ratio between rats and humans and a is the coefficient form AS The unit of CL is mL/min	[97]
QSAR <sup>3</sup>	$\begin{aligned} \log CL_{pred} = & 0.433 \cdot \log(CL_{rat}) \\ & + 1.0 \cdot \log(CL_{dog}) \\ & - 0.00627 \cdot MW + 0.189 \cdot Ha \\ & - 0.00111 \cdot \log(CL_{dog}) \cdot MW \\ & + 0.0000144 \cdot MW^2 \\ & - 0.0004 \cdot MW \cdot Ha - 0.707 \end{aligned}$	(88)	The unit of observed and predicted CL value is mL/min/kg	[98]

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$$\begin{aligned}
 & \text{LogCL}_{\text{po,pred}} \\
 &= -0.5927 + 0.7386 \log(\text{CL}_{\text{po,rat}}) \\
 &+ 0.5040 \log(\text{CL}_{\text{po,dog}}) \\
 &+ 0.06014 \text{clogP} \\
 &- 0.1862 \log(\text{CL}_{\text{po,dog}}) \times \text{clogP} \\
 &+ 0.02893 \text{MW} \times \text{clogP} \\
 &+ 0.02893 \text{MW} \times \text{clogP} \\
 &+ 0.02551 \log(\text{CL}_{\text{po,rat}}) \\
 &\times \log(\text{CL}_{\text{po,rat}}) \text{clogP} \\
 &- 0.03029 \log(\text{CL}_{\text{po,rat}}) \times \log(\text{CL}_{\text{po,dog}}) \\
 &\times \text{Ha} \\
 &- 0.03051 \log(\text{CL}_{\text{po,rat}}) \times \text{MW} \times \text{clogP} \\
 &+ 0.08461 \log(\text{CL}_{\text{po,dog}}) \times \log(\text{CL}_{\text{po,dog}}) \\
 &\times \log(\text{CL}_{\text{po,dog}}) \\
 &- 0.2510 \log(\text{CL}_{\text{po,dog}}) \times \log(\text{CL}_{\text{po,dog}}) \\
 &\times \text{MW} \\
 &+ 0.06061 \log(\text{CL}_{\text{po,dog}}) \times \log(\text{CL}_{\text{po,dog}}) \\
 &\times \text{Ha} \\
 &+ 0.04607 \log(\text{CL}_{\text{po,dog}}) \times \text{clogP} \times \text{clogP} \\
 &- 0.003596 \text{clogP} \times \text{clogP} \times \text{Ha} \\
 &+ 0.0005963 \text{clogP} \times \text{Ha} \times \text{Ha}
 \end{aligned}
 \tag{89}$$

The unit of observed and predicted oral CL value is mL/min/kg [99]

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\* Each comment corresponds to all the equations within each major section of the table defined by horizontal lines.

<sup>1</sup> The maximum life-span potential (MLP) is calculated by the equation  $\text{MPL (years)} = 185.4 \text{BW}^{0.636} \text{W}^{-0.225}$  [100].

<sup>2</sup> Fraction unbound intercept correction method

<sup>3</sup> Quantitative structure activity relationship (QSAR) consist of physicochemical properties, such as molecular weight (MW), partition coefficient (cLogP), and number of hydrogen-bound acceptors (Ha).

#### 4.2.1. Two-Term Method

Boxenbaum and Fertig [17] have developed the two-term method. In their work, the intrinsic clearance of antipyrine was predicted using a two-term allometric equation including brain weight (BW) and weight (W) based on Equation (77). The equation was in good agreement with the relationship between antipyrine intrinsic clearance and physiological variables, BW and W. However, in their article, only a single drug, antipyrine, was tested. Further investigation revealed that this two-term equation was limited to general conditions.

