Example Problem 2: IVIVE for Compound X in Human Liver (Good Agreement Scenario)

Of course. Here is a second example for a hypothetical "Compound X," where the simple IVIVE model successfully predicts the observed in vivo data. This example demonstrates a scenario where passive diffusion and protein binding are the dominant mechanisms of distribution.

Objective

A research team is investigating a new chemical, "Compound X," which is believed to distribute throughout the body primarily via passive diffusion with no significant active transport in the liver. They have observed its steady-state concentration in human blood and liver tissue. The goal is to validate if a simple in vitro experiment can accurately predict the observed in vivo liver concentration, which would support the passive distribution hypothesis.

Methodology

The team will follow the standard IVIVE workflow. They will determine the in vitro partition coefficient ($PC_{\text{in vitro}}$) for Compound X using human hepatocytes. This value will then be used in the in silico model to predict the liver tissue concentration ($C_{\text{tissue,pred}}$) from the observed blood concentration. Finally, this prediction will be compared to the observed liver concentration ($C_{\text{tissue,obs}}$).

Part 1: The In Vitro Experiment

An experiment is conducted using the same setup as before, but with Compound X.

Given In Vitro Parameters

- Hepatocytes: 0.5×10^6 cells
- Mean Hepatocyte Volume ($V_{\text{cell,single}}$): $3400\,\mu\text{m}^3$
- Medium Volume (V_{medium}): $1.0\,\text{mL}$
- Total Compound X added: $1.0\,\text{nmol}$

After incubation, the distribution of Compound X shows significantly less accumulation in the cells compared to PFOA:

- Mass in Cells ($M_{\text{cell,vitro}}$): $0.02\,\text{nmol}$
- Mass Bound to Protein in Medium ($M_{\text{bound,medium}}$): $0.78\,\text{nmol}$
- Mass Free in Medium ($M_{\text{free,medium}}$): $0.20\,\text{nmol}$

Step 1.1: Calculate In Vitro Concentrations

Total Cell Volume ($V_{cell,vitro}$):

$$V_{cell,vitro} = (0.5 \times 10^6 \text{ cells}) \times (3400\,\mu m^3/\text{cell}) = 1.7 \times 10^9\,\mu m^3 = 1.7 \times 10^{-6}\,L$$

Free Compound X Concentration in Medium ($C_{free,medium}$):

$$C_{free,medium} = \frac{M_{free,medium}}{V_{medium}} = \frac{0.20\,\text{nmol}}{1.0 \times 10^{-3}\,L} = 200\,\text{nmol}\,L^{-1}$$

Total Compound X Concentration in Cells ($C_{cell,vitro}$):

$$C_{cell,vitro} = \frac{M_{cell,vitro}}{V_{cell,vitro}} = \frac{0.02\,\text{nmol}}{1.7 \times 10^{-6}\,L} \approx 11,765\,\text{nmol}\,L^{-1}$$

Step 1.2: Calculate the In Vitro Partition Coefficient ($PC_{\text{in vitro}}$)

Equation Used:

$$PC_{\text{in vitro}} = \frac{C_{cell,vitro}}{C_{free,medium}}$$

Calculation:

$$PC_{\text{in vitro}} = \frac{11,765\,\text{nmol}\,L^{-1}}{200\,\text{nmol}\,L^{-1}} \approx 58.8$$

This much lower partition coefficient (58.8 vs. 653.6 for PFOA) reflects the lower tendency of Compound X to accumulate in cells.

Part 2: The In Silico Prediction of In Vivo Concentration

We now use this new $PC_{\text{in vitro}}$ value to predict the liver concentration.

Given In Vivo and Physiological Parameters

- Observed Compound X in Blood ($C_{blood,obs}$): $17.5\,\mu g\,L^{-1}$
- Observed Compound X in Liver ($C_{tissue,obs}$): $14.0\,\mu g\,kg^{-1}$
- Molar Mass of Compound X: $350.0\,g\,mol^{-1}$
- Affinity Constant (K_a) of Compound X for Albumin: $1.0 \times 10^5\,L\,mol^{-1}$
- Total Protein in Blood ($C_{protein,blood}$): $600\,\mu mol\,L^{-1}$
- Total Protein in ISF ($C_{protein,ISF}$): $200\,\mu mol\,L^{-1}$
- V_{blood}: 0.05 L, V_{ISF}: 0.15 L, V_{cell}: 0.80 L, V_{tissue}: 1.0 L

