Supplemental data:

Correction for non-specific binding to various components of ultrafiltration apparatus and impact on estimating *in vivo* rat clearance for a congeneric series of 5-ethyl, 5-n-alkyl barbituric acids.

**Drug Metabolism and Disposition**

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**Derivation of an equation to correct for non-specific binding to components of the ultrafiltration apparatus.**

Consider the scheme depicting various parts of the ultracentrifuge apparatus (Supplemental Figure 1). Assume that the drug concentration is below the association constant of the protein and let \( \theta = \frac{C_b}{C_u} \), where \( C_b \) and \( C_u \) are the bound and unbound concentrations, respectively. Now consider the events in each part of the apparatus.

1. **Addition of protein-free drug solution to the top of the ultrafiltration reservoir, without filtration.**

Then the mass balance can be expressed as:

\[
Total = V_M \cdot C_{tot} = V_M \cdot C_u + A_R \tag{Equation A1}
\]

where \( V_M \) is the volume of the added solution, \( C_{tot} \) and \( C_u \) are the total and unbound concentrations of compound in the reservoir, and \( A_R \) is the amount adsorbed onto the walls of the reservoir.

Let \( K_{pr} = \frac{A_R}{C_u} \)
Then
\[ V_M \cdot C_{\text{tot}} = V_M \cdot C_{\text{uR}} + Kp_R \cdot C_{\text{uR}} \]  
\text{Equation A2}

Therefore
\[ f u_R = \frac{C_{\text{uR}}}{C_{\text{tot}}} = \frac{V_M}{V_M + Kp_R} \]  
\text{Equation A3}

2. \textit{Addition of microsomal protein solution into the top reservoir of the ultrafiltration tube without filtration.}

If non-specific binding to the reservoir is absent, then it follows that the mass balance is:
\[ V_M \cdot C_{\text{mic}} = V_M \cdot C_{b,mic} + V_M \cdot C_{u_{mic}} \]  
\text{Equation A4}

where \( C_{\text{mic}}, \ C_{b,mic} \) and \( C_{u_{mic}} \) are the total, microsomal bound and unbound drug concentrations, so that:
\[ V_M \cdot C_{\text{mic}} = V_M \cdot \theta_{\text{mic}} \cdot C_{u_{mic}} + V_M \cdot C_{u_{mic}} \]  
\text{Equation A5}

Therefore, rearranging gives:
\[ f u_{\text{mic}} = \frac{C_{u_{mic}}}{C_{\text{mic}}} = \frac{1}{1 + \theta_{\text{mic}}} \]  
\text{Equation A6}

If the drug also binds non-specifically to the reservoir then the mass balance becomes:
\[ V_M \cdot C_{\text{mic}} = V_M \cdot C_{b,mic} + V_M \cdot C_{u_{mic}} + A_R \]  
\text{Equation A7}

where the apostrophe denotes the situation where there is binding to the reservoir.

Now \( \theta_{\text{mic}} = \frac{C_{b,mic}'}{C_{u_{mic}'}} \) and \( A_R = Kp_R \cdot C_{u_{mic}'} \)

Therefore
\[ V_M \cdot C_{\text{mic}} = \left( V_M \cdot \theta_{\text{mic}} + V_M + Kp_R \right) \cdot C_{u_{mic}'} \]  
\text{Equation A8}
Rearranging gives:

\[ C_{mic} = \left[ \theta_{mic} + \frac{1}{fu_{mic}} \right] Cu_{mic} \]  

Equation A9

So that

\[ fu_{mic} = \frac{Cu_{mic}}{C_{mic}} = \frac{1}{\theta_{mic} + \frac{1}{fu_{mic}}} \]  

Equation A10

However, we actually require an expression for \( fu_{mic} \)

where

\[ \theta_{mic} = \frac{1}{fu_{mic}} - \frac{1}{fu_{R}} \]  

Equation A11

which when substituted into Equation 10 and rearranging gives:

\[ fu_{mic} = \frac{1}{1 + \left( \frac{1}{fu_{mic}} - \frac{1}{fu_{R}} \right)} \]  

Equation A12

Therefore, to calculate \( fu_{mic} \), \( Cu'_{mic} \) has to be estimated by relating it to the concentration measured after filtration (i.e. accounting for losses to membrane and collection cup).

3. **Events after filtration.**

Consider first the loss on the membrane as unbound drug passes through it. Drug concentration in the ultrafiltrate (\( Cu_{UF} \)) is then related to \( Cu'_{mic} \) via Equation A13.

\[ Cu_{UF} = Cu'_{mic} (1 - f_{mem}) \]  

Equation A13

where \( f_{mem} \) is the fraction of the filtered drug that remains on the membrane.

However, it is not possible to measure \( Cu_{UF} \) directly, but the unbound concentration in the collection cup after any binding to the cup (\( Cu_{C} \)) is related to \( Cu_{UF} \) via the mass balance described in Equation A14.

\[ V_{UF} \cdot Cu_{UF} = V_{UF} \cdot Cu_{C} + A_{C} \]  

Equation A14

where \( V_{UF} \) is the volume of ultrafiltrate and \( A_{C} \) is the amount of drug adsorbed to the collection cup.

Rearranging Equation A14 in an analogous manner to Equation  and Equation  gives:
\[ fu_c = \frac{Cu_C}{Cu_{UF}} = \left( \frac{V_{UF}}{V_{UF} + Kp_c} \right) \]  \hspace{1cm} \text{Equation A15}

where \( fu_c \) is the fraction of drug unbound to the collection cup and \( Kp_c = \frac{A_c}{Cu_c} \).

Therefore, substituting Equation A15 into Equation A13 gives:

\[ Cu_{mic} = \frac{Cu_c}{fu_{mem} \cdot fu_c} \]  \hspace{1cm} \text{Equation A16}

where, in this case, \( Cu_c \) is the unbound fraction of drug in the collection cup after filtration.

The fraction unbound to collection cup can be determined by adding protein-free media directly to the cup through Equation A17, i.e. by assuming \( Cu_{UF} = C_{tot} \).

\[ fu_c = \frac{Cu_c}{C_{tot}} \]  \hspace{1cm} \text{Equation A17}

Now, the fraction of drug bound to membrane can be estimated from a control filtration containing no protein in the supernatant since, under these conditions \( Cu_{mic} = Cu_R \).

Therefore, substituting into Equation A16 gives:

\[ fu_{mem} = \frac{Cu_c}{Cu_R \cdot fu_c} \]  \hspace{1cm} \text{Equation A18}

Consequently, \( Cu_c \) can be determined in an ultrafiltrate containing protein in the sample reservoir and therefore, by assuming that the fraction of non-specific binding to sample reservoir, membrane and collection cup does not alter with barbiturate or microsomal concentration, \( fu_{mic} \) can be estimated by rearrangement of Equations A10, A12 and A16 to give:

\[ fu_{mic} = \frac{1}{1 + \left( \frac{C_{mic} \cdot fu_{mem} \cdot fu_c}{Cu_c} - \frac{1}{fu_R} \right)} \]  \hspace{1cm} \text{Equation A19}
or

\[ f_{\text{mac}} = \frac{1}{1 + \left[ \frac{f_{\text{mem}} \cdot f_{\text{c}}}{f_{\text{o}} - 1} \right]} \]  

Equation A20

where \( f_{\text{o}} \) is the observed ratio \( C_{u,c}/C_{\text{mic}} \).