#### 4.2.2. Rule of Exponent

Mahmood and Balian [6,89,101–106] have contributed to numerous works on AS. In their studies, they compared the CL value of antiepileptic drugs using four different allometric equations: a simple AS in Equation (74), the product of CL and MPL in Equation (75), the Boxenbaum's two-term power function in Equation (77), and the product of CL and BW in Equation (76), a novel equation developed by Mahmood and Balian.

It is well known that the simple AS adequately predicts the CL of a drug, which is mainly cleared by renal excretion. However, under general circumstances, simple AS was not adequate to predict CL. When Boxenbaum's two-term method was used to predict the CL of antiepileptic drugs, the prediction failed. When MLP and brain weight were incorporated in simple AS, the predicted values showed good agreement with the observation values [89].

Mahmood and Balian extended their studies to another work [105] by applying three of four AS methods (except for the two-term power method) to drugs with various physiochemical and PK properties.

Moreover, the ROE, established by Mahmood and Balian [105], provides a guide for the selection of appropriate AS methods for the prediction of drug clearance. The choice of method depends on the exponent determined by simple AS. If the exponent lies between 0.55 to 0.7, the simple AS method is reasonable. If the exponent lies between 0.71 to 1.0, clearance can be predicted reasonably well using CL product MLP method. If the exponent is larger than 1.0, clearance can be predicted using CL product BW method [105]. Mahmood [103] also tested the ROE to predict oral clearance by the same approach. Results showed that the ROE also predicted oral clearance.

Some researchers have expressed concerns about AS since correction factors such as MLP and BW have no clear biological rationale [107]. The fact that three or more species are needed for a reliable prediction of CL [108] is time consuming and costly in the drug development process. However, considering that various experiments are conducted during the drug discovery and development process, at least two animals in an in vivo study are necessary for the non-clinical study [109].

The ROE method could be applied to predict a human CL for biliary excreted drugs. Correction factors are derived based on bile flow with normalization by body weight and liver weights. After the appropriate method is selected by ROE, the CL in a given species is divided by the calculated bile flow correction factor and scaled. The predictability of human CL is significantly improved using the bile flow correction factor [110].

#### 4.2.3. One or Two Species Method

A few studies have reported AS using only one or two species [90,94,95]. As presented in Equations (80) to (85), these empirically determined equations provide valuable information about predicted human CL. Results using these methods are in good agreement. Especially, considering the limited available data in the early phase of drug discovery and development process, these methods are useful tools for the prediction of human CL and provide evidences for go or no-go decision-making.

#### 4.2.4. Liver Blood Flow

Liver blood flow is used as a correction factor in AS. Liver blood flow is used to predict human CL using Equation (86). It has been suggested that the simple liver blood flow-based scaling is the best method and that monkey liver blood flow (MLBF) is superior to predict human CL from rats, dogs, and monkeys' CL data [96].

The advantage of this method is clear in that it only needs a single species to scale up from animal data. In addition, the MLBF method is particularly applicable to drugs that are not readily metabolized and/or renally excreted when administered intravenously [101]. However, Mahmood [101] has raised an issue about the MLBF method reported by Nagilla and Ward [96]. Mahmood [101] claimed that the reported MLBF method had statistical flaws and that the dataset used in their work [96] should be clarified. Furthermore, the bile flow used in the study did not match with the bile flow rate reported by Davies and Morris [35] which they cited. Furthermore, the MLBF method assumes that data from rats, dogs, and monkeys are always available. However, this is not true. In addition, this method is based on only three species.

#### 4.2.5. Incorporation of in Vitro Data

Lave et al. [93] have investigated in vitro data with AS to predict hepatic clearance for 10 drugs that are extensively metabolized. They determined the rate of metabolism in various animal species via in vitro experiments, including human liver microsomes and hepatocytes. The authors concluded that correcting clearance with in vitro metabolic rates significantly improved the prediction of human CL compared with direct scaling or correction with BW.

Although this in vitro correction method provided a rationale based on physiological factors, Mahmood [102] demonstrated that MLP corrected AS produced the same results. However, this in

vitro correction method showed a clear disadvantage in that in vitro CL from several species must be determined in the MLP correct AS method, which is time consuming and costly [22].

