The Theoretical Basis of IVIVE: An Explanation for the Lack of Success of IVIVE and the Lack of Success of Endogenous Markers and "Cocktail" Approaches to Predict the Clearance of A Drug in a Patient

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First, let me apologize for not being with you in person today. This would have been my 9th trip to India and I have always enjoyed the hospitality, good fellowship and scientific interactions, in addition to visiting many outstanding cultural and historical sites.

However, my responsibilities at UCSF and primarily a memorial service for my very good friend Professor Robert Gibson, which was held earlier today (Friday in San Francisco) prevented me from attending.

Thank you for giving me the opportunity to present our recent work via video and Skype.

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)

Hypothesis: Transporter effects for Class 2 drugs would make IVIVE predictions based on microsomal/hepatocyte incubations less accurate than those for Class 1 drugs where transporter effects should be negligible.

Our Hypothesis was Correct

Using less than a 2-fold difference between predicted and measured clearance as a success criterion

81.9 % of Class 2 drugs were poorly predicted, while 62.3% of Class 1 drugs were poorly predicted

But why are IVIVE predictions so poor?

But first I summarize our analysis

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.

Another approach to predicting human drug PK for an NME has been frequently considered. More than 40 years ago Garrett proposed dosing a 'cocktail' to define the metabolic characteristics of a patient, allowing the selection of appropriate doses of drugs metabolized by the same enzyme pathway as the probe compounds within the cocktail.

Many clinical pharmacologists have been intrigued with the possibility of predicting the effects of the most prominent enzyme CYP3A using measures of endogenous cortisol metabolism or administration of the erythromycin breath test.

We decided to go back to first principles to try to understand the poor IVIVE predictive rate and recognized that the theoretical basis for the methodology employed has never been evaluated leading to some surprising and controversial findings, of which our first paper was published last March.

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

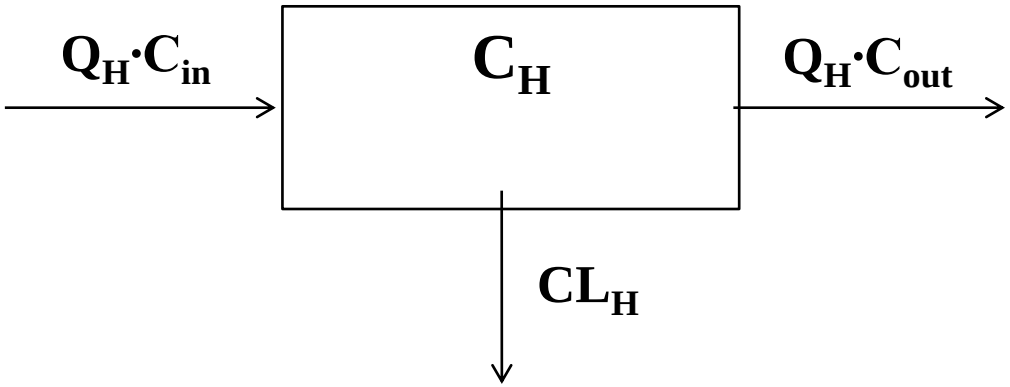
L.Z. Benet, S. Liu and A.R. Wolfe

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

Clearance is a mass balance relationship

It is defined as the rate of elimination from the body/organ at steady-state divided by some measured steady-state concentration, and clearance is defined in terms of the concentration measured. If we measure blood concentration it is the blood clearance; if we measure unbound plasma concentration it is the unbound clearance.

$$CL_X = \frac{Rate\ of\ Elimination}{C_X}$$



So in a perfused organ, such as the liver, at steady-state, rate of elimination equals $Q_H \cdot C_{in} - Q_H \cdot C_{out}$ and CL_H will be defined by where we measure the steady-state concentration.

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:

