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Method optimization for fathead minnow (Pimephales promelas) liver S9 isolation

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Standard protocols have been proposed to assess metabolic stability in rainbow trout liver S9 fractions. Using in vitro substrate depletion assays, in vitro intrinsic clearance rates can be calculated for a variety of study compounds. Existing protocols suggest potential adaptations to these experimental methods in order to allow the use of other fish species by taking into account aspects of fish husbandry and incubation mixture temperature. Small fish species, especially species with diffuse livers, represent a unique challenge in liver S9 isolation. Small fish species require the pooling of dozens of individuals in order to create liver S9 pools large enough to accommodate enzymatic characterization. Dissections involving diffuse livers are even further time consuming, and may therefore compromise enzymatic activity. Here, we examined three dissection techniques, including dissection and immediate flash freezing in liquid nitrogen. The percent recovery of the S9 fraction was described using glucose-6-phosphatase (G6P) activity as a marker for microsomal protein. Enzymatic activity was characterized for each liver S9 pool, and in vitro substrate depletion assays were conducted using the pharmaceutical propranolol. We observed a higher percent recovery of S9 activity in the liver samples that were flash frozen in liquid nitrogen prior to S9 isolation than for any other dissection techniques. However, this elevated enzymatic recovery did not correspond to significantly higher intrinsic clearance rates of propranolol. These observations are important, as fish are commonly collected in the field and tissues are dissected and flash frozen in order to characterize enzymatic activity (e.g. EROD activity) in the laboratory.

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