The Universally Unrecognized Assumption in Calculating Organ Clearance and Extraction Ratio: Implications for BA/BE Determinations

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Amman **September 25, 2018**

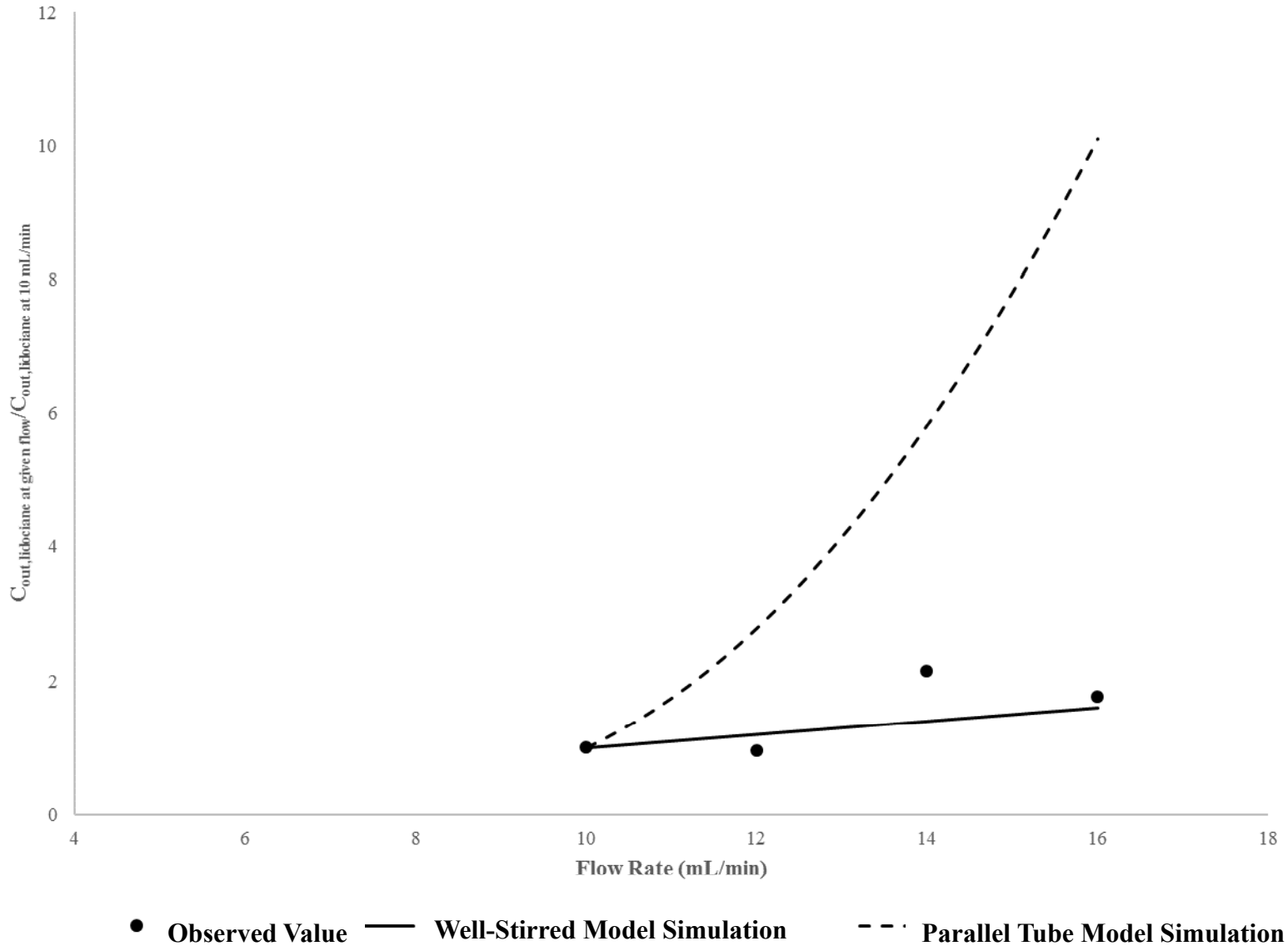
Richard Feynman
Nobel Prize in Physics 1965

"It doesn't matter how beautiful your theory is, it doesn't matter how smart you are. If it doesn't agree with experiment, it's wrong."

In this presentation I will address two well known, quite old, pharmacokinetic/drug metabolism approaches where the experiments do not agree with the theory,

First: Models of hepatic elimination and the extraction ratio;

Second : IVIVE (in vitro - in vivo extrapolation) to predict in vivo clearance from in vitro measures of hepatic elimination; and then review our recent data investigating the variability of the variability measures in referenced scaled bioequivalence determination for narrow therapeutic index drugs.



Plot of the observed and simulated lidocaine concentrations in the effluent perfusate of rat livers for the well-stirred and parallel-tube models [from Pang and Rowland, 1977].

We decided to go back to first principles to try to understand this poor predictability and recognized that the theoretical basis for the methodology employed had never been evaluated leading to some surprising and controversial findings, of which our first paper was published this March

"The Universally Unrecognized Assumption in Predicting Drug Clearance and Organ Extraction Ratio"

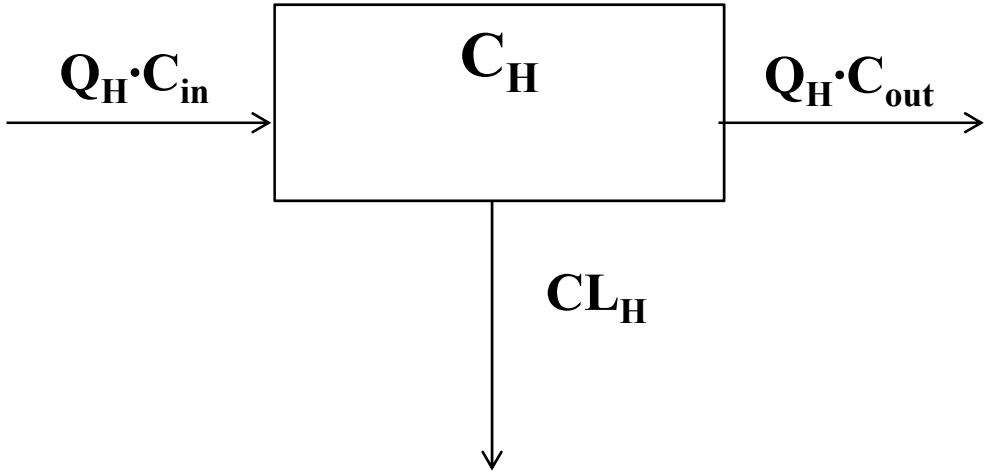
L.Z. Benet, S. Liu and A.R. Wolfe

***Clin. Pharmacol. Ther.* 103, 521-525 (2018)**

In 1972 Rowland proposed that organ clearance could be calculated as the fraction of the entering drug concentration that is lost (the extraction ratio) multiplied by organ blood. That is:

$$CL_H = Q_H \cdot ER = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} \qquad \text{(Eq. 1)}$$

