Example Problem: IVIVE for PFOA in Human Liver

Objective

A research team has observed the steady-state concentration of Perfluorooctanoic acid (PFOA) in human blood and liver tissue samples. They want to determine if a simple in vitro experiment using human hepatocytes can accurately predict the observed in vivo liver concentration.

Methodology

The team will follow the IVIVE workflow. First, they will conduct an in vitro experiment to determine the free partition coefficient ($PC_{\text{in vitro}}$). Then, they will use this value in an in silico model, starting with the observed blood concentration, to predict the liver tissue concentration ($C_{\text{tissue,pred}}$). Finally, they will compare this prediction to the observed liver concentration ($C_{\text{tissue,obs}}$) to validate the model.

Part 1: The In Vitro Experiment

An experiment is conducted using cryopreserved human hepatocytes.

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 PFOA added: $1.0\,\text{nmol}$
- Protein in Medium: 4% Bovine Serum Albumin (BSA)
- Molar Mass of BSA: $66\,500\,\text{g/mol}$

After incubation to reach equilibrium, the distribution of PFOA is measured:

- Mass in Cells ($M_{\text{cell,vitro}}$): $0.10\,\text{nmol}$
- Mass Bound to Protein in Medium ($M_{\text{bound,medium}}$): $0.81\,\text{nmol}$
- Mass Free in Medium ($M_{\text{free,medium}}$): $0.09\,\text{nmol}$

Step 1.1: Calculate In Vitro Concentrations

First, we calculate the necessary volumes and concentrations.

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

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

Converting to Liters: $1\,\text{L} = 10^{15}\,\mu\text{m}^3$, so

$$V_{\text{cell,vitro}} = 1.7 \times 10^{-6}\,\text{L}$$

Free PFOA Concentration in Medium ($C_{\text{free,medium}}$):

$$C_{\text{free,medium}} = \frac{M_{\text{free,medium}}}{V_{\text{medium}}} = \frac{0.09\,\text{nmol}}{1.0 \times 10^{-3}\,\text{L}} = 90\,\text{nmol/L} = 90\,\text{nM}$$

Total PFOA Concentration in Cells ($C_{\text{cell,vitro}}$):

$$C_{\text{cell,vitro}} = \frac{M_{\text{cell,vitro}}}{V_{\text{cell,vitro}}} = \frac{0.10\,\text{nmol}}{1.7 \times 10^{-6}\,\text{L}} \approx 58824\,\text{nmol/L} = 58824\,\text{nM}$$

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

The key parameter from this experiment is the partition coefficient between the free concentration in the medium and the total concentration in the cells.

Equation Used: This corresponds to the definition in Equation (2) of the methodology document.

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

Explanation: This ratio describes how PFOA partitions between the external fluid and the cell interior at equilibrium. It is a unitless value. Note that the original document has this ratio inverted in some places, but the definition $C_{\text{cell}}/C_{\text{free}}$ is the one that correctly leads to the final equations.

Calculation:

$$PC_{\text{in vitro}} = \frac{58824\,\text{nM}}{90\,\text{nM}} \approx 653.6$$

This unitless partition coefficient will now be used in the in silico model.

Part 2: The In Silico Prediction of In Vivo Concentration

Now, we use the $PC_{\text{in vitro}}$ value to predict the concentration in a hypothetical 1 kg sample of human liver tissue.

Given In Vivo and Physiological Parameters

- Observed PFOA in Blood ($C_{\text{blood,obs}}$): $20.7\,\mu\text{g/L}$
- Observed PFOA in Liver ($C_{\text{tissue,obs}}$): $50.1\,\mu\text{g/kg}$
- Molar Mass of PFOA: $414.07\,\text{g/mol}$
- Affinity Constant (K_a) of PFOA for Albumin: $1.2 \times 10^5\,\text{L/mol}$
- Total Protein (Albumin) in Blood Plasma ($C_{\text{protein,blood}}$): $600\,\mu\text{mol}$
- Total Protein (Albumin) in ISF ($C_{\text{protein,ISF}}$): $200\,\mu\text{mol}$
- Physiological Fractions for $1\,\text{kg}$ Liver Tissue:
  - Residual Blood Volume (V_{blood}): 5% = $0.05\,\text{L}$
  - Interstitial Fluid Volume (V_{ISF}): 15% = $0.15\,\text{L}$
  - Cellular Volume (V_{cell}): 80% = $0.80\,\text{L}$
  - Total Tissue Volume (V_{tissue}): $1.0\,\text{L}$ (assuming density of $1\,\text{kg/L}$)

Step 2.1: Convert Units and Calculate Free Concentration in Blood

Convert Observed Blood Concentration to Molar:

$$C_{\text{blood,obs}} = \frac{20.7 \times 10^{-6}\,\text{g/L}}{414.07\,\text{g/mol}} = 5.0 \times 10^{-8}\,\text{mol/L} = 50\,\text{nM}$$

Calculate Free PFOA Concentration in Blood ($C_{\text{free,blood}}$):
Equation Used: This is Equation (11) from the methodology document.

$$C_{\text{free,blood}} = \frac{C_{\text{blood,obs}}}{1 + K_a \cdot C_{\text{protein,blood}}}$$