#### 4.2.6. Protein Binding

Theoretically, only unbound drugs can be distributed to the hepatocytes where the metabolism occurs and/or kidney excretes. Since the protein binding properties of a drug vary between species [111], disposition of the drug may be variable in different species. Chiou et al. [112] have reported the effect of protein binding on prediction of human clearance in AS for 15 extensively metabolized drugs. In their work, consideration of protein binding to correct inter-species differences in AS tended to improve the prediction of human clearance. Although it is theoretically feasible to use AS with unbound clearance ( $CL_u = CL/f_u$ ) based on protein binding, in practice,  $f_u$  does not significantly improve its predictability [22], [113].

Mahmood [104] has investigated the role of protein binding in the prediction of CL using 20 randomly selected drugs. Furthermore, Mahmood [6] has compared total CL and  $CL_u$  and found that for drugs excreted renally or via extensive metabolism,  $CL_u$  could not be predicted any better than total CL.

#### 4.2.7. QSAR Approach

Wajima et al. [98,99] have tried to predict human CL based on physicochemical properties of drugs. In their method, human CL was predicted using descriptors including MW, cLogP, and Ha. Observed rat and dog data were incorporated into their analysis. Their method facilitated prediction of human clearance.

#### 4.2.8. Fraction Unbound Intercept Correction Method (FCIM)

FCIM was developed by Tang et al. [97] for prediction of human CL. In this method, water-octanol partition coefficient and the ratio of  $f_p$  between rats and human ( $Rf_u$ ) are considered. The authors concluded that the new method significantly improved the prediction, even better than ROE. Furthermore, this method improved the prediction of vertical allometry.

However, when Mahmood [106] performed comparative analysis using ROE and FCIM for drugs with various PK properties (i.e., extensively metabolized, renally excreted and/or secreted and biliary excreted), the results showed that both methods facilitated the prediction of human clearance. In some cases, one of these two methods could be more suitable for predictions. However, the author expressed concern about FCIM since it uses a fixed exponent of 0.77 and a constant of 33.35 while exponents of AS are dependent on the species used in the scaling. Furthermore, FCIM is not suitable for renally secreted and biliary excreted drugs. Despite such concerns, when both methods are considered, it is possible to predict CL in a wide range of drugs.

#### 4.2.9. Multiexponential Allometric Scaling (MA)

Goteti et al. [92] have developed a new method for animal scale up using MA. In this method, the human CL is estimated by the equation below:

$$CL = aW^b + cW^d \quad (90)$$

where  $a$  and  $b$  represent coefficient and exponent obtained from simple AS, respectively, and  $c$  and  $d$  are coefficient and slope from MA, respectively.

The slope of MA (i.e., the value of  $d$  in Equation (90)) is determined by plotting blood flow rate, organ volume, and organ weights of liver and kidney in non-clinical species against  $W$ . As a result, the slopes of liver and kidney were found very similar. The value of  $d$  is fixed as 0.9. The coefficient of  $c$  is the function of the coefficient of  $a$  from AS. The final MA equation is derived as shown in Equation (78).

The MA method can successfully predict human clearance. Their results indicate that monkey is an important species for scaling. When the exponent of simple AS was greater than 0.7, MA showed better prediction of human CL than the simple AS method.

#### 4.3. Prediction of Volume of Distribution by AS

##### 4.3.1. Volume of Distribution in PKs

There are three types of volume of distribution ( $V_d$ ) and generally estimated in PKs.

- Volume of distribution of central compartment ( $V_c$ ).
- Volume of distribution at steady state ( $V_{ss}$ )
- Volume of distribution by area ( $V_{area}$ ), also known as  $V_\beta$

$V_c$  is used as a correlation factor for the concentration and number of drugs in the body by the following equation:

$$X = V_c \times C \quad (91)$$

where  $X$  and  $C$  refer to the amount of drugs in the body and concentration in the blood, respectively. Of these three types of volume of distribution,  $V_c$  is generally predicted from animal data. Its predictability is better than the others [114].