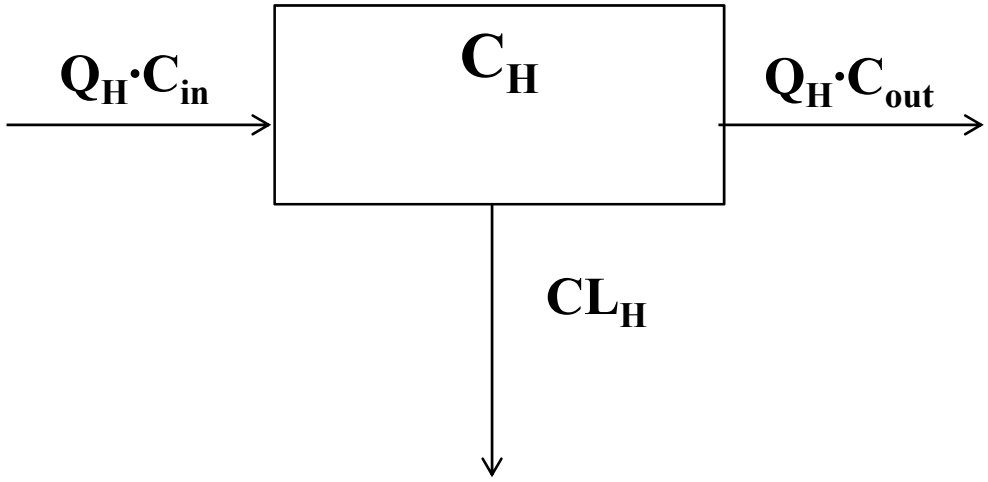
and this has been universally accepted as the definition of organ clearance ever since. But consider the implication.



At steady-state in the body as a whole, rate in = rate out and rate out equals the clearance term with respect to where we measure

Apply this principle to an organ $Q_H (C_{in} - C_{out}) = CL_H \cdot C_{H,ss}$

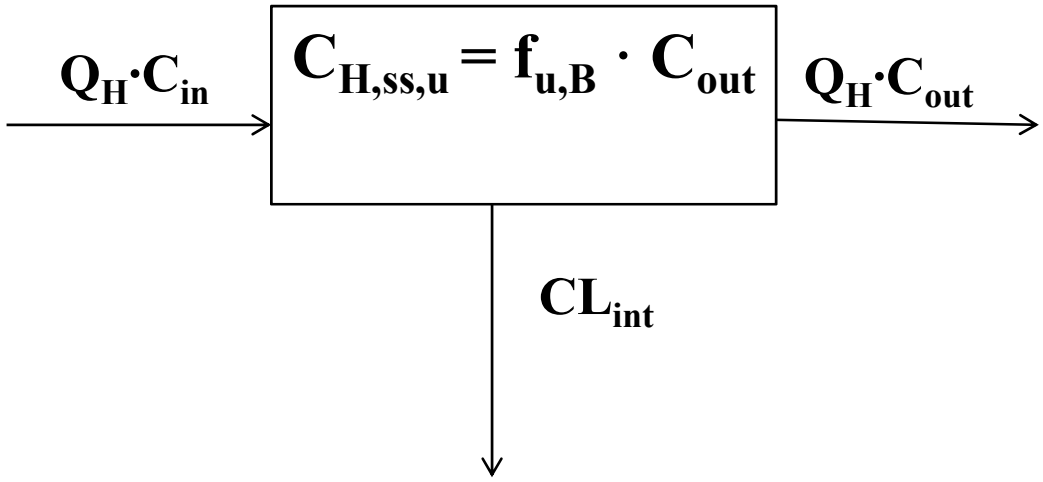
$$CL_H = Q_H \cdot ER = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} \qquad \text{(Eq. 1)}$$



$$Q_H\,(C_{in} - C_{out}) = CL_H \cdot C_{H,ss}$$

If $C_{H,ss}$ is set equal to C_{in}, we have assumed that the driving force concentration for elimination in the liver at steady-state is always equal to the entering concentration. That is, there is no incremental metabolism, and we have assumed the well-stirred model of hepatic elimination. Thus, there is no justification in testing other models of hepatic metabolism for experiments measuring concentrations entering and exiting an isolate organ because clearance was calculated assuming the well-stirred model.

You are used to seeing the organ clearance relationship in terms of intrinsic clearance, CL_{int}



$$Q_H\,(C_{in} - C_{out}) = CL_H \cdot C_{in} = CL_{int} \cdot C_{H,u} = CL_{int} \cdot f_{u,B} \cdot C_{out}$$

But the relationship above is only correct for the well-stirred model, and Rowland, Benet and Graham (1973) when they first presented the intrinsic clearance concept did not recognize this.

Thus, there is no justification in testing other models of hepatic metabolism for experiments measuring concentrations entering and exiting an isolate organ because clearance and intrinsic clearance were calculated assuming the well-stirred model.