Explanation: This equation calculates the unbound (free) concentration of a substance based on its total concentration, its affinity for a binding protein, and the total concentration of that protein. It assumes protein binding sites are in large excess.
Calculation:

$$C_{\text{free,blood}} = \frac{50\,\text{nM}}{1 + (1.2 \times 10^5\,\text{L/mol}) \cdot (600 \times 10^{-6}\,\text{mol/L})}$$

$$C_{\text{free,blood}} = \frac{50\,\text{nM}}{1 + 72} \approx 0.685\,\text{nM}$$

Step 2.2: Apply IVIVE Hypothesis and Calculate Cellular Concentration

Core IVIVE Hypothesis: The free concentration of PFOA in the blood is in equilibrium with the free concentration in the interstitial fluid.

$$C_{\text{free,ISF}} = C_{\text{free,blood}} = 0.685\,\text{nM}$$

Calculate Cellular PFOA Concentration (C_{cell}):

Equation Used: This uses the rearranged definition of the partition coefficient.

$$C_{\text{cell}} = C_{\text{free,ISF}} \cdot PC_{\text{in vitro}}$$

Explanation: We assume the partitioning behavior observed in vitro ($PC_{\text{in vitro}}$) is the same as the partitioning between the ISF and the liver cells in vivo. We use the free concentration in the ISF to drive the partitioning into the cells.

Calculation:

$$C_{\text{cell}} = 0.685\,\text{nM} \cdot 653.6 \approx 447.7\,\text{nM}$$

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

We now sum the mass of PFOA in each sub-compartment (blood, ISF, cells) and divide by the total tissue volume.

Equation Used: This is the corrected version of Equation (7).

$$C_{\text{tissue,pred}} = \frac{M_{\text{blood}} + M_{\text{ISF}} + M_{\text{cell}}}{V_{\text{tissue}}}$$

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

Explanation: This equation performs a mass balance. The total concentration in the tissue is the sum of the masses in the residual blood, the interstitial fluid, and the cells, all divided by the total tissue volume.

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

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

$$C_{\text{total,ISF}} = 0.685\,\text{nM} \cdot (1 + (1.2 \times 10^5\,\text{L/mol}) \cdot (200 \times 10^{-6}\,\text{mol/L}))$$

$$C_{\text{total,ISF}} = 0.685\,\text{nM} \cdot (1 + 24) = 0.685\,\text{nM} \cdot 25 = 17.125\,\text{nM}$$

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

Numerator (Total Mass in nmol):

$$M_{\text{blood}} = 50\,\text{nM} \cdot 0.05\,\text{L} = 2.5\,\text{nmol}$$

$$M_{\text{ISF}} = 17.125\,\text{nM} \cdot 0.15\,\text{L} \approx 2.57\,\text{nmol}$$

$$M_{\text{cell}} = 447.7\,\text{nM} \cdot 0.80\,\text{L} \approx 358.16\,\text{nmol}$$

$$\text{Total Mass} = 2.5 + 2.57 + 358.16 = 363.23\,\text{nmol}$$

Final Calculation:

$$C_{\text{tissue,pred}} = \frac{363.23\,\text{nmol}}{1.0\,\text{L}} = 363.23\,\text{nM}$$

Convert Predicted Concentration to μg/kg:

$$C_{\text{tissue,pred}} = (363.23 \times 10^{-9}\,\text{mol/kg}) \cdot (414.07\,\text{g/mol}) = 1.50 \times 10^{-4}\,\text{g/kg} = 150.4\,\mu\text{g/kg}$$

Part 3: Comparison, Analysis, and Conclusion

Comparison of Results

Parameter	Observed Value	Predicted Value
Liver Concentration	50.1 µg/kg	150.4 µg/kg
Tissue:Blood Ratio	2.42 (50.1 / 20.7)	7.27 (150.4 / 20.7)

Analysis

The in silico model, parameterized with the in vitro partition coefficient, predicted a liver tissue concentration of 150.4 µg/kg. This is approximately three times higher than the observed in vivo concentration of 50.1 µg/kg. Consequently, the predicted tissue-to-blood ratio (7.27) is also significantly higher than the observed ratio (2.42).

This discrepancy indicates that the simple IVIVE model, based solely on passive partitioning and protein binding, is insufficient to describe the distribution of PFOA in the human liver.

Conclusion

The hypothesis that a simple in vitro experiment can predict the in vivo liver concentration of PFOA is not supported by this example. The model significantly over-predicted the accumulation in the liver.

This result strongly suggests that other biological processes, which were not included in this simple model, are playing a significant role in vivo. Based on the literature (such as the "Assumptions" document), the most likely missing factors are active transport processes. Specifically, there may be efflux transporters on the hepatocyte membrane that actively pump PFOA out of the cells and back into the ISF or blood, thereby limiting its net accumulation.

Therefore, to create a more accurate and predictive model for PFOA in the liver, the model must be refined to include terms for active transport (e.g., CL_{eff} for efflux), as referenced in the Li et al. (2019) paper. The initial IVIVE approach served as a valuable first step, demonstrating that passive processes alone cannot explain the observed phenomena.