The following equations show that  $V_d$  is clearly different from the actual tissue volume where drugs are distributed in the body:

$$\begin{aligned} X &= V_{bl} \cdot C + \sum_{i=1}^n V_i \cdot C_i \\ &= V_{bl} \cdot C + \sum_{i=1}^n V_i \cdot K_i \cdot C \\ &= \left( V_{bl} + \sum_{i=1}^n V_i \cdot K_i \right) \cdot C \\ &= V_d \cdot C \end{aligned} \quad (92)$$

where  $V_{bl}$  is the volume of blood,  $V_i$  is the volume of organ,  $C_i$  is the concentration in the organ, and  $K_i$  is the partition coefficient ( $K_i = C_i/C$ ). In this equation, the greater the tendency to distribute to tissues from blood (i.e., the greater  $K_i$ ), the greater is the  $V_d$ .

##### 4.3.2. Prediction of $V_d$

Various methods have been developed to predict  $V_d$ , and the equations are presented in Table 4. In general,  $V_d$  is well correlated with body weight, indicating that the exponent of  $V_d$  is around 1 (usually between 0.8 and 1.1) [115]. Furthermore, for the prediction of  $V_d$ , the two species in AS are acceptable compared to the use of three or more species. In the study of Mahmood and Balian [108], the average exponents using the simple AS for the prediction of  $V_d$  are 0.89 and 0.90 in case of 3 and 2 species, respectively.

The effect of protein binding on the prediction of  $V_d$  by AS has been investigated. As mentioned above, it is well known that protein binding properties vary between species. Furthermore, only unbound drugs penetrate blood vessels and biological membranes. For a drug with low binding affinity to plasma and tissue protein or drugs that are only distributed in the extracellular space, they can be scaled since total body water and extracellular water shows inverse correlation with animal size in AS [116].

Sawada et al. [117] have reported that considering the unbound fraction in the prediction of  $V_d$  may increase the accuracy of prediction results than the volume against unbound fraction in the plasma. In another study of Sawada et al. [118], the authors investigated the prediction of disposition of beta-lactam antibiotics and reported large differences in free volume of distribution between species. However, additional work revealed no advantage in consideration of the unbound

fraction when Sawada et al.'s work was re-evaluated by adding six more drugs from the study of Mahmood [115].

**Table 4.** Methods for prediction of volume of distribution ( $V_d$ ).

Method	Equation	Comment *	Ref.
Simple AS	$V = a(W)^b$ (93)	The prediction of $V_d$ is well predicted equally with using two species in AS	[108]
Average fraction unbound in tissue <sup>1</sup>	$V = V_{\text{plasma}}(1 + R_{E/I}) + f_u \cdot V_P \left( \frac{V_E}{V_P} - \frac{V_R \cdot f_u}{\alpha_R} \right)$ (94)	It is useful to analyze and predict an alteration in apparent $V_d$ then identify the cause of alteration. It is particularly useful for drugs with low $V_d$ (<15 L or 0.2 L/kg)	[119]
Proportionality	$V_{\text{human,pred}} = \frac{V_{\text{animal}} \cdot f_{u,\text{human}}}{f_{u,\text{animal}}}$ (95)	It is assumed that the volume of distribution at a steady state of free drug is identical between species	[120]
One species AS	$V_{\text{human,pred}} = -0.35V_{\text{rat}}^{0.91}$ (96)	Statistical modeling is applied in this model	[121]
QSAR	$\begin{aligned} \log(V_{d_{ss,\text{human}}}) = & 0.1859 \\ & \cdot \log(V_{d_{ss,\text{rat}}}) \times \log(V_{d_{ss,\text{rat}}}) \\ & - 0.3887 \\ & \cdot \log(V_{d_{ss,\text{rat}}}) \times \log(\text{MW}) \\ & + 0.3089 \\ & \cdot \log(V_{d_{ss,\text{dog}}}) \times \log(\text{MW}) \\ & + 0.003306 \cdot \log(\text{MW}) \times c \log P \\ & + 1.71 \end{aligned}$ (97)	$V_{d_{ss,\text{human}}}$ (mL/kg) is predicted by QSAR modeling with quadratic term descriptors	[122]

\* Each comment corresponds to all the equations within each major section of the table defined by horizontal lines.