Here's what is being done. Organ clearance is calculated as

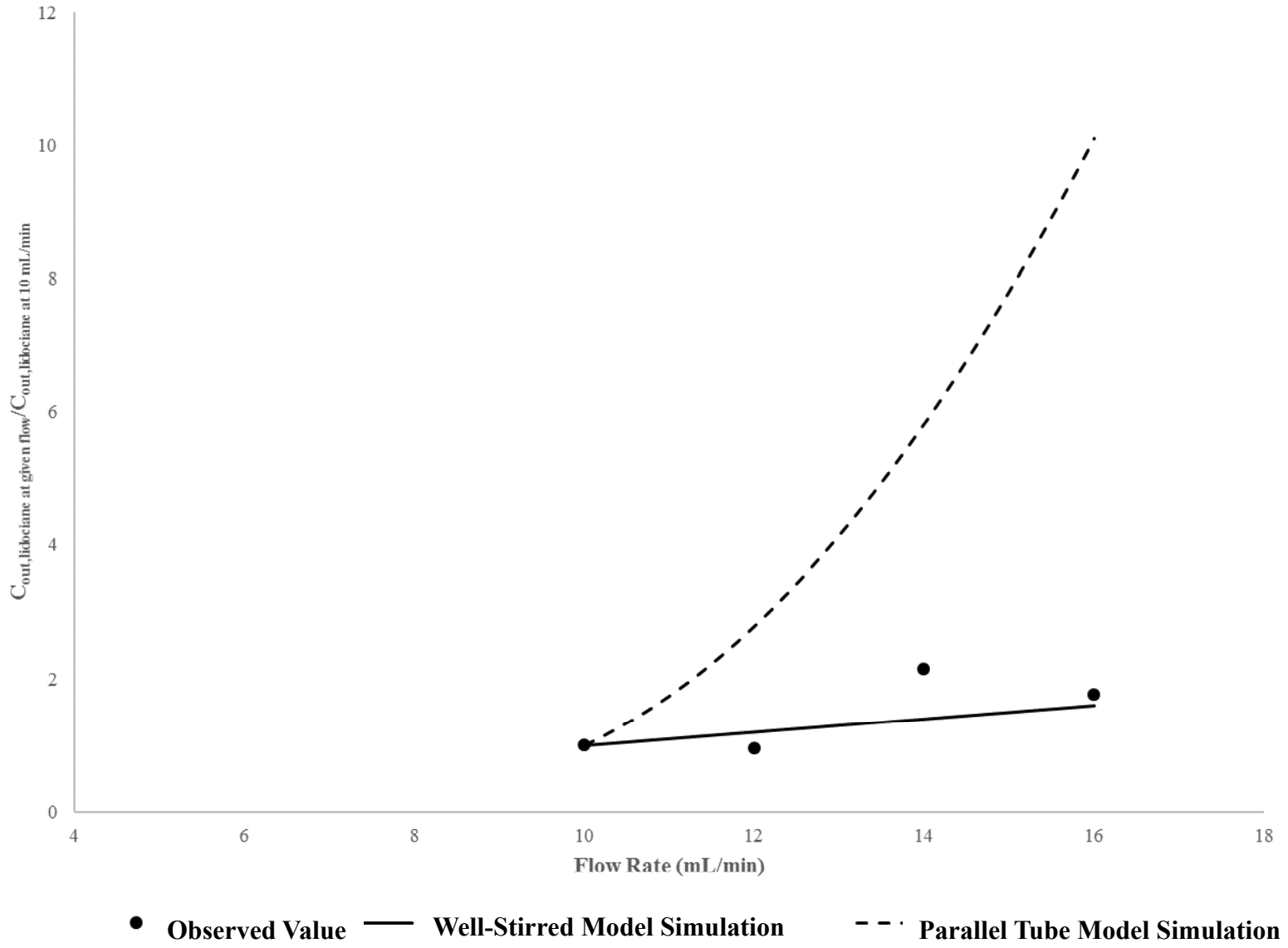
$$CL_H = Q_H \cdot ER = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}}$$

not recognizing that the relationship is only consistent with the well-stirred model.

Then using this ER or CL term one calculates CL_{int} in the parallel tube (PT) or dispersion model by the following equation

$$CL_{H,PT} = Q_H \cdot ER = Q_H \frac{C_{in} - C_{out}}{C_{in}} = Q_H \cdot (1 - e^{-\frac{f_{u,B} \cdot CL_{int}}{Q_H}})$$

But, unknowingly what the field has done is calculate clearance by the well-stirred model, and then used this clearance to attempt to calculate an intrinsic clearance that is from a parallel tube or dispersion model. Experimentally, one changes Q_H or $f_{u,B}$ and then measures the new CL or ER. But then when the results are tested the best fit is always consistent with the well-stirred model, because the measurements are only consistent with the well-stirred model.



Plot of the observed and simulated lidocaine concentrations in the effluent perfusate for the well-stirred and parallel-tube models [from Pang and Rowland, 1977].

Now, obviously, this is very controversial. For 46 years our field, including me, have believed that

$$CL_H = Q_H \cdot ER = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} \quad \text{(Eq. 1)}$$

and that this equation was model independent.

Thus in determining bioavailability (F), the first pass hepatic loss can be predicted by

$$F_H = 1 - ER = 1 - \frac{CL_H}{Q_H} \quad \text{(Eq. 2)}$$

But now we are saying that F_H is only consistent with the well-stirred model.

Rowland and Pang disagree with our mass balance analysis and provided a Commentary in which they argue that Eq. 1 "simply express[es] proportionality between observed rate of elimination and a reference concentration" and it is not model dependent.

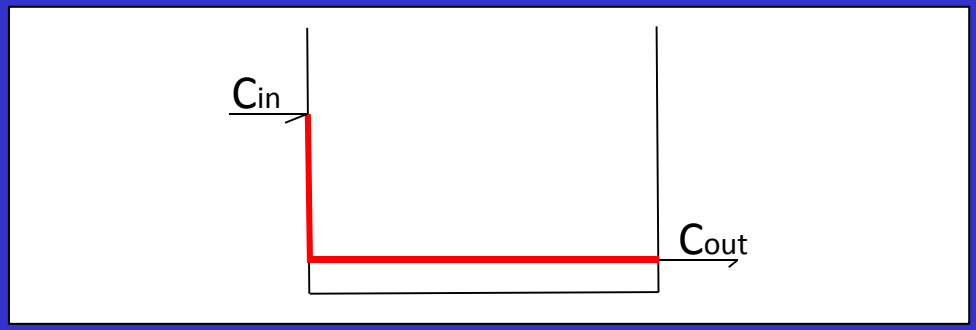
$$CL_H = Q_H \cdot ER = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} \qquad \text{(Eq. 1)}$$

We disagree with Rowland and Pang for three reasons: First, there are no inherent truths in pharmacokinetics, all relationships can be derived based on mass balance.