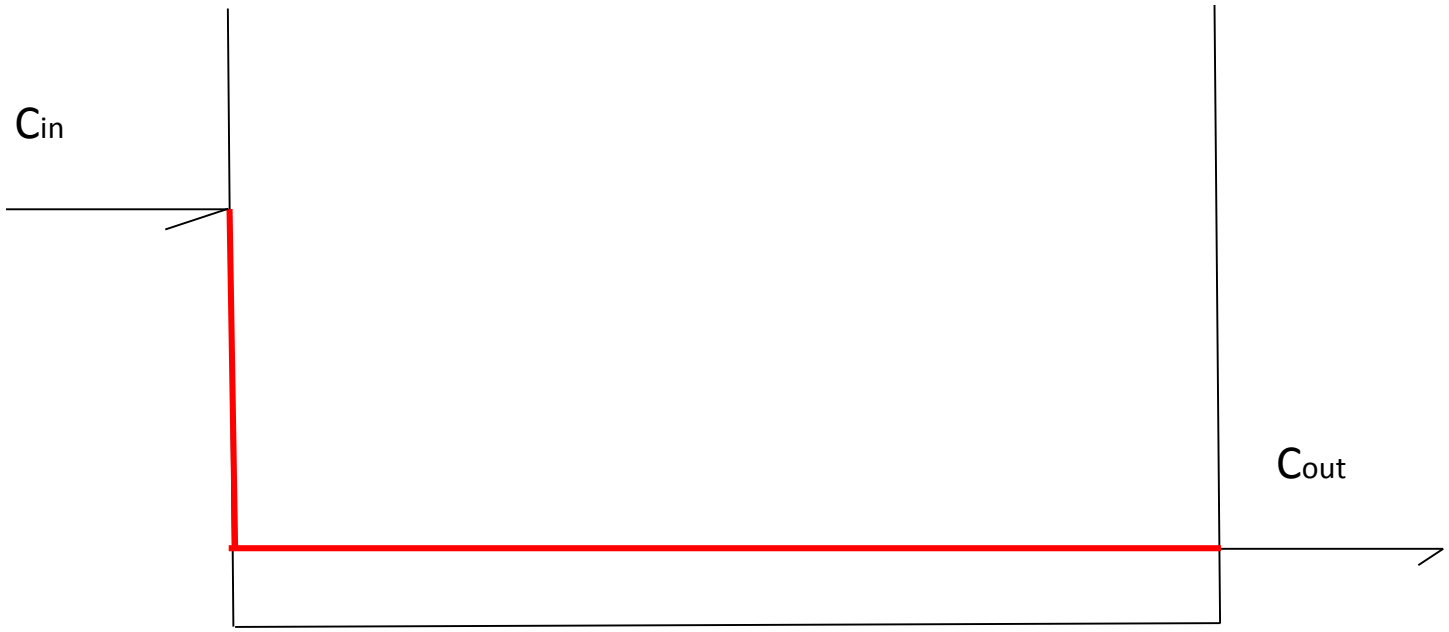
$$CL_H = Q_H \cdot ER = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} \qquad \text{(Eq. 1)}$$

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

$$CL_X = \frac{Rate\ of\ Elimination}{C_X}$$

In Eq. 1 the measured steady-state concentration is not a concentration in the liver, but rather the concentration coming into the liver, C_{in} . That's the well-stirred model. So for 46 years our field has believed that Eq. 1 is a universal relationship, not recognizing that it is only consistent with the well-stirred model.

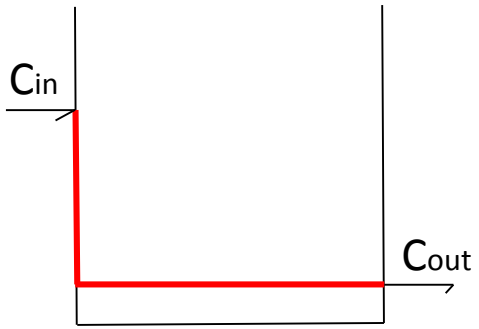
Here's the well-stirred model



The steady-state mixed flow reactor of Chemical Engineering, adapted to Pharmacokinetics as the well-stirred model

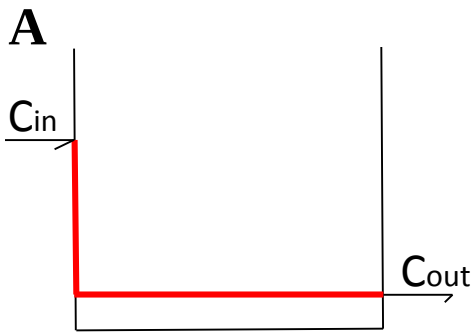
Let's look again pictorially at the organ relationship for Eq. 1

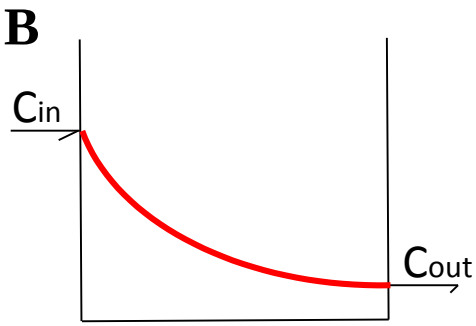
$$Q_H \cdot (C_{in} - C_{out}) = CL_H \cdot C_{in}$$



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

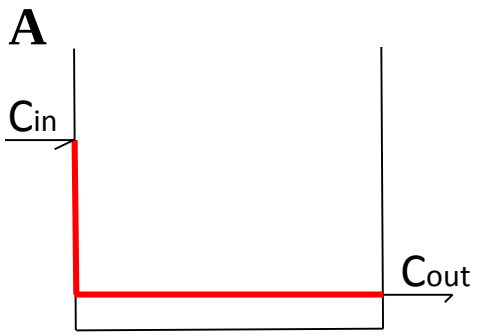
And so if CL is calculated using Eq. 1, measuring only C_{in} and C_{out}, the clearance values will only be consistent with the well-stirred model.

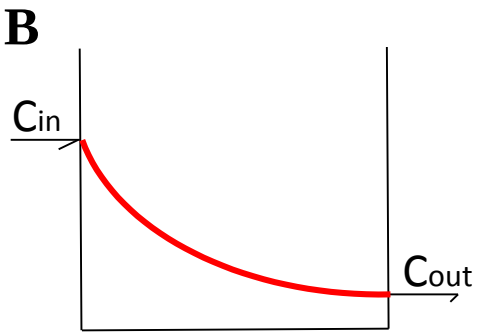




A. The well-stirred model

B. The steady-state plug flow reactor of Chemical Engineering, adapted to Pharmacokinetics as the parallel-tube model





A. **The well-stirred model**
B. **The steady-state plug flow reactor of Chemical Engineering, adapted to Pharmacokinetics as the parallel-tube model**

What is the difference between the two models? C_{in} and C_{out} are the same, but the amount of time that drug molecules are in the reactor/model is different.

