Enhancing ERA for High log$P_{O/W}$ Substances: Partitioning into Air ($K_{O/A}$), Cell Membranes ($K_{M/W}$), and Bovine Serum Albumin ($K_{BSA/W}$)

Background

Octanol–Water partition coefficients (log$P_{O/W}$) underpin environmental modelling providing a rudimentary, overly generic value to estimate a substance's behavior. The end-point of an OECD 107, 117 or 123 is incorporated into modelling tools and assessments to predict a molecule's behavior in a wide range of situations, whether it is affinity to an environmental compartment matrix, rate of transfer to water resources or bioaccumulation. To varying degrees, Log*P* values derived from these regulatory studies are a fundamental yet superficial input for persistance, bioaccumulation, mobility and toxicology trigger assessments.

High Log*P* (>4, OECD 123) substances are particularly challenging to evaluate in environmental risk assessments (ERA). Their strong hydrophobicity drives extensive sorption and partitioning processes that are not fully captured by conventional testing, e.g. OECD 123. Smithers has begun development of a customizable program of partition coefficient studies investigating partition behavior across three environmentally and biologically relevant simulations: partition between air ($K_{O/A}$), cell membrane ($K_{M/W}$), and bovine serum albumin ($K_{BSA/W}$).

Partition from Octanol into Air ($K_{O/A}$),

Method (Static):

Test vessels consist of a glass container sealed with either a septum or a valve, to allow sampling at specified timepoints. The octanol phase is dosed directly using a long needled syringe.

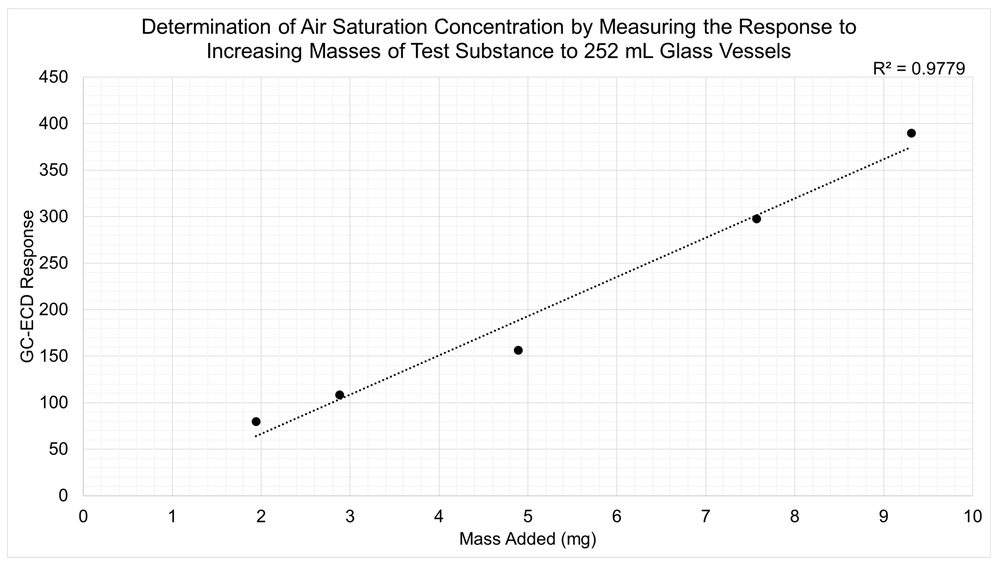
Step 1: Within the test vessel, the ratio of octanol to air must be optimised to achieve the highest accuracy and precision possible.

Step 2: The time taken to reach equilibrium is measured by repeat analysis of samples over the course of the test.

Step 3: Partitioning is evaluated at three concentrations to confirm concentration-independent behaviour and absence of non-ideal effects.

Result: Definitive test (Step 3) is conducted on duplicate samples at each concentration and in general achieved RSD of < 5 % for log$K_{O/A}$.

Pass criteria include ensuring both gas and liquid phase are not saturated, and a total mass balance within the vessel greater than 90%.



In the above graph, the air saturation concentration was not reached as there is no plateau after 24 hours equilibrium. A weight of up to 9.3 mg of substance could be used to generate air phase stocks.

Partition through a Lipid Bilayer ($K_{M/W}$)

Method:

A Sovicell Transil Intestinal Absorption kit and the recommended procedure was modified and used. The kit is in a 96 well plate format, each well contains a specified volume of phosphotidylcholine membranes supported on silica beads.