Second, mass balance as expressed in the rearranged Eq. 1

$$Q_H \cdot (C_{in} - C_{out}) = CL_H \cdot C_{in}$$

sets elimination, $Q_H \cdot (C_{in} - C_{out})$, equal to CL_H multiplied by C_{in} and only C_{in} as depicted here.

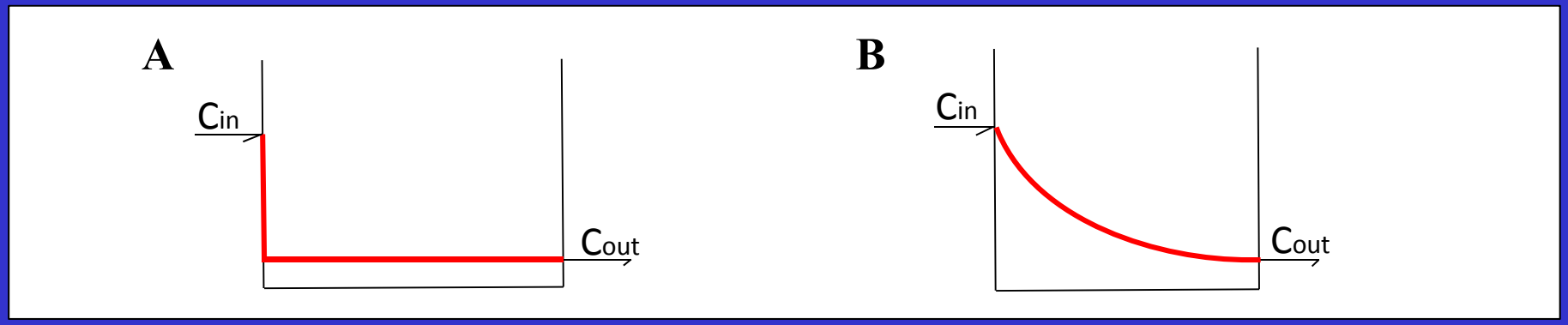


Only C_{in} drives clearance. No concentration within the organ has any effect on CL_H.

Third, Rowland and Pang have ignored a further mass balance pharmacokinetic equation

$$CL = V_{ss} \div MRT \qquad \text{(Eq. 3)}$$

where V_{ss} is the volume of distribution steady-state and MRT is the mean residence time of drug in the system. In Model B, the parallel tube model of drug elimination, drug concentrations decrease exponentially as drug passes through the organ



It is obvious that $MRT_B >> MRT_A$

Mean residence time may not be a familiar concept. Let me try to explain it in terms of popcorn makers, where we will measure the mean residence time of unpopped corn kernels in the reactor (popper)

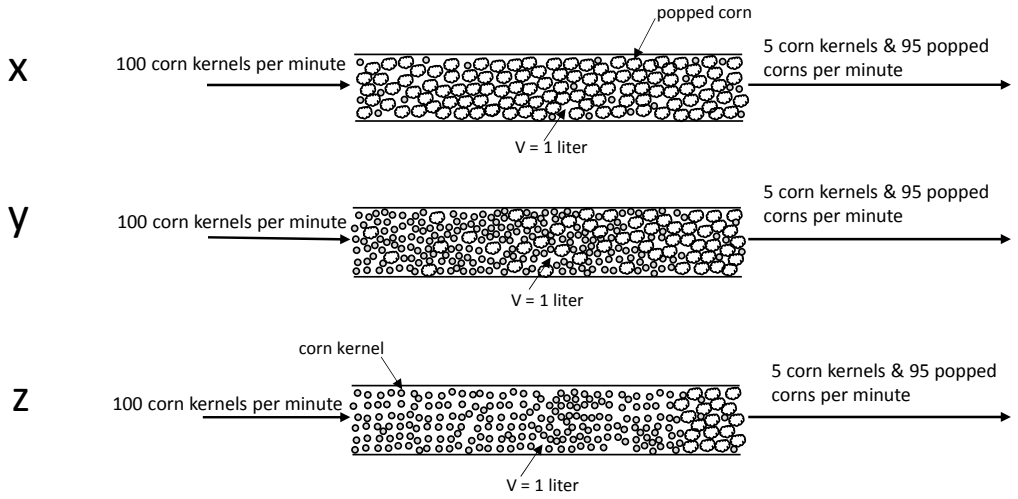
Three Steady-State Popcorn Makers

In all three reactors (poppers) unpopped corn kernels enter the reactors at 100 corn kernels per minute and leave the reactors at 5 corn kernels per minute and 95 popped corn per minute.

In reactor X all of the popping that will occur takes place at the front end of the reactor.

In reactor Y the popping occurs throughout the reactor.

In reactor Z the popping occurs at the back end of the reactor.