One outcome is $AUC_B > AUC_A$

That's the clearance argument: CL is inversely related to AUC

There is a second argument: the mean residence time is markedly different.

$$MRT_B > MRT_A$$

Rowland and Pang provided a Commentary that disagrees with our derived conclusion in which they argue that Eq. 1 “simply express[es] proportionality between observed rate of elimination and a reference concentration” and that Eq. 1 is not model dependent.

But in a more recent invited Opinion submitted to *Clin. Pharmacol. Ther.*, L.Z. Benet, “Clearance Revisited”, we point out that Rowland and Pang are ignoring a second mass balance pharmacokinetic equation

$$CL = \frac{V_{ss}}{MRT} \qquad \text{(Eq. 2)}$$

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)

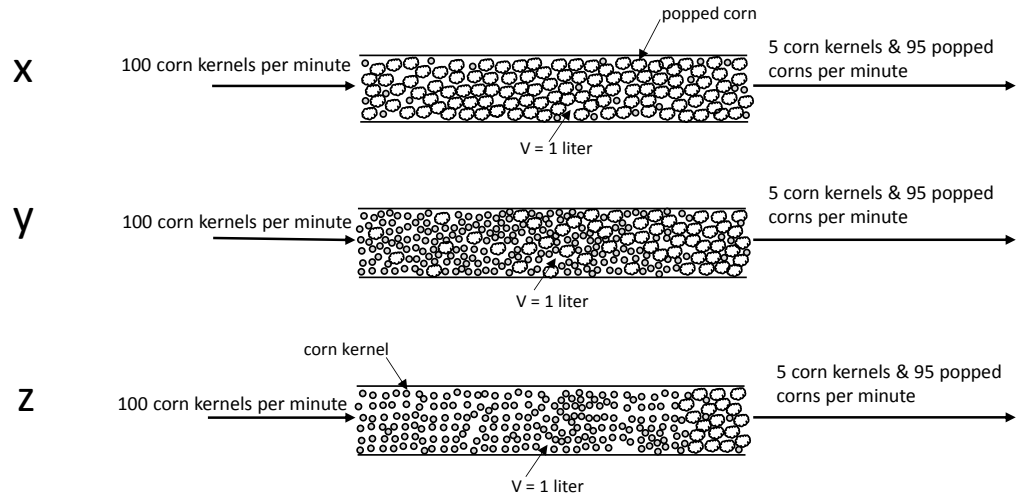
Three Steady-State Popcorn Makers

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.