<sup>1</sup> Where  $V_d$  is apparent volume of distribution,  $V_{\text{plasma}}$  is plasma volume,  $V_E$  is extracellular space minus the plasma,  $V_R$  is physical volume into which the drug distributes minus the extracellular space,  $f_u$  is the fraction unbound in plasma, and  $R_{E/I}$  is the ratio of distributed albumin in the extravascular space to that in the intravascular space. It is 1.4.  $\alpha_R$  equals to  $C_u/C_R$  where  $C_u$  is unbound drug concentration at distribution equilibrium and  $C_R$  is concentration in  $V_R$ .

#### 4.3.3. Prediction of Elimination Half-Life by AS

Elimination half-life ( $t_{1/2}$ ) is one of the most important PK parameter determining the dosage regimen and drugability. Predicted CL cannot estimate the  $t_{1/2}$  since the  $V_d$  and CL are required for the estimation of  $t_{1/2}$  as presented by the equation:

$$t_{1/2} = \frac{0.693V_d}{CL} \quad (98)$$

Because of the hybrid nature of the  $t_{1/2}$ , this parameter has been poorly estimated by AS [89,114]. Instead of direct scaling of  $t_{1/2}$ , Mahmood [89] has suggested the calculation of  $t_{1/2}$  as a secondary parameter using Equation (98). Another approach for prediction of human  $t_{1/2}$  is based on the mean residence time (MRT) [114]. The MRT represents the average staying time of the drug in a

body organ or compartment as the molecules diffuses in and out [28] and the parameter is estimated by the following equation:

$$MRT = \frac{V_{ss}}{CL} \quad (99)$$

where CL is calculated by the Equation (1). Since  $V_{ss}$  is the summation of the volume of the central compartment ( $V_c$ ) and peripheral compartment ( $V_p$ ) in a two-compartment PK model, MRT can also be expressed with the equation below combined with Equation (2) [28]:

$$MRT = \frac{V_c + V_p}{k \cdot V_c} \quad (100)$$

Mahmood [114] investigated the prediction of MRT by AS. Results showed good agreement. Therefore, the  $t_{1/2}$  was predicted using the predicted MRT by following equation:

$$t_{1/2} = \frac{MRT}{1.44} \quad (101)$$

## 5. Prediction of Absorption Related PK Parameters

Absorption rate constant ( $k_a$ ) is generally expressed by first or zero order constant. It could be estimated from various PK models. However,  $k_a$  is originally an apparent parameter that can be best estimated through first-order loss of drug from the gastrointestinal tract, not through first-order appearance of drug in the plasma [52].

AS was applied to predict turn-over parameters. Turn-over rate refers to the amount that a compound is secreted or synthesized per unit time [52]. Therefore, in general, neither is  $k_a$  scaled from animal data, nor is the affinity parameter (i.e.,  $K_m$ ) in Michaelis-Menten equation applied by body weight scaling. [88]. Although AS equation for scaling the first order kinetic parameter (i.e.,  $k$ ) has been suggested by Kenyon [88] as shown in Equation (102) in Table 5, further evaluation is required.

Various empirical relationships of effective permeability ( $P_{eff}$ ) with physicochemical properties or Caco2 in vitro data have been reported. They are shown in Table 5. The  $k_a$  was estimated with the predicted  $P_{eff}$  combined with Equation (112). Another way to predict  $k_a$  is to use the mean value of absorption parameters from animal data. Liu et al. [123] have reported a method for human PK projection of imiglitin using IVIVE, AS, and PK/PD modeling. In their study, the absorption parameter was applied as the mean value in non-clinical animal models such as rats, dogs, and monkeys.

