Incremental improvements to the trout S9 biotransformation assay

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In vitro substrate depletion methods have been used in conjunction with computational models to predict biotransformation impacts on chemical accumulation by fish. There is a consistent trend, however, toward overestimation of measured chemical residues resulting from controlled whole-animal exposures. One possible reason for this discrepancy is that in vitro methods underestimate in vivo rates of metabolic activity. The goal of this effort was to evaluate a well-known in vitro assay employing the trout liver S9 fraction, and determine whether and how assay performance can be improved. This work was performed using several polycyclic aromatic hydrocarbons (PAHs) as model substrates. In vitro intrinsic clearance was inversely related to substrate concentration, consistent with saturable (Michaelis-Menten) kinetics. However, the activity of the S9 system was found to decrease steadily over time. Combining these results, it is suggested that first-order clearance in assays performed over longer time periods (> 1 hr) may be an artifact of these competing influences. These factors may also explain the curvilinear nature of data reported by several authors. Acetone used as a spiking solvent had negative effects on PAH clearance at concentrations greater than 0.5% (v/v), as did alamethicin (25 µg/mL), which is often added to support Phase II UGT activity. Increasing the amount of S9 protein had unexpected effects on substrate depletion (tending to increase it) and intrinsic clearance (which tended to remain constant). This finding challenges current dogma which suggests that enzymes operate against the free chemical concentration in solution. Collectively, these results underscore the importance of conducting preliminary studies to optimize assay performance. Intrinsic clearance rates are maximized by decreasing substrate concentration and the amount of spiking solvent. S9 protein content can be “tailored” to a degree to optimize the rate of substrate depletion. All of these factors must be balanced by limitations on analytical sensitivity and the need for measurable activity.