Three Steady-State Popcorn Makers

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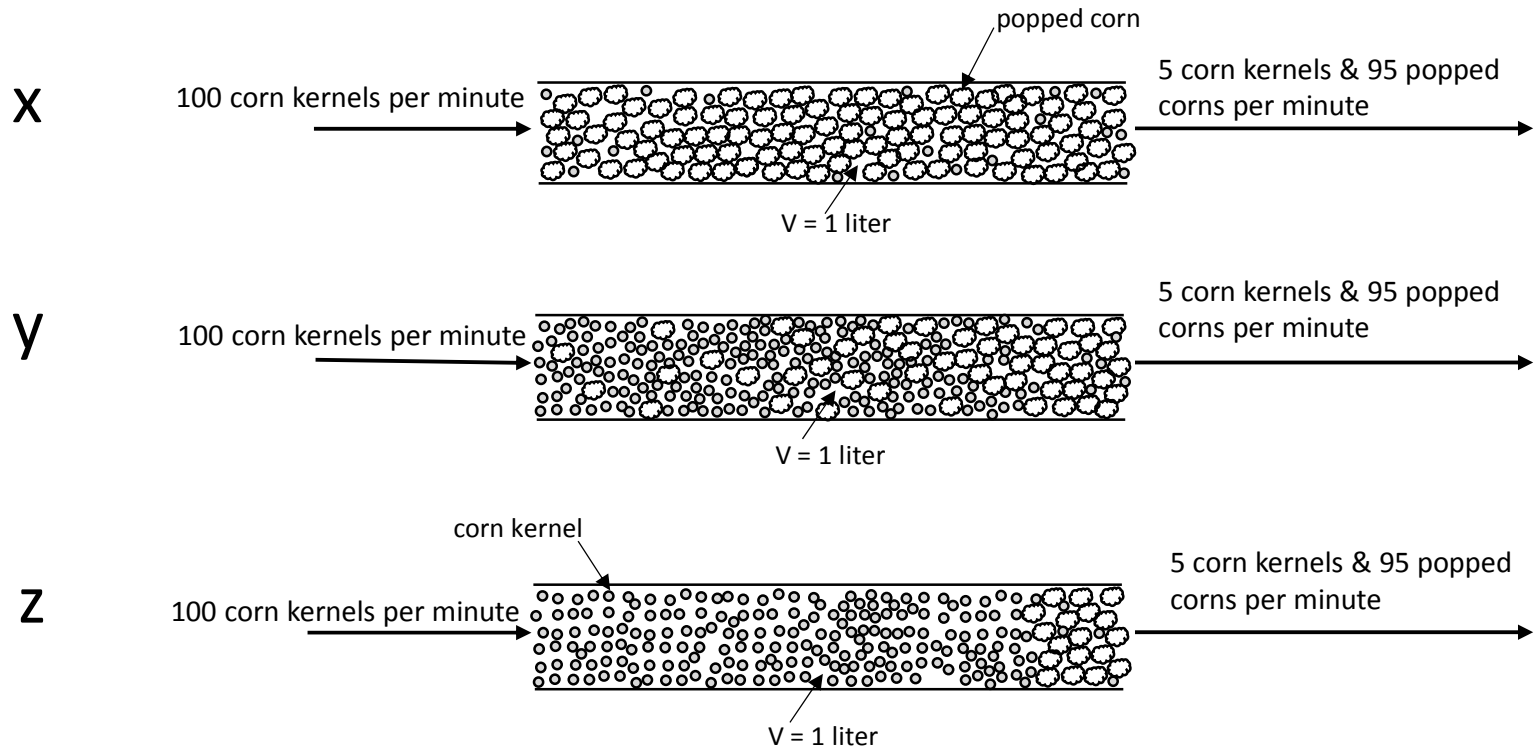
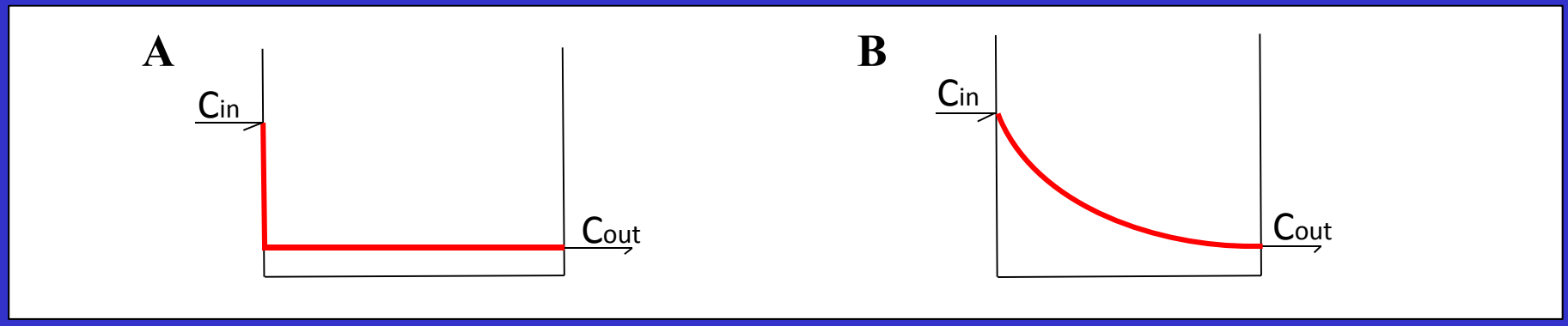


The mean residence time of unpopped corn kernels will be $MRT_Z > MRT_Y > MRT_X$

Poppers X and Y are respectfully, representative of the well-stirred model A where all of the clearance occurs as the drug enters the organ (infinite mixing rate) and model B where clearance occurs exponentially as drug passes through the organ (zero mixing rate). The pharmacokinetic dispersion models represent intermediate mixing rates.



Since $MRT_B >> MRT_A$ how can the clearance of drug in models A and B be identical?

It is hard to imagine how clearance in Eq. 1 is model independent when *MRT* is model dependent. To do so, one must hypothesize that V_{ss} in Eq. 3 is also model dependent and changes exactly as *MRT* from model to model.

$$CL = V_{ss} \div MRT \qquad \text{(Eq. 3)}$$

Yet, it is well recognized in pharmacokinetics that volume of distribution is drug dependent and not a function of *CL* nor *MRT*. The supposition that Eq. 1 is model independent is not supported either by pharmacokinetic theory or by experimental data.

Clearance Revisited. L.Z. Benet. Clin. Pharmacol. Ther., submitted.

In Vitro-In Vivo Extrapolation (IVIVE)

How is it done?

Assumption: $CL_{int,in\ vivo} = SF \cdot CL_{int,in\ vitro}$

where *SF*, the scaling factor, is the ratio of metabolic enzymes in vivo to the metabolic enzymes in the in vitro incubation.

Then the $CL_{int,in\ vivo}$ term is put into a model of hepatic elimination to estimate $CL_{in\ vivo}$

$$CL_{in\ vivo} = \frac{Q \cdot f_{u,B} \cdot CL_{int,in\ vivo}}{Q + f_{u,B} \cdot CL_{int,in\ vivo}}$$

where Q is organ blood flow and fu,B is fraction unbound in blood

So how successful is the IVIVE methodology?

Hepatic Clearance Predictions from In Vitro-In Vivo Extrapolation and the Biopharmaceutical Drug Disposition Classification System

Christine M. Bowman and Leslie Z. Benet

Drug Metab. Dispos. 44:1731-1735 (2016)

We evaluated 11 different data sets using human microsomes and hepatocytes to search for trends in accuracy, extent of protein binding, and BDDCS class and the original papers when data in the 11 sets was taken from published studies.