Equations for predicting the fraction of absorption ( $F_a$ ) have been reported by a few investigators. In Equation (115),  $F_a$  is predicted by a mechanism-based model using equilibrium solution for  $k_a$ . Other relationships between  $F_a$  and  $P_{eff}$  are presented in Equation (116). The empirical equation could be used for prediction of  $F_a$  using in vitro permeability data.

**Table 5.** Methods for prediction of absorption parameters.

Method	Equation	Comments *	Ref.
AS	$k_a = \text{animal } k_a \times \left(\frac{W_{\text{human}}}{W_{\text{animal}}}\right)^{-0.25}$ (102)	The unit of $k_a$ is h in time <sup>-1</sup>	[88]
QSAR <sup>1</sup>	$\log P_{eff} = -2.883 - 0.01PSA + 0.192\log D_{5.5} - 0.239HBD$ (103)	The choice of model for prediction depends on the availability of descriptor data [124] Effective permeability in 10 <sup>-4</sup> cm/s	[124]
	$\log P_{eff} = -2.546 - 0.011PSA - 0.278HBD$ (104)		
	$\log P_{eff} = -3.067 + 0.162\log P - 0.01PSA - 0.235HBD$ (105)		

Use of Caco2 data <sup>2</sup>	$P_{\text{eff, human}} = 0.4926 \log P_{\text{eff, Caco2}} - 0.1454 \text{ (at pH = 7.4)}$	(106)	All tested drugs	[125]
	$P_{\text{eff, human}} = 0.6532 \log P_{\text{eff, Caco2}} - 0.3036 \text{ (at pH = 6.5)}$	(107)		
	$P_{\text{eff, human}} = 0.6836 \log P_{\text{eff, Caco2}} - 0.5579 \text{ (at pH = 7.4)}$	(108)	Only passively diffused drugs	
	$P_{\text{eff, human}} = 0.7254 \log P_{\text{eff, Caco2}} - 0.5441 \text{ (at pH = 6.5)}$	(109)		
	$P_{\text{eff, human}} = 0.4898 \log P_{\text{eff, Caco2}} + 0.3311 \text{ (at pH = 7.4)}$	(110)	Only carrier-mediated drugs	
	$P_{\text{eff, human}} = 0.542 \log P_{\text{eff, Caco2}} + 0.06 \text{ (at pH = 6.5)}$	(111)		
Sinko et al. <sup>5</sup>	$k_a = \frac{2P_{\text{eff}}}{R}$	(112)	The absorption rate constant is proportional to the $P_{\text{eff}}$	[126]
Mechanism based modeling <sup>3</sup>	$F_{a, \text{pred}} = 0.884F_{a, \text{exp}} + 7.47$	(113)	$F_a$ is expressed as percent unit The equation is the result of the correlation between $F_{a, \text{pred}}$ and $F_{a, \text{exp}}$	[127]
	$k_{a, \text{eq}} = \frac{P_m S}{V_c}$	(114)	$k_{a, \text{eq}}$ is expressed as the unit of min <sup>-1</sup>	
	$F_a = \frac{k_{a, \text{eq}}}{k_i + k_{a, \text{eq}}}$	(115)	$k_{a, \text{eq}}$ is a key determinant for $F_a$ and can be used as PK modeling	
Compartmental absorption and transit model <sup>4</sup>	$F_a = 1 - (1 + 0.54P_{\text{eff}})^{-7}$	(116)	$F_a$ is expressed as the fractional value.	[128]

\* Each comment corresponds to all the equations within each major section of the table defined by horizontal lines.