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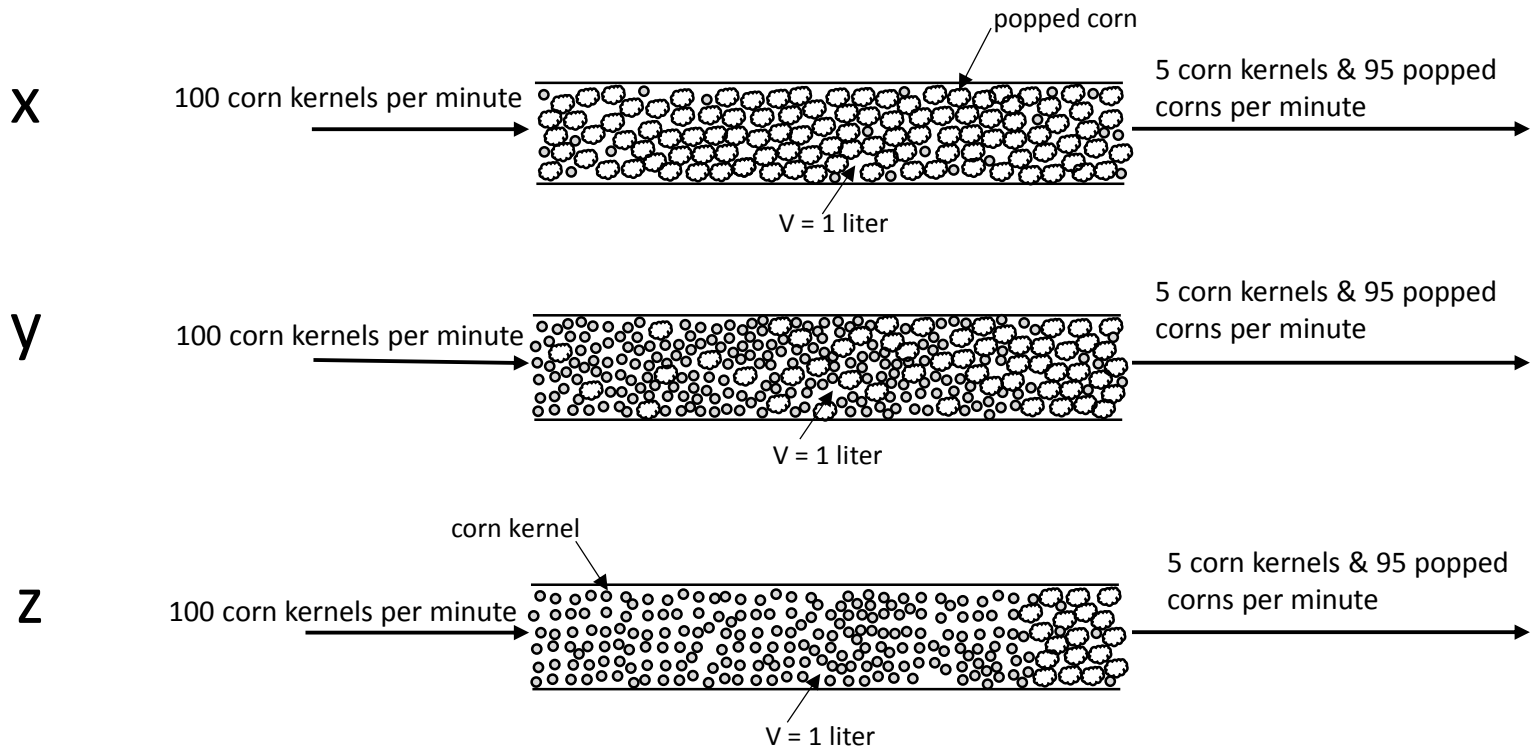
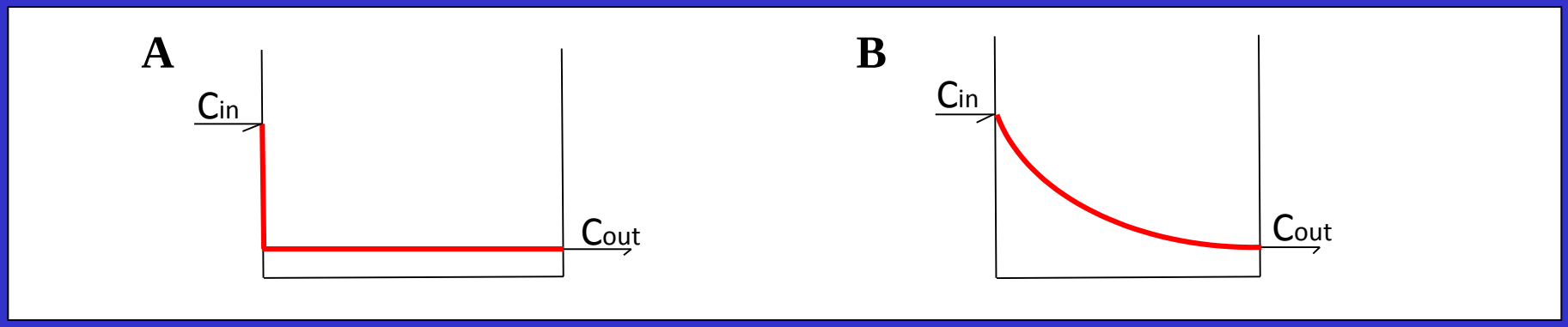


The mean residence time of unpopped corn kernels will be $MRT_Z > MRT_Y > MRT_X$

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?

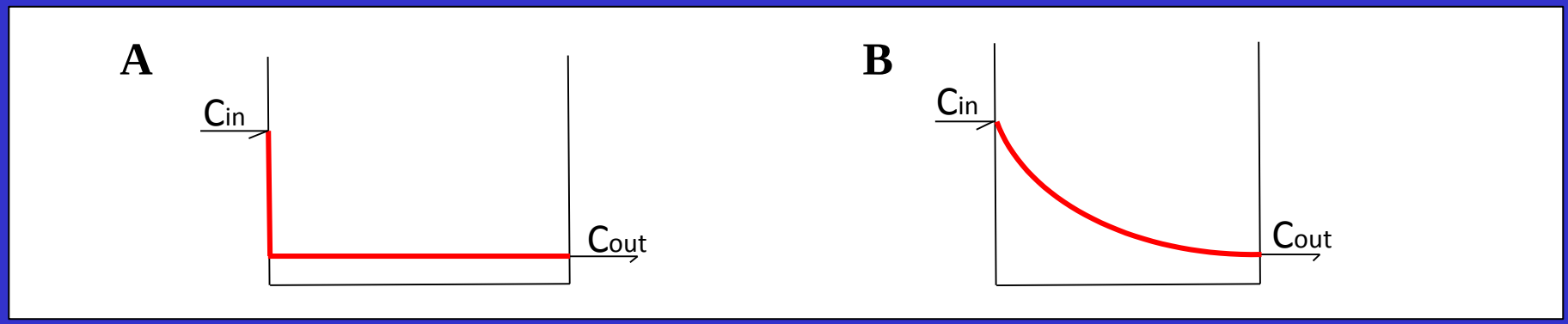
It is hard to imagine how clearance in Eq. 1 is model independent when MRT is model dependent. To do so, one must hypothesize that V_{ss} in Eq. 2 is also model dependent and changes exactly as MRT from model to model.

$$CL = \frac{V_{ss}}{MRT} \qquad \text{(Eq. 2)}$$

Yet, it is well recognized in pharmacokinetics that volume of distribution is drug dependent and not a function of CL nor MRT.