Five human microsome data sets, some with multiple IVIVE scaling options, were included for a total of 332 values. Six human hepatocyte studies also coincidentally included 332 values.

Every data set examined had ≥ 41% inaccuracy (more than 2-fold IVIVE error) and average fold error values as high as 21.7. The weighted average inaccurate results were 66.8% for microsomes and 66.2% for hepatocytes.

It has been reported that IVIVE predictions for human liver microsomes under-predict in vivo metabolic clearance by ~9 fold and human hepatocytes (cryopreserved) by 3~6 fold. In our analysis we did not see this great a difference between microsomes (avg. 5 fold under-prediction) and hepatocytes (avg. 4 fold under-prediction), but significant differences from drug to drug do exist.

But, what became obvious to us, and others who have reviewed these analyses, is that the field does not know why IVIVE on average under-predicts and is different from drug to drug.

Many, many papers have investigated the potential corrections in the predictive equation

$$CL_{in\ vivo} = \frac{Q \cdot f_{u,B} \cdot CL_{int,in\ vivo}}{Q + f_{u,B} \cdot CL_{int,in\ vivo}}$$

primarily investigating alternative methodology related to protein binding terms and corrections for the potential pH difference intra- and extra-cellularly as we recently reviewed

CM Bowman & LZ Benet "An Examination of Protein Binding and Protein-Facilitated Uptake Relating to In Vitro-In Vivo Extrapolation" Eur J Pharm Sci, 123: 502-514 (2018)

But no one has investigated the theoretical basis of the experimental approach

Let's go back and review the methodology

How is it done?

Assumption: $CL_{int,in\ vivo} = SF \cdot CL_{int,in\ vitro}$

How is $CL_{int,in\ vitro}$ **determined?**

The drug is incubated with either microsomes or hepatocytes and over time the half-life of drug loss is determined, which is converted to a rate constant $\left(\frac{0.693}{t_{1/2}} = \frac{V_{max}}{K_m}\right)$**, which is then multiplied by the volume of the in vitro incubation mixture and divided by the** $f_{u,inc}$**, the fraction unbound in the incubation mixture, to obtain** $CL_{int,in\ vitro}$

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We asked why should the volume of the *in vitro* incubation mixture, selected by the investigator, when multiplied by the measured rate constant of elimination to determine the *in vitro* clearance measure yield a clinically relevant *in vivo* clearance?

Why would this drug independent volume term be clinically relevant?

And then with respect to the half-life of the *in vitro* incubation, which will always yield a single exponential value if no saturation occurs, why will it be relevant for an *in vivo* liver where lipophilic regions not containing the metabolic enzymes will most likely result in a multiexponential process?

Will the one compartment in vitro incubation measure of hepatic elimination predict the in vivo rate constant of elimination when correcting for differences in metabolic enzymes?

Fig. 1 Homogeneous In Vitro Incubation

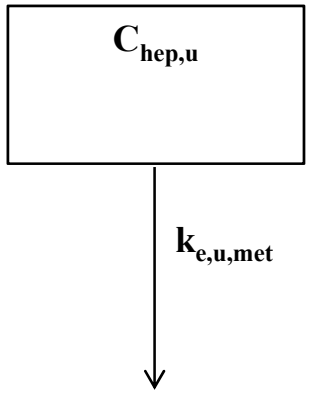
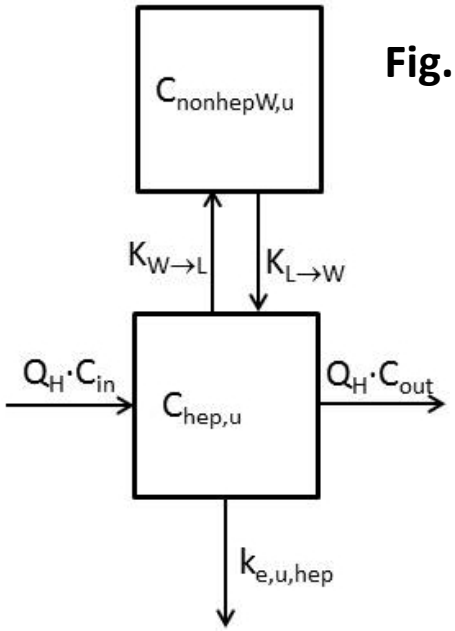


Fig. 2 Heterogeneous Liver Model



Yes. That is the IVIVE assumption.

But we are not predicting rate constants, because we wouldn't know how to get V_{hep}, the drug volume of distribution in vivo in contact with the enzymes since $CL_{int,in\ vivo} = k_{e,u,hep} \cdot V_{hep}$. The best we could do is get $V_{ss,liver}$, the total drug volume of distribution in the liver.

So we believe that the poor IVIVE predictions are in good part related to ignoring the differences in drug volumes of distribution *in vitro* vs *in vivo*, which will be different for each drug molecule.

Leading to our deriving the relationship

The Theoretical Derivation of IVIVE:
An Explanation for the Lack of Success of IVIVE and the Lack of Success of Using Endogenous Substance Kinetics to Predict the Clearance of A Drug in a Patient

L. Z. Benet, C. M. Bowman and J. K. Sodhi

in preparation

Our extensive derivation, which could not be presented here, proposes that

$$CL_{int,in\ vivo} = \mathrm{SF} \cdot \frac{rate\ constant\ of\ elimination\ for\ total\ drug_{\ in\ vitro} \cdot V_{in\ vitro}}{f_{u,in\ vitro}} \cdot \frac{V_{ss,H}}{V_{hep}}$$

where we designate $R_{ss} = \frac{V_{ss,H}}{V_{hep}}$

the steady-state volume of distribution of drug in the whole liver ($V_{ss,H}$) to the volume of distribution of drug in water/fluid in contact with the metabolic enzymes in the liver (V_{hep}). Drug can distribute throughout $V_{ss,H}$, but the metabolic enzymes are restricted to V_{hep}.

Thus Far

- We have presented a theoretical basis for why *ER* is a well-stirred model concept and that when only concentrations entering and exiting an elimination organ are measured, only the well-stirred model may describe the clearance measures. Therefore, F_H calculations also assume the well-stirred model.
- We have presented a theoretical basis as to why we believe that present IVIVE methodology and all of the many modifications proposed would not be expected to provide a useful solution for the majority of NMEs.
- Will this cover all of the pharmacokinetic aspects that are related to bioavailability and its application to bioequivalence?

