Report for 2002KY1B: Evaluating site remediation success using a sensitive biochemical indicator in fish

- Articles in Refereed Scientific Journals:
    - Brammel, B.F., J.S. McLain, J. T. Oris, and A.A. Elskus, Submitted, Use of Rainbow Trout as a Biomonitoring Tool for Evaluating the Effectiveness of a PCB Remediation Project, Environmental Toxicology and Chemistry.
- Conference Proceedings:
    - Brammel, B. F., D. J. Price, X. Arzuaga, W. J. Birge, and A. A. Elskus, 2003, CYP1A Expression in Longear Sunfish as a Biomarker of PCB Exposure, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 1.
    - B. F. Brammel, J. S. McLain, J. T. Oris, and A. A. Elskus, 2003, Use of rainbow trout as a biomonitor for evaluating the effectiveness of a PCB remediation project, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 3.
    - Arzuaga, X. And A. A. Elskus, 2003, Resistance to Polychlorinated Biphenyl (PCB) and Polyaromatic Hydrocarbon (PAH) Mediated Induction of CYP1A in a PCB Resistant Population of Fundulus Heteroclitus, 2003, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 7.
    - Price, D. J. and W. J. Birge, 2003, The Stoneroller Minnow as an In Situ Monitor of PCB Contamination in Freshwater Systems, in Proceedings Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, p. 5.

Report Follows:

Problem and Research Objectives

Polychlorinated biphenyls (PCBs) are ubiquitous aquatic pollutants with significant toxic effects in both humans and fish, including altered reproduction, immunosuppression, carcinogenesis, and neurotoxicity. Significant levels of environmental PCBs in Kentucky have led to the posting of fish advisories in several waterways (Kentucky Division of Water). The focus of the present study was the Town Branch-Mud River (TB/MR) system in Kentucky, a PCB-contaminated site currently under remediation. This project addressed several needs identified by the *Water Science and Technology Board* [3], including the need to understand the impact of contaminants on higher organisms, to monitor the time course of recovery following contamination, and to evaluate the effectiveness of management efforts to improve water quality. Water quality in Kentucky is evaluated based on contaminant concentrations in water, sediment or biota, and/or on biological indices of species diversity. Contaminant concentrations alone provide no information on organism response, and diversity indices do not distinguish between response to contaminants, habitat disturbance, or natural stressors. For example, there is no information on whether exposure to PCBs in the TB/MR system is producing sublethal effects in fish populations in that system, and/or whether present remediation efforts are reducing those effects. The enzyme, CYP1A, is strongly and rapidly induced in animals exposed to toxic organic pollutants, including PCBs. We hypothesized that CYP1A levels in TB/MR resident fish reflect organic contaminant levels at their site of collection. Research objectives were: 1) To use CYP1A levels in caged fish to evaluate the effectiveness of bioremediation efforts in the TB/MR system, and 2) to determine if CYP1A levels in resident fish in the TB/MR system reflect expected habitat contamination level.

Methodology

As our first step in evaluating PCB-remediation success in the TB/MR system, we measured CYP1A response in naive fish caged in the system. This was necessary because resident fish may not adequately reflect conditions at the site of capture as some species may move between remediated and unremediated areas, while others may have developed resistance to PCB induction of CYP1A. For these reasons, we first evaluated the CYP1A1 response of hatchery-reared, rainbow trout caged at each study site to provide a site-to-site evaluation of the effectiveness of bioremediation efforts in the TB/MR system. Rainbow trout were caged at reference, remediated and unremediated sites in the TB/MR waterway for two weeks in Spring 2002 and evaluated for CYP1A expression level (messenger RNA, CYP1A protein, CYP1A enzyme activity), and gut contents were analyzed to determine what organisms were consumed by the caged trout. To evaluate the response of resident fish species, we measured hepatic CYP1A expression in fish collected from reference, remediated and unremediated sites in the TB/MR waterway. Species were selected based on known sensitivity to CYP1A inducers and on our ability to collect statistically-sufficient numbers of individuals at each site to distinguish site differences in CYP1A response. Resident green sunfish, longear sunfish, creek chub, yellow bullhead, rock bass, spotted bass, and common carp were collected in Fall 2002 and PCB body burdens and CYP1A1 activity evaluated.