But not only is it obvious that MRT differs between models A and B, but one can easily see that AUC differs between models A and B.

$$AUC_B > AUC_A$$



Therefore $CL_A \neq CL_B$

The supposition that Eq. 1 is model independent is not supported either by pharmacokinetic theory nor by experimental data.

What is the relevance in finding 46 years later that Eq. 1 is only consistent with the well-stirred model?

- It is not appropriate to calculate CL_{int} for the parallel tube or dispersion models from CL measurements that are only consistent with the well-stirred model.
- Calculating hepatic bioavailability as $F_H = 1 - \frac{CL_H}{Q_H}$ assumes the well-stirred model.
- All of the extended clearance concept derivations and equations are only consistent with the well-stirred model

"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.*, 2018 submitted.

We present a number of concepts in that paper, but the theme of this talk relates to the relevance of understanding volume of distribution with respect to clearance models and IVIVE. Thus, I will present here a volume related issue with the extended clearance concept and it use in PBPK.

Recently, a number of excellent papers have been published, many by the famous investigators speaking here, examining the use of physiologic based pharmacokinetic (PBPK) modeling to characterize the critical parameters in oral DDIs and the use of the bottom-up approach to predict these DDIs from in vitro measures. But there appears to be a deficiency in the present PBPK software that needs to be updated.

The present PBPK models, and the investigators using them, do not appear to recognize that significant transporter drug interactions can result in volume of distribution changes in addition to clearance changes. And since half-lives are a function of both clearance and volume of distribution attempting to explain changes in concentration-time curves will not provide accurate predictions if potential volume changes are not considered.

Let's examine some of the transporter interaction studies from our laboratory.

Ratio of Pharmacokinetic Parameters Control Phase/IV Rifampin Phase

Drug	$\frac{CL/F^{Con}}{CL/F^{Rif}}$	$\frac{V_{ss}/F^{Con}}{V_{ss}/F^{Rif}}$	$\frac{MRT^{Con}}{MRT^{Rif}}$	$\frac{t_{1/2,z}^{Con}}{t_{1/2,z}^{Rif}}$
Atorvastatin	7.7	17.7	2.3	2.7
Rosuvastatin	3.6	12.2	3.4	1.6
Glyburide (noninduced)	2.2	3.1	1.4	1.3
Glyburide (induced)	2.2	2.3	1.0	1.1

Note: The control glyburide induced CL/F was 2.9 fold higher than the noninduced control glyburide CL/F

Concern: If these DDIs can be modeled by presently employed PBPK programs, such as Simcyp, what are the implications?

clearance concept AUC_R measurements as we define in our Pharm Res submitted paper.

$$\frac{AUC}{Dose_{oral}} = \frac{F_{abs} \cdot F_G \cdot (CL_{H,int} + PS_{eff,int})}{PS_{inf,int} \cdot f_{u,B} \cdot CL_{H,int}} \quad \text{(Eq. 3)}$$

where $CL_{H,int}$ is the sum of the intrinsic metabolic and biliary clearances, PS_{int} parameters are the sum of the intrinsic efflux and influx active and passive transporter clearances.

independent of the other parameters in the equation. That is, there is no need to determine the β factor to predict the relevance of OATP inhibition or to assume that metabolism is not an important pathway of elimination or to assume that $PS_{eff,int}$ is negligible.

confirm that protein binding interactions, either in the blood or the liver ($f_{u,H}$), have negligible effects on the unbound concentrations driving both efficacy or toxicity, since unbound drug concentrations are independent of protein binding changes for drugs given orally, as Benet and Hoener reported in 2002.

Now, finally, let's return to IVIVE

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

Earlier in this presentation, I detailed the poor predictability of presently employed IVIVE approaches and predictions from endogenous metabolic markers, with the following conclusion:

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

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

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

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

CM Bowman and 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).

Although protein binding errors may explain part of the poor IVIVE predictability, we investigated another aspect.

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

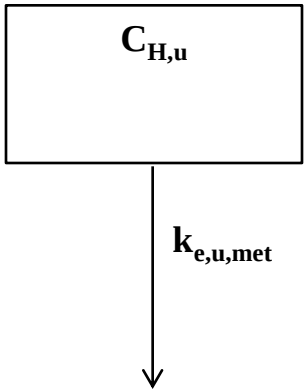
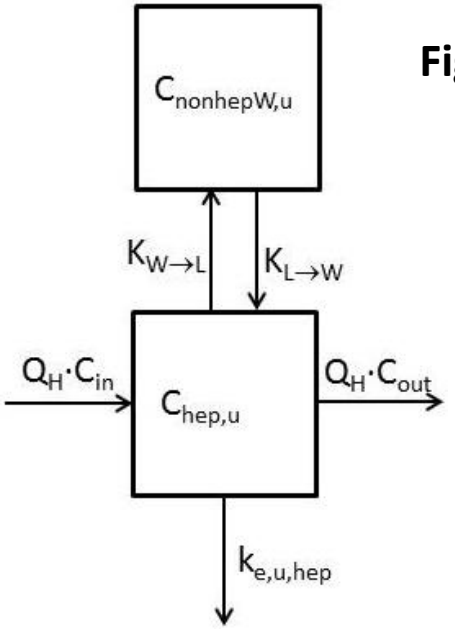


Fig. 2 Heterogeneous Liver Model



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 I will present here (very rapidly), proposes that

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

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}.