But

- We have only addressed predictions of hepatic metabolism (trying to understand initially why we have been so unsuccessful in past IVIVE attempts)
- We have not yet addressed transporters, nor transporter-enzyme interplay, or oral drug administration predictions and recognized that once again the theoretical basis for the Extended Clearance Concept has not previously been presented.

Therefore, we recently submitted a paper entitled:

"The Extended Clearance Concept Following Oral and Intravenous Dosing: Theory and Critical Analyses"

L.Z. Benet, C.M. Bowman, S. Liu and J.K. Sodhi, Pharm Res.

To determine the extent of availability, we measure exposure and in bioequivalence evaluations we compare this measure as AUC. What are the potential variables in AUC determinations following oral dosing for a hepatically eliminated drug?

$$\frac{AUC}{Dose_{oral}} = \frac{F_{abs} \cdot F_G \cdot (CL_{H,int} + PS_{eff,int})}{PS_{inf,int} \cdot f_{u,B} \cdot CL_{H,int}} \quad \text{(Eq. 4)}$$

where F_{abs} is the fraction of drug dose absorbed, F_G is the fraction of the absorbed dose that leaves the gut unchanged, $CL_{H,int}$ is the sum of the metabolic and biliary intrinsic hepatic clearances, $PS_{inf,int}$ and $PS_{eff,int}$ are the intrinsic hepatic influx and efflux transport clearances (fluxes), respectively, and $f_{u,B}$ is the fraction unbound in blood

In Eq. 4, the only term relating to the dosage form is F_{abs} and in addition, Eq. 4 is only valid for the well-stirred model of hepatic elimination and hepatic first pass loss. So I am sympathetic with Professor Amidon's long held belief that measures of AUC may not be the best evaluator of dosage form equivalence. There are 6 other subject specific variables and a model of hepatic elimination that we must assume are constant and valid in comparing AUC measures in bioequivalence evaluations.

Bioequivalence Requirements for Highly Variable and Narrow Therapeutic Index Drugs

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Schools of Pharmacy and Medicine
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2nd MENA Regulatory Conference on Bioequivalence, Biowaivers, Bioanalysis, Dissolution and Biosimilars

Amman **September 16, 2015**

Highly Variable (HV) Drugs ($CV_{within} \geq 30\%$)

One problem with our previous regulations was that the safest drugs, those exhibiting high within subject variability, were the hardest to prove that a generic was bioequivalent to the innovator.

Highly variable drugs are the safest, since by definition, HV approved drugs must have a wide therapeutic index, otherwise there would have been significant safety issues and lack of efficacy during Phase 3.

Highly variable narrow therapeutic index drugs are dropped in Phase 2 since it is not possible to prove either efficacy or safety.

Drugs with High Variability BE Measures: Approach Now Recommended by OGD

This approach is Mixed Scaled Average BE

Normal non-scaled average bioequivalence for CV < 30%

Reference-scaled average bioequivalence (ABE) for CV ≥ 30%

Protocol for Reference-Scaled ABE Approach

BE study uses a three-period, reference-replicated, crossover design with sequences of TRR, RTR, & RRT

A four-period design is also acceptable (sequences of TRTR and RTRT) T = test product; R = reference product

Usual pharmacokinetic sampling to determine Cmax, AUC(0-t), and AUC(0-inf)

At least 24 subjects should be enrolled

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Protocol for Reference-Scaled Average Bioequivalence (RSABE) Approach

a. Reference replicate data analyzed for determination of σ_{wR}
b. If $\sigma_{wR} < \sigma_{w0}$ then data analyzed using unscaled average BE method
c. If $\sigma_{wR} \geq \sigma_{w0}$ then data analyzed using scaled average BE and point-estimate criteria

Drugs with HV BE Measures: RSABEApproach

BE limits, upper, lower = EXP $\pm$ 0.223 σ_{wR} / σ_{w0}

- **Where σ_{w0} = 0.25**

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- **The point estimate (Test/Reference geometric mean ratio must fall within [0.80-1.25]**
- **Both conditions must be passed by the test product to conclude BE to the reference product**
- **If test variability is higher than reference variability then product is less likely to be declared BE to reference**

Highly Variable Conclusions

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- **Highly variable drugs on the market are the safest drugs because marked swings in systemic drug levels have been shown to not affect safety and efficacy in individual patients.**
- **High variability can result from a number of environmental and genetic factors, none of which appear to require any special considerations not already found in the labeling of the innovator drug.**
- **The HV drug guidance is a strong advance leading to significant cost and human subject exposure savings with no increased potential for safety and lack of efficacy issues related to the methodology.**

Returning to today's talk, for highly variable drugs the variability of the 6 other subject specific parameters in Eq. 4 besides F_{abs} are of little clinical relevance and have little impact on bioequivalence determinations since we reference-scale them out. These highly variable drug are very safe.

But what about narrow therapeutic index drugs?

Narrow Therapeutic Index Drugs BE Measures: Approach Now Recommended by OGD

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Protocol for RSABE NTI Drug Approach

BE study uses a four-way crossover fully replicated design
i.e., Test product given twice. Reference product given twice
This design will provide the ability to:
--Scale a criterion to the within-subject variability of the reference product, and
--Compare test and reference within-subject variance to confirm that they do not differ significantly

Usual pharmacokinetic sampling to determine Cmax, AUC(0-t), and AUC(0-inf)
At least 24 subjects should be enrolled

The FDA draft guidance on Warfarin (recommended Dec 2012) details the methodology recommended.

Recommended BE Limits for Generic NTI Drugs

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- **BE limits will change as a function of the within-subject variability of the reference product (reference scaled average bioequivalence as for HV drugs)**
- **If reference variability is ≤ 10%, then BE limits are reference-scaled and are narrower than 90.0-111.11%.**
- **(Lamotrigine example of Prof. Polli yesterday, but the USP potency limits for lamotrigine tablets is 90-110%)**
- **If reference variability is > 10%, then BE limits are reference-scaled and wider than 90.0-111.11%, but are capped at 80-125% limits.**
- **The Agency believes that this recommendation encourages development of low-variability formulations.**

However, the Warfarin draft recommended guidance for NTI drugs contains a new requirement

Sponsors must calculate the 90 % confidence interval of the ratio of the within subject standard deviation of test product to reference product σ_{WT}/σ_{WR}. The upper limit of the 90% confidence interval for σ_{WT}/σ_{WR} will be evaluated to determine if σ_{WT} and σ_{WR} are comparable. The proposed requirement for the upper limit of the 90% equal-tails confidence interval for σ_{WT}/σ_{WR} is less than or equal to 2.5.