Principal Findings and Significance

Our first principal finding suggests that bioremediation efforts to remove polychlorinated biphenyl contamination in the Town Branch waterway were not completely successful. We have three pieces of evidence supporting this conclusion; biological response in fish caged at the site, PCB levels in sediments at the site, and PCB levels in the flesh of resident fish from this site. Rainbow trout caged at the remediated Town Branch site had significantly elevated levels of the biomarker enzyme, CYP1A1, in both their gills and their liver, relative to fish caged upstream at a reference site. Elevated CYP1A expression occurs in response to exposure to inducing chemicals, typically PAHs and PCBs [5, 18]. That CYP1A levels were elevated in the gills of the caged fish, together with evidence of low food consumption by these fish, suggests exposure to contaminants in this system is waterborne. Although not part of our original proposal, we also evaluated levels of PCBs in sediments and resident fish at these sites. Samples of sediment and resident fish were collected by doctoral students in my laboratory, Ben Brammell and Xabier Arzuaga, and by David Price, a doctoral candidate in the laboratory of Dr.Wesley Birge, (Dept of Biology at UK), and analyzed by David Price. These analyses indicated that PCBs are present and bioavailable in the TB/MR system. Highly elevated levels of PCBs remain in the sediments from the remediated section of Town Branch (up to 40 ppm total Aroclor dry wt basis) and have been bioaccumulated to extraordinarily high levels by resident fish collected at this site (up to 98 ppm wet edible flesh, with a median concentration of 24 ppm). These levels are at the high end of those measured in fish from New Bedford Harbor, Massachusetts, considered one of the most highly contaminated PCB Superfund sites in the US. Median PCB levels in edible flesh ranged from 5.5 - 7.4 ppm for New Bedford Harbor flounder species up to 24 ppm median PCB for American eel [17]. PCB levels in fish from the unremediated section of the Mud River ranged from non-detectable up to 20 ppm (median 3.89 ppm), indicating that this site is also a significant source of PCBs for resident species. In comparison, Town Branch reference fish collected upstream of the remediated site had PCB body burdens that were up to 100 times lower than fish from the remediated site, ranging from non-detectable to < 3 ppm (median = 0.56 ppm). Our second main finding was that resident species appear to have developed resistance to at least some of the biological effects of PCBs. Since inducing chemicals are present in this system, as demonstrated by induction of CYP1A in the caged trout, lack of induction in resident species suggests they have developed resistance to the contaminants present in this habitat. Moreover, their PCB body burdens are similar to, and in some cases well above, those known to induce CYP1A in other fish species [1, 8]. The ability of fish to develop resistance to halogenated hydrocarbons, including PCBs and dioxins, has been demonstrated in resident populations of fish in several contaminated sites [2, 6, 7, 12, 15, 16]. To confirm our suspicion that resident species have acquired resistance to PCBs, we collected green sunfish and yellow bullhead catfish in March 2003 and are currently depurating them for use in challenge experiments with PCBs to be conducted in July 2003 (half life of PCBs in fish is 4 months, [13]). By treating depurated resident fish with PCBs we expect to find little to no induction of CYP1A if the fish have developed resistance relative to similarly treated reference fish. In addition to the lack of CYP1A1 inducibility in resistant fish, these animals have also consistently demonstrated resistance to the harmful effects of these chemicals (reviewed in [6, 9]). If resident fish demonstrate resistance to CYP1A induction in our PCB challenge experiments, we will conduct further studies to explore whether these fish have also developed resistance to the toxic effects of PCBs, specifically, the well-characterized ability of PCBs to alter thyroid hormones [4, 10, 11, 14]. To address this question, we plan to conduct studies examining PCB effects on thyroid hormone in resistant fish under our 2003/2004 USGS 104B grant.

References cited

1. Addison RF, Zinck ME and Willis DE, Induction of hepatic mixed-function oxidase (MF) enzymes in trout (Salvelinus fontinalis) by feeding Aroclor 1254 or 3-methylcholanthrene. *Comparative Biochemistry and Physiology* **61C:** 323-325, 1978.