In Vivo Hepatic Drug Metabolism

In a model independent manner at steady-state the amount lost between entrance into and exit from the liver will equal the steady-state amount in the liver, $A_{ss,H}$, multiplied by some steady-state rate constant, k_{ss}.

$$Q_H \cdot C_{in} - Q_H \cdot C_{out} = k_{ss} \cdot A_{ss,H} \qquad \text{(Eq. 1)}$$

where Q_H is the liver blood flow, C_{in} and C_{out} are the drug concentrations in blood flowing into and out of the liver, respectively. Converting the steady-state amount into a concentration, $C_{ss,H}$, by multiplying and dividing the right hand side of Eq. 1 by the steady-state volume of distribution of the liver, $V_{ss,H}$, yields

$$Q_H \cdot C_{in} - Q_H \cdot C_{out} = k_{ss} \cdot V_{ss,H} \cdot C_{ss,H} \qquad \text{(Eq. 2)}$$

Assuming the well – stirred model where $C_{H,ss}$ equals C_{out}

$$Q_H \cdot C_{in} - Q_H \cdot C_{out} = k_{ss} \cdot V_{ss,H} \cdot C_{out} = k_{ss,u} \cdot V_{ss,H} \cdot f_{u,B} \cdot C_{out} \quad \textbf{(Eq. 3)}$$

where $f_{u,B}$ is the ratio of the unbound concentration of drug in the plasma to the whole blood concentration.

Dividing all of the terms in Eq. 3 by C_{in} will give the relationship in Eq. 4.

$$CL_{H,in\ vivo} = Q_H \cdot \frac{C_{in} - C_{out}}{C_{in}} = Q_H - Q_H \cdot \frac{C_{out}}{C_{in}} = k_{ss,u} \cdot V_{ss,H} \cdot f_{u,B} \cdot \frac{C_{out}}{C_{in}} \quad \textbf{(Eq. 4)}$$

where $CL_{H,in\ vivo}$ is the experimentally measured in vivo hepatic metabolic clearance. Solving the 3rd and 4th terms of Eq.4 for $\frac{C_{out}}{C_{in}}$ and substituting this relationship into the 4th term in Eq. 4 yields:

$$CL_{H,in\ vivo} = \frac{Q_H \cdot f_{u,B} \cdot k_{ss,u} \cdot V_{ss,H}}{Q_H + f_{u,B} \cdot k_{ss,u} \cdot V_{ss,H}} \quad \textbf{(Eq. 5)}$$

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Defining the product of $k_{ss,u}$ and $V_{ss,H}$ as the intrinsic clearance of unbound drug in the liver via metabolism, i.e.,

$$CL_{int,in\ vivo} = k_{ss,u} \cdot V_{ss,H} \quad \text{(Eq. 6)}$$

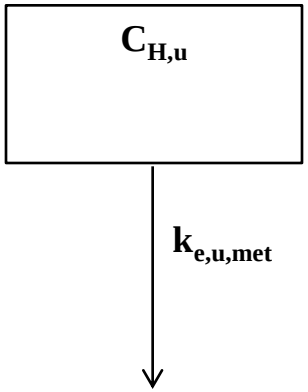
Substituting Eq. 6 into Eq. 5 yields

$$CL_{H,in\ vivo} = \frac{Q_H \cdot f_{u,B} \cdot CL_{int,in\ vivo}}{Q_H + f_{u,B} \cdot CL_{int,in\ vivo}} \quad \text{(Eq. 7)}$$

the well-stirred model definition of clearance.

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



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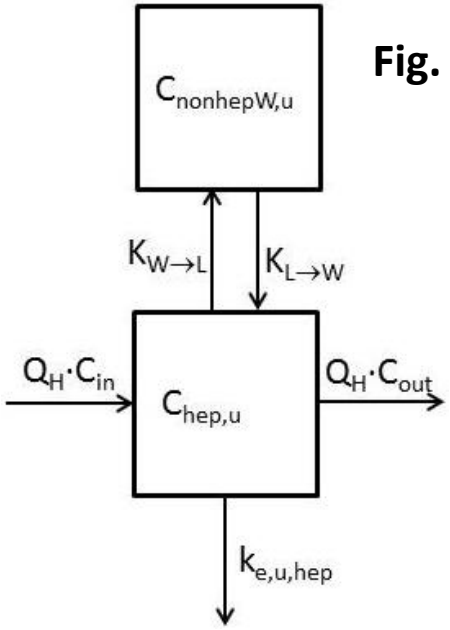

$$CL_{int,in\ vivo} = k_{u,ss} \cdot V_{ss,H}$$

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In Vitro Hepatic Metabolism

Now consider in vitro measures of metabolism. Since in this initial derivation we are considering the non-permeability limited condition, for in vitro microsomal or hepatocyte incubations there is no possibility of transporter effects at the hepatocyte membrane. Then the rate of drug elimination in the in vitro incubation will be given by Eq. 8.

$$-\frac{dA_{in\ vitro}}{dt} = k_{e,u,in\ vitro} \cdot A_{u,in\ vitro} \qquad \text{(Eq. 8)}$$

where $A_{in\ vitro}$ is the total amount of drug in the incubation media, $A_{u,in\ vitro}$ is the total amount of unbound drug in the incubation media, and $k_{e,u,in\ vitro}$ is the metabolic rate parameter for unbound drug in the incubation media with units of time^{-1} per mass of metabolic enzyme in the incubation system. Equation 8 can then be changed into a concentration relationship

$$-\frac{dC_{in\ vitro}}{dt} = k_{e,u,in\ vitro} \cdot C_{u,in\ vitro} \qquad \text{(Eq. 9)}$$

The in vitro intrinsic clearance can be defined as

$$CL_{int,in\ vitro} = k_{e,u,in\ vitro} \cdot V_{in\ vitro} \qquad \text{(Eq. 10)}$$

where $V_{in\ vitro}$ is the volume of distribution for the drug in the in vitro incubation.

