End of Project Report - December 11, 1996

Principal Investigator: O.E. Rhodes, Jr.

Institution: Purdue University

Project Title: Assessment of Genetic Damage in Bullhead Catfish in a Contaminated Reservoir in the Great Lakes Basin Region Using the Alkaline DNA Unwinding Method

Project Objectives

The primary goal of this research is to quantify differences in molecular level genetic damage to bullhead catfish in the heavily contaminated Lake George reservoir versus levels of damage in catfish from the Jasper Pulaski reservoir, an area of virtually uncontaminated water quality, using the alkaline DNA unwinding method. DNA unwinding is a method of quantifying single strand DNA breaks. Elevated numbers of single strand DNA breaks and subsequent reductions in DNA repair efficiency, caused by toxicants such as heavy metals, can lead to increased rates of cell abnormalities and carcinogenesis. In turn, evidence of such DNA damage may be indicative of the presence of environmental toxicants that pose a health risk to consumers of fish such as humans and wetland wildlife species. A second goal of this research is to determine the utility, based on measures of molecular level genetic damage, of using bullhead catfish as a sentinel species for assessment of toxicological risks to indigenous fish species and consumers of fish in the Great Lakes and Great Lakes Basin region.

Progress Report

I am pleased to report that we have all necessary equipment and supplies in place to complete this Sea Grant funded pilot project and have begun our preliminary examinations of the DNA unwinding procedure in bullhead catfish. Unfortunately, our collaborators experienced some difficulties in obtaining catfish samples and delivering them to our lab and we did not receive our control samples until the first week of November 1996. However, having now received all of the tissues from our study organisms, a Ph.D. level graduate student and I are optimizing the alkaline DNA unwinding procedure for bullhead catfish. I anticipate that we will begin to analyze our study samples in January of 1997 and that the project will be completed in April 1997.

Results To Date

Thus far in the pilot project we have identified several critical areas in the DNA unwinding procedure where sample handling and DNA extraction protocols must be performed with great care. First, it is clear that several grams of tissue must be collected to ensure that large quantities of DNA can be obtained for analysis. This has been a problem to date in that our collaborators failed to collect enough tissue from some of our study animals to obtain adequate amounts of DNA for subsequent examinations. In order to rectify this problem we have now modified our DNA extraction protocols, using commercially available kits, to obtain as high a yield of good quality DNA as possible from every milligram of sample material available to us. This experience will ensure that future sample collection activities for our genotoxicological analyses will properly address our total tissue volume needs. In addition, the switch to commercially produced kits for DNA extraction was also motivated by our discovery that standard protocols for Phenol-Chloroform extractions of DNA from catfish liver often generated poor quality DNA for analysis in our laboratory. Thus, we have moved to commercial DNA extraction kits that provide both a high yield of DNA as well as high quality DNA. Currently, we are extracting DNA of excellent quality from gill tissues of our study catfish in hopes of also examining this tissue type for evidence of DNA damage within the framework of this and future research efforts in my laboratory.

Anticipated Results

It is anticipated that at least 1 publication will result from the research undertaken in this pilot project. In addition, this preliminary work has facilitated the development of linkages between our laboratory at Purdue University and research laboratories performing similar types of genotoxicological assays in other regions of the United States.