2. Bello SM, Franks DG, Stegeman JJ and Hahn ME, Acquired resistance to Ah receptor agonists in a population of Atlantic killifish *(Fundulus heteroclitu*s) inhabiting a marine superfund site: In vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes. *Toxicol. Sci.* **6**0(1**):** 77-91, 2001.

3. Board WSaT, Envisioning the agenda for water resources research in the 21st century. pp. 49 pp. National Research Council, Washington, D.C., 2000/1.

4. Brown SB, Fisk AT, Brown M, Villella M, Muir DCG, Evans RE, Lockhart WL, Metner DA and Cooley HM, Dietary accumulation and biochemical responses of juvenile rainbow trout (Oncorhynchus mykiss) to 3,3',4,4',5-pentachlorobiphenyl (PCB 126). *Aquatic Toxicology* **59:** 139-152, 2002.

5. Buchneli T and Fent K, Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. *Critical Reviews in Environmental Science and Technology* **25:** 201-268, 1995.

6. Elskus AA, Toxicant resistance in wildlife: fish populations. In: *PCBs: Recent Advances in Environmental Toxicology and Health Effects* (Eds. Robertson LW and Hansen LG), pp. 273-276. The University Press of Kentucky, Lexington, 2001.

7. Elskus AA, Monosson E, McElroy AE, Stegeman JJ and Woltering DS, Altered CYP1A expression in Fundulus heteroclitus adults and larvae: a sign of pollutant resistance? *Aquatic Toxicology* **4**5(2-3**):** 99-113, 1999.

8. Elskus AA and Stegeman JJ, Induced cytochrome P-450 in *Fundulus heteroclitus* associated with environmental contamination by polychlorinated biphenyls and polynuclear aromatic hydrocarbons. *Mar. Environ. Res.* **27:** 31-50, 1989.

9. Hahn M, Mechanisms of innate and aquired resistance to dioxin-like compounds. *Reviews in Toxicology* **2:** 395-443, 1998.

10. Leatherland JF and Sonstegard RA, Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon (Oncorhynchus kisutch), by dietary mirex and PCBs. *Journal of the Fisheries Research Board of Canada* **35:** 1285-1289, 1978.

11. Leatherland JF and Sonstegard RA, Effects of dietary Mirex and PCBs in combination with food deprivation and testosterone administration on thyroid activity and bioaccumulation of organoclorines in rainbow trout Salmo gairdneri Richardson. *Journal of Fish Diseases* **3:** 115-124, 1980.

12. Nacci D, Coiro L, Champlin D, Jayaraman S, McKinney R, Gleason TR, Munns WR, Specker JL and Cooper KR, Adaptations of wild populations of the estuarine fish *Fundulus heteroclitus* to persistent environmental contaminants. *Mar. Biol.* **13**4(1**):** 9-17,

1999.
13. Niimi AJ and Oliver BG, Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout *(Salmo gairdner*i). *Can. J. Fish. Aquat. Sci.* **40:** 1388-1394, 1983.
14. Porterfield SP, Vulnerability of the Developing Brain to Thyroid Abnormalities - Environmental Insults to the Thyroid System. *Environmental Health Perspectives* **102:** 125-130, 1994.
15. Prince R and Cooper KR, Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on chemically impacted and nonimpacted subpopulations of Fundulus heteroclitus: I. TCDD toxicity. *Environ. Toxicol. Chem.* **14:** 579-587, 1995a.
16. Prince R and Cooper KR, Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on chemically impacted and nonimpacted subpopulations of Fundulus heteroclitus: II. metabolic considerations. *Environ. Toxicol. Chem.* **14:** 589-595, 1995b.
17. Weaver G, PCB contamination in and around New Bedford, Mass. *Environ. Sci. Technol.* **18:** 22A-27A, 1984.
18. Whyte JJ, Jung RE, Schmitt CJ and Tillitt DE, Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Critical Reviews in Toxicology* **30(**4**):** 347-570, 2000.