Predicting $CL_{int,in\ vivo}$ from $CL_{int,in\ vitro}$

When attempting to predict in vivo intrinsic clearance from in vitro intrinsic clearance measurements in microsomal or hepatocyte incubations, the field has universally applied chemical principles to scale the in vitro measure to yield comparable turnover numbers (k_{cat}) for the in vitro and in vivo data. What the field has done is set $k_{e,u}$ equal to V_{max}/K_m, assuming that the in vivo and in vitro V_{max} values are equivalent when correcting for differences in metabolic enzyme mass. Since k_{cat} equals V_{max} divided by enzyme concentration then it has been assumed that $k_{e,u}$ equals $k_{cat} \cdot enzyme\ conc/K_m$. Then the in vitro and in vivo rate constants for unbound drug elimination are given by

$$k_{e,u,in\ vitro} = \frac{k_{cat} \cdot mg\ enzyme\ in\ vitro\ incubation}{K_m \cdot V_{in\ vitro}} \qquad \text{(Eq. 11)}$$

and $$k_{ss,u,in\ vivo} = \frac{k_{cat} \cdot mg\ enzyme\ in\ whole\ liver}{K_m \cdot V_{hep}} \qquad \text{(Eq. 12)}$$

where V_{hep} is the volume of distribution of total (unbound plus bound) drug in the hepatocyte water in the liver to give the concentration of drug in the liver to which we assume the metabolic enzymes are exposed.

Thus, although drug can distribute throughout the liver, the enzymes are only present in the hepatocyte water. We maintain that this is a critical difference that has not been recognized when attempting IVIVE predictions. In chemistry, we set k_{cat} values equal, but k_{cat} is a function of enzyme concentration, and in pharmacokinetics, the apparent volume of distribution of the drug will often not be equal to the apparent volume of distribution of the enzymes.
Dividing Eq. 12 by Eq. 11 gives

$$\frac{k_{ss,u,in\ vivo}}{k_{e,u,in\ vitro}} = \frac{mg\ enzyme\ in\ whole\ liver}{mg\ enzyme\ in\ vitro\ incubation} \cdot \frac{V_{in\ vitro}}{V_{hep}} \qquad \text{(Eq. 13)}$$

That is, the ratio of in vivo to in vitro rate constants is a function of the drug and the enzyme concentration ratios in vivo to in vitro, which are most often not the same. Taking the ratios of the definitions of in vivo and in vitro intrinsic clearance (Eq. 9 divided by Eq. 10) and substituting in the ratio of the in vivo and in vitro unbound rate constants (Eq. 13) yields Eq. 14.

$$\frac{CL_{int,in\ vivo}}{CL_{int,in\ vitro}} = \frac{mg\ enzyme\ in\ whole\ liver}{mg\ enzyme\ in\ vitro\ incubation} \cdot \frac{V_{ss,H}}{V_{hep}} \qquad \text{(Eq. 14)}$$

If one assumes that $V_{ss,H} = V_{hep}$, which is what the field has been unknowingly doing, one is not accurately determining the concentration of enzyme to which the drug is exposed in the whole liver in vivo. And obviously, enzyme proteins are not drugs. As noted above, drugs distribute into tissues, enzyme proteins do not, consistent with the use of V_{hep} in Eq. 12

Let SF, the Scaling Factor, equal the ratio of enzyme in the whole liver to that of the in vitro incubation

$$SF\ (Scaling\ Factor) = \frac{mg\ enzyme\ in\ whole\ liver}{mg\ enzyme\ in\ vitro\ incubation} \qquad \text{(Eq. 15)}$$

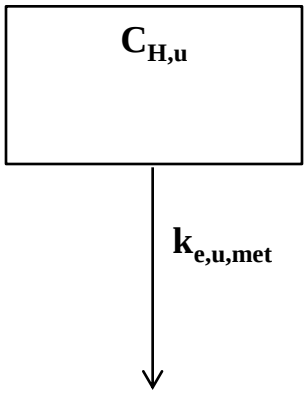
Substituting Eq. 15 into Eq. 14 and rearranging gives

$$CL_{int,in\ vivo} = SF \cdot CL_{int,in\ vitro} \cdot \frac{V_{ss,H}}{V_{hep}} \qquad \text{(Eq. 16)}$$

or $$CL_{int,in\ vivo} = SF \cdot CL_{int,in\ vitro} \cdot R_{ss} \qquad \text{(Eq. 17)}$$