<sup>1</sup> In this equation, passive intestinal absorption in humans was predicted. Abbreviations are:  $P_{\text{eff}}$ , effective permeability; PSA, polar surface area;  $\log D_{5.5}$ , octanol/water distribution coefficient at pH 5.5; HBD, number of hydrogen bond donors;  $\text{clogP}$ , calculated  $\log P$  value.

<sup>2,5</sup>  $P_{\text{eff}}$  is calculated by the equation of  $P_{\text{eff}} = Q(1 - C_{\text{out}}/C_{\text{in}})/2\pi RL$ , where  $P_{\text{eff}}$  is effective permeability,  $Q$  is perfusion rate (mL/min),  $C_{\text{out}}$  and  $C_{\text{in}}$  are outlet and inlet drug concentration, respectively,  $R$  is the radius of human jejunum (1.75 cm) [129], and  $L$  is the length of perfusion segment (10 cm). Caco2 permeability and human effective permeability are expressed with values of  $\times 10^{-6}$  cm/s and  $\times 10^{-4}$  cm/s, respectively.

<sup>3</sup>  $k_{a, \text{eq}}$  is the equilibrium solution for  $k_a$ ,  $P_m$  is drug permeability across intestinal mucosa ( $\times 10^{-6}$  cm/s),  $S$  is the absorptive surface area which is set at 200 m<sup>2</sup>,  $V_c$  is the volume of distribution in well-perfused organs,  $k_i$  is the rate constant of intestinal transit, which is set to be  $5.025 \times 10^{-3} \text{ min}^{-1}$  as an inverse value of the average transit time [130] in human small intestine (approximately 199 min).

<sup>4,5</sup>  $P_{\text{eff}}$  is human effective permeability in cm/h.

## 6. Conclusion

Investigating the CL pathway is a significantly important issue in drug development. Drug CL parameters have an impact on the determination of dosing regimens for both normal and special populations, such as pediatric, elderly, and patients with renal or hepatic impairment, drug–drug interactions, and so on.



Prediction of PK parameters from non-clinical studies is essential in the drug discovery and development process. Over the last five decades, numerous translational approaches have been developed to predict human PK parameters. Both IVIVE and AS methods provide insight based on non-clinical studies for decision-making in the drug discovery and development process.

In the overall prediction of total clearance, AS represents a powerful method for the use of non-clinical data from single or multiple species. However, it is difficult to determine the variation in transporters and/or enzyme expression, affinity, and specificity with AS.

The proposed ECM model combined with the prediction of contribution could represent a breakthrough in AS and conventional IVIVE methodology.

Integration of the in vitro data and in vivo animal data is recommended for accurate prediction of specific ADME processes in humans [7] and recently, the combined methods were applied to a drug development process [123,131]. Since the choice of method depends on data availability and each method has advantages and disadvantages, the designing of an overall non-clinical study to generate appropriate data for scaling is one of the key steps in the practice of investigation.

Despite its uncertainty, ongoing refinement of IVIVE and AS methods will increase the accuracy of predictability and increase our understanding of the underlying rationale into mechanisms of extrapolation from in vitro or in vivo to human.

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#### Appendix: Abbreviation list in alphabetical order

Abbreviation	Description
ADME	absorption, distribution, metabolism, excretion
AS	allometric scaling
AUC <sub>0-inf</sub>	area under the concentration-time curve from zero to infinity
BDDCS	biopharmaceutics drug disposition classification system
BW	brain weight
CB	drug concentration in blood
CL	clearance
CL <sub>H</sub>	hepatic clearance
CL <sub>int</sub>	intrinsic clearance
CL <sub>int, H</sub>	hepatic intrinsic clearance
CL <sub>int, H, human</sub>	human hepatic intrinsic clearance
CL <sub>int, H, pred</sub>	predicted hepatic intrinsic clearance
CL <sub>int, in vitro</sub>	in vitro intrinsic clearance
CL <sub>int, met</sub>	intrinsic metabolic clearance
CL <sub>int, sec</sub>	intrinsic secretory clearance
CL <sub>met</sub>	metabolic clearance
cLogP	partition coefficient
clogP	calculated logP
CL <sub>org</sub>	organ clearance
C <sub>P</sub>	drug concentration in plasma
C <sub>R</sub>	concentration in VR
C <sub>RBC</sub>	drug concentration in red blood cells