The guidance recommends that the within-subject variability of an NTI reference drug be used to set the acceptable bioequivalence interval. However, we recognized that no one had ever investigated the variability of the within-subject variability (WSV). To do that the reference formulation must be dosed more than twice

Evaluating Within-Subject Variability for Narrow Therapeutic Index Drugs

P. Jayanchandra, H. Okochi, L. A. Frassetto, W. Park, L. Fang, L. Zhao and L.Z. Benet

***Clin. Pharmacol. Ther.*, submitted**

Our research studies were undertaken to address three scientific question:

First: Is it possible for normal WSV to lead to non-equivalence of an NTI drug with itself using the new RSABE approach?

Second : Without a preset 90% confidence interval for an NTI drug, warfarin, is it possible that the bioequivalence interval could be less than the USP content uniformity limits of ± 5%?

Third: Will the upper limit of the 90% confidence interval of the ratio of the within-subject standard deviation of the reference product to reference product need to be ≤ 2.5 in order for equivalent WSV to be declared?

To address these questions, we designed an un-blinded, cross-over clinical study to measure the WSV of warfarin pharmacokinetic parameters for 10 healthy volunteers with similar CYP2C9 and VKORC1 alleles who received the reference listed drug (RLD) Coumadin® on three different occasions.

This allows the WSV to be determined for Reference doses 1 and 2 (R1-R2), Reference doses 1 and 3 (R1-R3), and Reference doses 2 and 3 (R2-R3).

Table 1 Inter-Subject Variability
C_{max} [ng/mL], AUC_{0-72} [ng•hr/mL]

	R-warfarin		*S-warfarin*	
	C_{max}	AUC_{0-72}	C_{max}	AUC_{0-72}
Mean	**612**	**21,450**	**659**	**14,760**
S.D.	**182**	**6,450**	**171**	**4,150**
% CV	**29.8**	**30.1**	**26.0**	**28.1**

Table 2 Intra-Subject Variability (%)

	R-warfarin		*S-warfarin*	
	C_{max}	AUC_{0-72}	C_{max}	AUC_{0-72}
Smallest	**3.65**	**4.27**	**5.39**	**2.51**
Largest	**15.0**	**16.2**	**19.1**	**11.9**
R1-R3	**12.10**	**9.45**	**14.2**	**6.83**
R2-R3	**8.73**	**12.4**	**8.69**	**9.99**
R1-R3	**9.19**	**10.8**	**15.4**	**6.97**
Range	**3.7-15.0**	**4.3-16.2**	**5.4-19.1**	**2.5-11.9**

Using the 3-period study data, we derived 1000 replicates of 4-period datasets by bootstrapping and built a WSV distribution of the 1000 replicates. We performed two BE tests to evaluate the mean comparison (criterion 1) and the variability comparison (criterion 2) obtained using the RSABE approach. Our clinical study goes beyond the current data limitation where repeated RLD treatments were given in three periods instead of two periods to each individual, to allow estimation of variability of WSV and the appropriateness of the proposed BE criteria for NTI drugs.

Table 3 Bioequivalence Testing on Bootstrap of Experimental Data

Passing Rate (%)

	R -warfarin				S -warfarin			
PK Metrics	Mean Compar-ison	Variabil-ity Compar-ison	Both Criteria		Mean Compar-ison	Variability Compar-ison	Both Criteria	
C_{max}	96.8	87.0	85.6		97.3	86.5	85.6	
AUC_{0-72}	95.1	84.2	82.6		96.2	85.7	84.5	

Using the mean comparison based on the WSV of the RLD product (Criterion 1), high bioequivalence pass rates (95-97%) are achieved. Using the new variability comparison (Criterion 2), however, bioequivalence pass rates are lower (84-87%). While one may argue that an overall bioequivalence pass rate of 83-86% is lower than would be expected for the RLD product tested against itself, the passing rate appears reasonable for both tests, given the small sample size of each bootstrap dataset (N = 10). Additionally, we believe the variability comparison provides further assurance on BE demonstration.

From the generated WSV distribution for the bootstrapped dataset for a fully-replicated 4-way crossover study we determined that the probability of the within subject standard deviation falling below 5% would only occur 0.1% of the time (1 in 1000 cases) answering question 2 concerning failing the USP ± 5% content uniformity limits.

In contrast to the RSABE approach that scales the bioequivalence limit to the WSV of the reference product, we also evaluated the bioequivalence passing rate if the 90% confidence interval is fixed to 90-111% for the bootstrapped 1000 replicates of the clinical data.

Table 3 Bioequivalence Testing on Bootstrap of Experimental Data

Passing Rate (%)

	R -warfarin				S -warfarin			
PK Metrics	Mean Compar-ison	Variabil-ity Compar-ison	Both Criteria	Fixed Limits	Mean Compar-ison	Variability Compar-ison	Both Criteria	Fixed Limits
C_{max}	96.8	87.0	85.6	92.8	97.3	86.5	85.6	75.3
AUC_{0-72}	95.1	84.2	82.6	85.6	96.2	85.7	84.5	99.9

Bioequivalence passing rates were obtained using the fixed limits of 86-99% for AUC_{0-72} and 75-93% for Cmax for the RLD product tested against itself. These rates are lower than for the RSABE approach.

Conclusions

- The proposed FDA mean bioequivalence comparison criterion based on the WSV of the RLD product (the RSABE approach appears to provide a better test that a fixed 90-111% bioequivalence limit for this NTI drug
- There is little concern that the low WSV determination will cause a warfarin formulation to fail the USP content labeling criterion.
- The present evaluation suggests that the variability criterion of the current FDA draft product-specific guidance for warfarin (and potentially other NTI drugs) may add further assurance on BE demonstration.

Thank you for your attention

A copy of the slides
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