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

Fig. 1 Homogeneous Liver Model



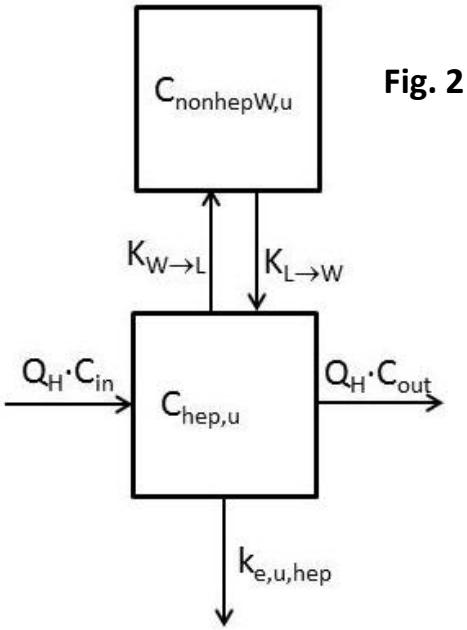


Fig. 2 Heterogenous Liver Model

We believe that a significant portion of poor IVIVE predictability outcomes results from the lack of recognition that the liver is a heterogeneous organ, with both aqueous and lipid components, so that at steady state the driving force concentration in the whole liver will not equal the concentration in contact with the metabolic enzymes (i.e., that $C_{hep,u}$ in Fig. 2 does not equal $C_{H,u}$ in Fig. 1) and that the rate constant in vivo ($k_{e,u,hep}$) is not directly scalable from the in vitro rate constant ($k_{e,u,met}$).

Fig. 1 Homogeneous Liver Model

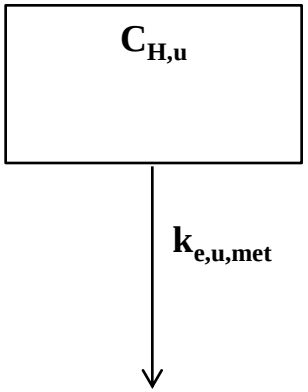
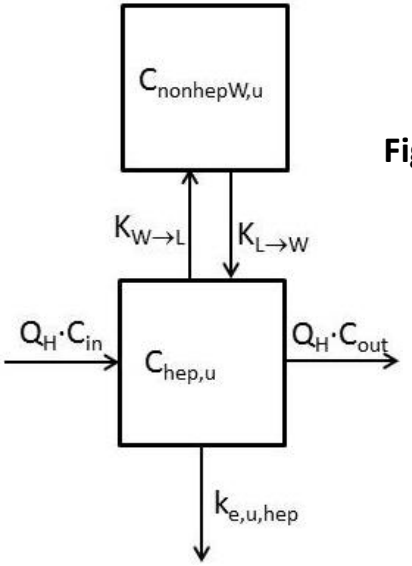


Fig. 2 Heterogenous Liver Model



That is, the direct scaling of the in vitro to the in vivo intrinsic clearance must consider the R_{ss} term as given above, the ratio of the steady-state volume of distribution in the liver to the volume of distribution for the drug in contact with the hepatocyte enzymes. Since this value will in the great majority of cases be greater than 1.0 and different from drug to drug, this is consistent with the in vivo/in vitro differences reported in the literature.

As pharmacokinetic volumes of distribution for individual drugs are usually greater than physiologic volumes it is understandable why the predicted in vivo clearances predominantly, markedly under-predict the measured in vivo clearance.

Drug Cocktails to Predict Clearance of an NME

A further implication of the R_{ss} concept is that drug cocktails (or endogenous metabolism of cortisol) will not quantitatively predict the clearance of an NME, even if the NME and a drug in the cocktail are metabolized by exactly the same enzyme(s). That is because R_{ss} is drug specific depending on the distribution characteristics of each particular drug. Thus, the values of R_{ss} of two drugs would not be expected to be the same just because they are both metabolized by the same enzyme, even if the two drugs are metabolized to a similar type of metabolic product (e.g., the clearance of one benzodiazepine in a patient will not predict the clearance of other benzodiazepines).

However, using a drug cocktail to predict a potential drug interaction would probably be expected to give a correct estimate of the in vivo interaction since this is equivalent to changing the reaction rate in the microsome/hepatocyte incubation.

Conclusions

- We have presented a theoretical basis for why $Q_H \cdot ER$ 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.
- 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 investigated.
- We have presented a theoretical basis as to why drug cocktails have not been successful in predicting clearance of an NME quantitatively, but why they could be predictive of the extent of a drug interaction.

Conclusions

- **We have presented the AUC relationship for the Extended Clearance Concept following oral dosing, with the only limitation being that it is a well-stirred model concept. We have shown that AUC outcomes related to hepatic uptake transporters require no assumption concerning the value of the β factor, the presence or absence of metabolic elimination or the relative value of the intrinsic hepatic clearance versus intrinsic efflux transport clearance.**
- **Surprisingly, all of these findings require recognition of the importance of volume of distribution measures and characteristics.**

We recognize that much of what we are proposing is very controversial and that leading scientists in our field do not agree with our hypotheses and conclusions.

However, since all of our proposals are based on mass balance derivations, we ask that where there is disagreement, show us the error of our derivations.

Some of our new findings and hypotheses will be readily accepted, while others may experience years of controversy.

So stay tuned!!

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