WATER QUALITY

Development of a Biological Indicator of Exposure to PAHs in Fishes of the Grand Calumet River System

Final Report

We have developed a rapid, sensitive assay for detecting the exposure of fishes to carcinogenic polycyclic aromatic hydrocarbons (PAHs) in contaminated aquatic ecosystems. Since fish metabolize PAHs and excrete them as metabolites, assays that quantify the concentration of enzymes involved in the metabolic breakdown have been used to quantify the exposure of fish to PAHs. Using caged brown bullhead at sites with PAH-contaminated sediments, we quantified the concentration of a protein (CYP1A) that is involved in the metabolic breakdown of PAH compounds. The concentration of CYP1A protein in caged brown bullheads appears to be sensitive to the amount of exposure that the fish received. We compared the concentration of the CYP1A protein to the concentration of metabolites in the same fish and it appears that the CYP1A protein may be a more sensitive measure of exposure to PAHs than metabolites.

The Grand Calumet River has sediments that are severely contaminated with pollutants and several sites are scheduled for dredging. To determine if this sediment remediation is successful, we need to know how "clean" the sediments are after dredging. Therefore, sensitive measures of PAH exposure could be valuable for assessing the effectiveness of dredging contaminated sediments. The CYP1A indicator in caged brown bullhead could be a valuable part of monitoring efforts for all remediation projects where PAH-contaminated sediments are a concern.

Objectives

We have developed a rapid, sensitive assay for detecting the exposure of fishes to carcinogenic polycyclic aromatic hydrocarbons (PAHs) in contaminated aquatic ecosystems. Since fish metabolize PAHs and excrete them as metabolites, assays that quantify the concentration of enzymes involved in the metabolic breakdown have been used to quantify the exposure of fish to PAHs. Using caged brown bullhead at sites with PAH-contaminated sediments, we quantified the concentration of a protein (CYP1A) that is involved in the metabolic breakdown of PAH compounds. The concentration of CYP1A protein in caged brown bullheads appears to be sensitive to the amount of exposure that the fish received. We compared the concentration of the CYP1A protein to the concentration of metabolites in the same fish and it appears that the CYP1A protein may be a more sensitive measure of exposure to PAHs than metabolites.

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Summary of Progress

During 1999-2000, we completed the development of the enzyme-linked immunosorbent assay (ELISA) and western blot protocol using a new monoclonal antibody for CYP1A. These assays were used to quantify the induction of CYP1A protein in brown bullhead catfish collected from caged exposures and from native yellow bullheads of the Grand Calumet Lagoons. We are currently analyzing data and preparing manuscripts for publication.

Accomplishments

The potential application of our results will come from using the caged bullhead-CYP1A protein quantification to assay the exposure of fish to PAH-contaminated sediments in polluted aquatic ecosystems. The biomarker technique could be valuable for determining the effectiveness of dredging contaminated sediments, especially in the Grand Calumet River.

Narrative Report

The objective of the project during 1999-2000 was to complete ELISA and western blot protocols using a new monoclonal CYP1A antibody and to assess the sensitivity of the CYP1A biological indicator assay to known PAH concentrations in sediments of the Grand Calumet Lagoons. The western blot and ELISA protocols used a monoclonal antibody (Biosense Laboratories) to quantify the relative concentration of CYP1A protein in brown bullhead. We sampled livers of caged brown bullhead catfish at three sites in the Grand Calumet Lagoons with high (WL5), intermediate (WL4) and no (WL2) concentrations of PAHs in sediments. Additionally, we sampled liver tissue from native yellow bullheads from the Grand Calumet Lagoons. Using a western blot assay and ELISA, we quantified the relative concentration of CYP1A protein in microsomal fractions of bullhead livers. To assess the sensitivity of the CYP1A assay, we correlated the protein concentrations of CYP1A with exposure to sediments (sites in the lagoon) and to concentrations of PAH metabolites in bile from livers of the same fish.

The concentration of CYP1A protein in livers of caged brown bullheads varied among sites and over the exposure period. CYP1A concentrations increased at all three sites in the Grand Calumet Lagoons over the 10-week exposure period. At 10 weeks of exposure the relative concentration of CYP1A protein was greater in fish from WL4 and WL5 than in those from WL2 and our control fish maintained in the laboratory. The range of CYP1A protein concentrations in native yellow bullhead was within the range of concentrations of caged brown bullheads, indicating that these fish were exposed to PAHs in the lagoons. The trends in concentration of CYP1A protein, determined by the ELISA and western blot analysis, differed from that of concentrations of PAH metabolites from the same fish. Whereas metabolite concentrations were initially high and leveled off, the CYP1A protein concentrations continued to increase over the 10-week exposure period. Metabolism and physiological processes may explain the differences in response of metabolites and CYP1A concentrations. Because the response of the CYP1A protein was consistent with exposure site and duration of exposure, ELISA and western blot assays for CYP1A appear to be a sensitive measure of exposure to PAHs in fish.

Brief Summary

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- **Completion Date**:February 28, 2000
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