Inter-laboratory comparison of clearance rates of xenobiotics by cryopreserved trout hepatocytes for the prediction of bioaccumulation potential.

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Hepatic biotransformation is an important determinant of chemical bioaccumulation in fish. Consequently, improvements to bioaccumulation models can be made using estimates of chemical biotransformation rates. Cryopreserved trout hepatocytes have previously been used to measure the clearance rates of some compounds. The use of this information within a regulatory context requires, however, that such results be reproducible across laboratories. In this study, three independent laboratories performed a round-robin study using cryopreserved rainbow trout (Oncorhynchus mykiss) hepatocytes. Six compounds were selected for testing: benzo[a]pyrene, 4-nonylphenol, di-tert butyl phenol, fenthion, methoxychlor and o-terphenyl. Pre-trial studies were performed to streamline the assay protocol, standardize hepatocyte counting procedures, and characterize potential sources of variability. The results confirmed first-order depletion kinetics for the selected compounds and highlighted the effects of assay temperature as well as lot variability. Each laboratory then conducted clearance assays for the six compounds using a substrate depletion approach. The analyses for each substrate were conducted at one institute to focus the comparison on the assay itself. Compounds determined to be poorly (o-terphenyl; <0.05 ml/h/10^6 cells) or rapidly metabolized (benzo[a]pyrene; >0.3 ml/h/10^6 cells) were similarly determined across laboratories. Coefficients of variation across the three laboratories were generally 30% or better, suggesting this method of determining intrinsic clearance is transferable and reproducible. This inter-laboratory comparison strongly supports the use of cryopreserved trout hepatocytes as a tool for estimating hepatic clearance for bioconcentration factor predictions.