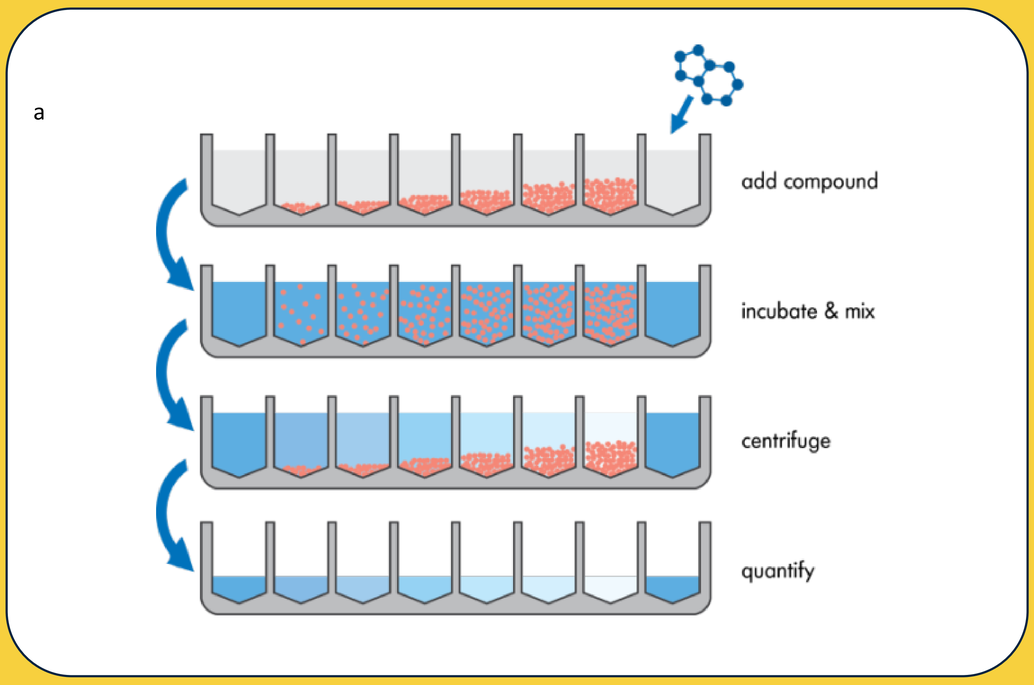
Step 1: Six wells of the same ratio are pooled into a HPLC vial and undergo washing with selected buffer, ensuring volumes of buffer are approximately equal.

Step 2: The procedure on the right is applied to the HPLC vials.

Step 3: The supernatant is analysed to determine the aqueous concentration, the beads can be extracted to calculate the mass balance and understand the concentration within the bilipid layer.

Result: The test can be modified for plastic-binding and volatile substances.

Supernatant analysis has resulted in RSD of < 12%, with ranges of < 0.3 log units.



Binding to Bovine Serum Albumin ($K_{BSA/W}$) - Still under development

Method 1: Partition Through a Membrane

An equilibrium device, a two chamber vessel separated by a cellulose wall, is used to understand the affinity of the substance to bovine serum albumin (BSA).

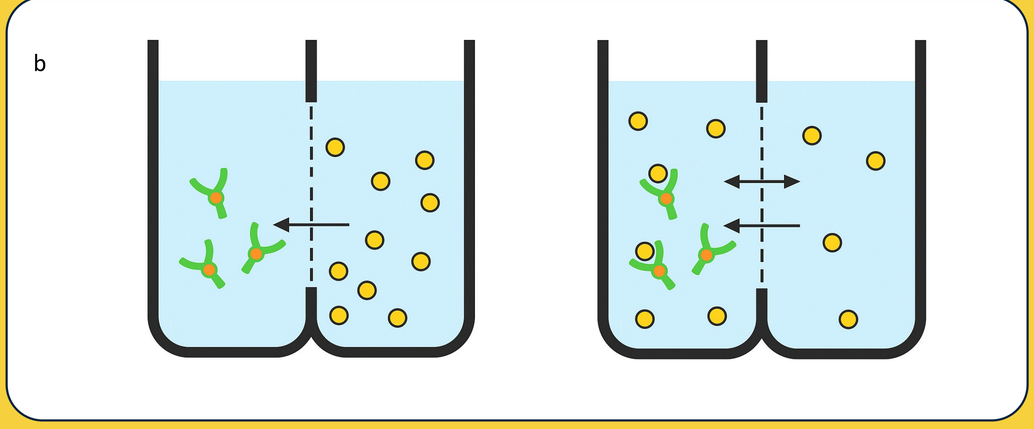
Step 1: Prepare and dose chambers. Buffered medium added to each chamber, one containing Bovine serum albumin (25 to 40 g/L).

Step 2: After dosing, equilibrate at test temperature (37 °C) until equilibrium is reached.

Step 3: Analyse the side without protein-ligand complex to determine binding constant.

Method 2: HPLC Simulation (Talking point!)

Operates on the principle of correlating literature-derived $K_{BSA/W}$ values with empirically determined retention times. Such a test would not be subject to difficult chemical properties such as volatility. Using an analytical column with BSA as a stationary phase, consistent with OECD Test Guideline 117 and 121.



Conclusion

It is possible to characterise the behaviour of high log$P_{O/W}$ with difficult-to-test properties such as low aqueous solubility and high volatility, with an acceptable degree of precision and accuracy. The summarised assays produce a single data point which can be added to ERA models and calculations to more accurately assess the impact to humans and the wider environs.

[a] Transil Intestinal Absorption Kit User Guide

[b] Plasma Protein Binding Dynamics: Equilibrium dialysis and Ultrafiltration Methods and Application in DDIs - IPHASE

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