Step 2.1: Convert Units and Calculate Free Concentration in Blood

Convert Observed Blood Concentration to Molar:

$$C_{blood,obs} = \frac{17.5 \times 10^{-6}\,g\,L^{-1}}{350.0\,g\,mol^{-1}} = 5.0 \times 10^{-8}\,mol\,L^{-1} = 50\,nmol\,L^{-1}$$

Calculate Free Compound X Concentration in Blood ($C_{free,blood}$):

$$C_{free,blood} = \frac{50\,nmol\,L^{-1}}{1 + (1.0 \times 10^{5}\,L\,mol^{-1}) \times (600 \times 10^{-6}\,mol\,L^{-1})}$$

$$C_{free,blood} = \frac{50\,nmol\,L^{-1}}{1 + 60} = \frac{50\,nmol\,L^{-1}}{61} \approx 0.820\,nmol\,L^{-1}$$

Step 2.2: Apply IVIVE Hypothesis and Calculate Cellular Concentration

IVIVE Hypothesis: $C_{free,ISF} = C_{free,blood} = 0.820\,nmol\,L^{-1}$

Calculate Cellular Concentration (C_{cell}):

$$C_{cell} = C_{free,ISF} \times PC_{in\ vitro} = 0.820\,nmol\,L^{-1} \times 58.8 \approx 48.2\,nmol\,L^{-1}$$

Step 2.3: Calculate Total Predicted Tissue Concentration ($C_{tissue,pred}$)

Equation Used:

$$C_{tissue,pred} = \frac{(C_{blood,obs} \times V_{blood}) + (C_{total,ISF} \times V_{ISF}) + (C_{cell} \times V_{cell})}{V_{tissue}}$$

First, find Total ISF Concentration ($C_{total,ISF}$):

$$C_{total,ISF} = C_{free,ISF} \times (1 + K_a \times C_{protein,ISF})$$

$$C_{total,ISF} = 0.820\,nmol\,L^{-1} \times (1 + (1.0 \times 10^{5}\,L\,mol^{-1}) \times (200 \times 10^{-6}\,mol\,L^{-1}))$$

$$C_{total,ISF} = 0.820\,nmol\,L^{-1} \times (1 + 20) = 0.820\,nmol\,L^{-1} \times 21 = 17.22\,nmol\,L^{-1}$$

Now, calculate $C_{tissue,pred}$:

Numerator (Total Mass in nmol):

$$M_{blood} = 50\,nmol\,L^{-1} \times 0.05\,L = 2.5\,nmol$$

$$M_{ISF} = 17.22\,nmol\,L^{-1} \times 0.15\,L \approx 2.58\,nmol$$

$$M_{cell} = 48.2\,nmol\,L^{-1} \times 0.80\,L \approx 38.56\,nmol$$

$$\text{Total Mass} = 2.5 + 2.58 + 38.56 = 43.64\,nmol$$

Final Calculation:

$$C_{tissue,pred} = \frac{43.64\,nmol}{1.0\,L} = 43.64\,nmol\,L^{-1}$$

Convert Predicted Concentration to $\mu g\,kg^{-1}$:

$$C_{tissue,pred} = (43.64 \times 10^{-9}\,mol\,kg^{-1}) \times (350.0\,g\,mol^{-1}) = 1.53 \times 10^{-5}\,g\,kg^{-1} = 15.3\,\mu g\,kg^{-1}$$

Part 3: Comparison, Analysis, and Conclusion

Comparison of Results

Parameter	Observed Value	Predicted Value
Liver Concentration	$14.0\,\mu g\,kg^{-1}$	$15.3\,\mu g\,kg^{-1}$
Tissue:Blood Ratio	0.80 (14.0 / 17.5)	0.87 (15.3 / 17.5)

Analysis

The in silico model, parameterized with the in vitro data for Compound X, predicted a liver tissue concentration of $15.3\,\mu g\,kg^{-1}$. This value is in excellent agreement with the observed in vivo concentration of $14.0\,\mu g\,kg^{-1}$. The predicted value is only about 9% higher than the observed value, which is well within typical experimental variability.

Conclusion

The hypothesis that a simple in vitro experiment can predict the in vivo liver concentration of Compound X is strongly supported by this example. The close match between the predicted and observed values indicates that the model, which only accounts for passive partitioning and protein binding, is sufficient to describe the distribution of Compound X in the liver. This result validates the initial assumption that Compound X is not a significant substrate for active uptake or efflux transporters in this tissue.

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