$C_u$	unbound drug concentration at distribution equilibrium
DMPK	drug metabolism and pharmacokinetic
$D_N$	dispersion number
ECM	extended clearance model
ER	extraction ratio
$ER_H$	hepatic extraction ratio
$ER_{H, obs}$	observed hepatic extraction ratio
$ER_{H, pred}$	predicted hepatic extraction ratio
F	fraction of absorption
$f_{IW}^i$	unbound fraction in the intracellular water of ionized compound
$f_{IW}^n$	unbound fraction in the intracellular water of neutral compound
$f_p^i$	unbound fraction in the plasma of ionized compound
$f_p^n$	unbound fraction in the plasma of neutral compound
$F_a$	fraction of absorption
$f_b$	unbound fraction in blood
FCIM	fraction unbound intercept correction method
$F_H$	hepatic availability
$F_I$	ionization factor
$f_{nH}$	fractional contribution of hepatic elimination
$f_{nmet}$	fractional contribution of metabolic elimination
$f_{nsec}$	fractional contribution of biliary elimination
$f_p$	unbound fraction in plasma
FPO	oral bioavailability
$f_{u, mic}$	non-specific binding factor to microsomes
$f_{u, liver}$	unbound fraction into the liver
$H_a$	number of hydrogen-bond acceptors
HBD	number of hydrogen-bond donor
Hct	hematocrit
HLM	human liver microsomes
IIV	inter-individual variability
ISEF	inter-system extrapolation factor
ISTD	internal standard
IVIVE	in vitro-in vivo extrapolation
IW	intracellular water
$k_a$	absorption rate constant
$k_{a, eq}$	equilibrium solution for $k_a$
$k_i$	the rate constant of intestinal transit
$K_m$	Michaelis constant
L	length of perfusion segment
MA	multiexponential allometric scaling
MLBF	monkey liver blood flow
MLP	maximum life-span potential
MPPGL	microsomal protein per gram of liver
MPR	microsomal protein recovery
MRT	mean residence time
MW	molecular weight
NCA	non-compartmental analysis
$P_{eff}$	effective permeability
PK	pharmacokinetic
PLR	plasma to whole liver concentration ratio

$P_m$	drug permeability across intestinal mucosa
PSA	polar surface area
$PS_{bile}$	biliary clearance
$PS_{efflux, total}$	apparent sinusoidal total efflux clearance from the intracellular side of hepatocytes back into blood
$PS_{inf, act}$	sinusoidal efflux from hepatocytes back into blood
$PS_{uptake, total}$	total apparent uptake clearance
$Q_H$	hepatic liver flow
QSAR	quantitative structure activity relationship
R	radius of human jejunum
RAF	relative activity factor
$R_{B/P}$	blood to plasma ratio
$R_{E/I}$	ratio of distributed albumin in the extravascular space to that in the intravascular space
$R_{fu}$	unbound fraction in plasma ratio between rats and humans
rhCYP	recombinant human CYP system
$R_N$	efficiency number
ROE	rule of exponent
S	absorptive surface area
$t_{1/2}$	half-life
V	volume of distribution
$V_{area}, V_{\beta}$	volume of distribution by area
$V_c$	volume of distribution of central compartment
$V_E$	extracellular space volume minus the plasma volume
$V_{max}$	maximal rate of the reaction
$V_{met}$	metabolic rate
$V_{plasma}$	plasma volume
$V_R$	physical volume into which the drug distributes minus the extracellular space
$V_{ss}$	volume of distribution at steady state
W	body weight
$\varepsilon^2$	variance for each sinusoid in the whole liver

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