DEVELOPMENT AND TESTING OF CRYOPRESERVED CARP HEPATOCYTE FOR THE ESTIMATION OF BIOCONCENTRATION IN FISH

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Abstract

Assessment of bioconcentration of xenobiotics in the aquatic environment is becoming increasingly important for regulatory approval in the Europe Union and other regions. Both subcellular and cellular fish hepatic *in vitro* systems have provided metabolic loss rates which have been successfully used to improve bioconcentration (BCF) estimates by via extrapolation models. While subcellular systems have the advantage that they can be cryopreserved and shipped to any laboratory for testing, most cellular systems are currently sourced from cultured fresh fish. The maintenance of fish cultures is both labor and time intensive. Carp and trout have been the most commonly used species for *in vivo* fish bioaccumulation studies. Cryopreserved trout hepatocytes have been developed and are being used for metabolism studies by Life Technologies and others. At P&G, fresh carp hepatocytes have been demonstrated to also have metabolizing capabilities. To facilitate studies with carp, P&G and Life Technologies located around the United States collaborated to develop cryopreserved carp hepatocytes. These cells were tested for their viability and metabolizing capabilities over time using various enzyme catalyzing systems.

Methods and Development

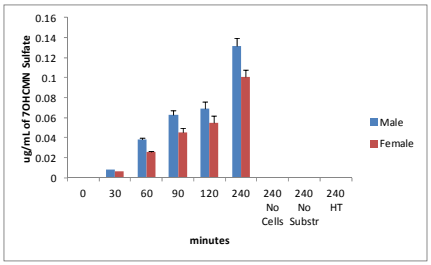
Common carp (*Cyprinus carpio*) were maintained under a natural photoperiod at approximately 22°C at P&G facilities. Fish were acclimated for at least 2 weeks prior to use. Fish were euthanized with MS-222. Carp have a non-capsulated liver that is intertwined with and attached to other organs so it was difficult to perform perfusion as used with mammals and trout to remove blood. Over multiple samplings, our sampling procedure evolved as described.

- **Previously, for fresh carp hepatocytes , P&G had done liver excision and *ex vivo* digestion of liver tissue with collagenase and trypsin** – *Fish culturing; 1-2 fish per test (no pooling)*
- ***In vivo* perfusion of liver with buffer to clear blood resulted in moderate success** – *difficulty with finding/ using veins and blood clotting*
- **Injection of heparin into caudal vein to facilitate clearing of blood before perfusion** – *Greater success in blood clearing ; good isolation*
- **No perfusion, realized that heparin injection resulted in blood washing out of liver into media during shipping** –
- *Less difficult and less time; good clearing and isolation*

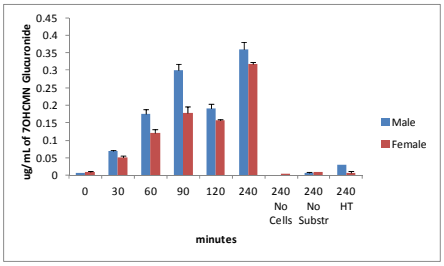
All liver perfusions/excisions were performed at P&G in Cincinnati, Ohio The tissue was placed in cold FRS and transported immediately to Life Technologies in Durham, N. Carolina packed in ice. Tissues were separated by sex for processing. All were washed with buffer by gentle rocking and centrifugation. Digestion followed with Type IV collagenase; after 30 minutes cells are removed and fresh collagenase was added to tissue for an additional 30 min (19.7°C). Isolated hepatocytes were cryopreserved with the method routinely used for mammalian and trout hepatocytes. Cells were then characterized at Life Technologies in Austin, Texas.

Results

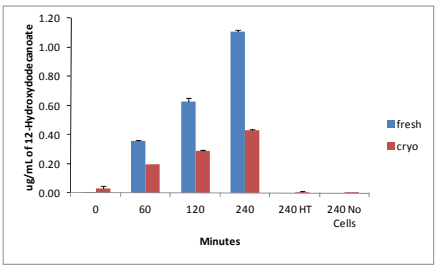
Sulfonation in Male vs. Female Fresh Carp Hepatocytes



Glucuronidation in Male vs. Female Fresh Carp Hepatocytes

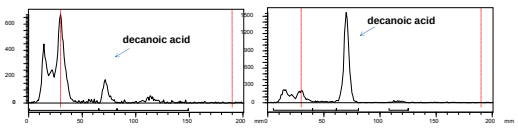


Lauric Acid Hydroxylation in Fresh vs. Cryopreserved



Decanoic Acid Metabolism

Fresh **Cryopreserved**



14C-Decanoic acid is routinely used by P&G as a positive control for metabolism. Incubations at 23°C for 120 minutes; Assay by TLC-RAD

Conclusions

- **Heparin facilitates the blood clearing from the liver tissue**
- **Cell viability after isolation (before cryopreservation) ranged form 66 – 99% and were graded B or above.**
- **Carp hepatocytes have been successfully cryopreserved with excellent viability**
- **Carp male hepatocytes have greater enzymatic capabilities than female**
- **The cryopreserved hepatocytes have less enzymatic capability to but indicate measurable activity**

Next Steps

- **Cryopreservation method will be optimized for carp**
- **Comparison of cryopreserved and fresh hepatocytes will be continued with various test chemicals**

Acknowledgments

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