Kentucky Water Resources Research Institute
Annual Technical Report
FY 2003

Introduction

The FY2003 Annual Technical Report for Kentucky consolidates reporting requirements of the Section 104(b) base grant award in a single technical report that includes: 1) a synopsis of each research project supported during the period, 2) a list of related reports, 3) a description of information transfer activities, 4) a sumarry of student support during the reporting period, and 5) notable achievements and awards during the year.

Research Program

The activities supported by Section 104(b) and the required matching are interwoven into the Kentucky Water Resources Research Institute's total program. Other elements of the Institute's program during FY2003 included: 1) the Environmental Systems Graduate Certificate, 2) the Environmental Protection Scholarship Program, and 3) Research, Service and Technolgy Transfer Activites funded by other sources.

Memorandum of Agreement projects with the Kentucky Division of Water included TMDL development for pH, nutrients, and pathogens in several Kentucky streams. Additional projects were funded by the Natural Resources and Environmental Protection Cabinet (Environmental Protection Scholarship), the Kentucky Cabinet for Health Services (Technical Support for the Maxey Flats Nuclear Disposal Site and the Paducah Gaseous Diffusion Plant), the Kentucky Deparmtnet of Military Affairs (Technical Support for Environmental Construction and Solid Waste Management), the Kentucky River Authority (Watershed Managemnt Services and Water Resources Planning), the National Institute of Environmental Health (Superfund Public Outreach Program), and east Kentucky PRIDE (Personal Responsibility in a Desirable Environment) Water Quality Assessment.

In addition, the Institute was instrumental in the development of the Kentucky Research Consortium for Energy and the Environment, a collaborative program of the University of Kentucky, Murray State University, and the University of Louisville. Creation of the consortium was directed by Lindell Ormsbee, Associate Director of the Kentucky Water Resources Research Institute. $5 million in Federal Funds (Department of Energy) have been secured for research on environmental assessment and cleanup at the Paducah Gaseous Diffusion Plant over the next several years.

Five research projects were selected for support provided by the 104(b) FY2003 program funding. Investigators included faculty at the University of Kentucky (Elskus, Coyne, and Yan), Western Kentucky University (Webb), and Northern Kentucky University (Boateng). Project synopses for the five investigations follow.

Biochemical and hormonal effects of incomplete site remediation: evaluating resident fish species

Basic Information

Title:	Biochemical and hormonal effects of incomplete site remediation: evaluating resident fish species
Project Number:	2003KY17B
Start Date:	3/1/2003
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Kentucky 6th
Research Category:	Biological Sciences
Focus Category:	Toxic Substances, Water Quality, Ecology
Descriptors:	PCBs, Endocrine Disruptors, Bioindicators
Principal Investigators:	Adria Anne Elskus

Publication

1. Brammell, B.F., D.J. Price, W.J. Birge, and A.A. Elskus, 2004, Apparent lack of CYP1A1 response to high body burdens in fish from a chronically contaminated PCB site, Marine Environmental Research , 58(2004), 251-255.
2. Brammel, B.F., D.J. Price, W.J. Birge, and A.A. Elskus, 2004, Pollutant response in species inhabiting chronically contaminated habitats: Two varieties of resistance?, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentukcy Water Resources Research Institute, Lexington, Kentucky, 3-4.
3. Arzuaga, Xabier, and Adria Elsksus, 2004, AHR/CYP1A mediates PCB induction of teratogenesis and CYP1A, but not oxidative stress: An in ovo vetebrate model, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 51-52.
4. Price, D.J., and W.J. Birge, 2004, Determining bioavilable PCB fractions in freshwater stream sediments using PCB body burdens in fish sentinel monitors, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 53-54.

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 Kentucky 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 work addresses several needs 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.

Results of our previous grant (2002KY1B) demonstrate that remediation of the PCB-contaminated TB/MR system has not been wholly successful. We found elevated levels of the biomarker enzyme, CYP1A1, in both gill and liver of trout caged in remediated sections of the TB/MR waterway, and preliminary data on PCB levels in sediments and resident fish confirmed the presence and bioavailability of these chemicals. Chronic exposure to PCBs can lead to the development of PCB-resistance in vertebrates, including fish, an effect associated with suppressed CYP1A1 expression. Exposure to PCBs can also disrupt endocrine systems in fish, including altering levels of thyroid hormone, a hormone involved in fish growth, reproduction, and the ability to cope with environmental stress. Both chemical resistance and endocrine disruption have population-level effects. Both CYP1A1 and UDP-GT, a key enzyme in TH metabolism and one that is altered by PCBs, are regulated through the aryl hydrocarbon receptor, suggesting there may be a common link between PCB effects on CYP1A1 and TH. We proposed to examine whether resident fish in the TB/MR have developed resistance to PCBs and whether resistant resident species also demonstrate resistance to disruption of thyroid hormone by PCBs

Methodology

Our first task was to make initial characterizations of resident populations in the TB/MR system as either PCB resistant or responsive, based on CYP1A1 expression level and PCB body burdens. In Fall 2002 and throughout 2003 we collected resident fish from the same TB/MR remediated, unremediated and reference sites we had used in our trout caging studies of 2002/3. Fishing Creek served as an additional reference site. Among the fish collected (green sunfish, longear sunfish, creek chub, yellow bullhead, rock bass, spotted bass, and common carp) were species known to develop resistance and those known to remain responsive when chronically exposed to PCBs [8, 19, 20]. We measured PCB body burdens and CYP1A1 activity in populations of these species from all three sites. Based on their CYP1A1 expression level relative to their PCB body burdens we classified resident populations as potentially resistant or responsive.

Our second task was to confirm PCB resistance and conduct studies to determine if PCB resistance was manifested by UDP-GT, another enzyme altered in resistant

mammals, and conferred resistance to the disruption of thyroid hormone by PCBs. To confirm resistance, we conducted laboratory-based PCB-challenge experiments and measured CYP1A1 response, a well-recognized test for resistance [4, 10]. Those populations that failed to respond to laboratory exposure to PCBs as measured by CYP1A1 induction were designated PCB-resistant and those that responded were designated PCB-responsive. Two of these resident populations, one resistant and one responsive, were then used in further laboratory PCB dose-response experiments and hepatic CYP1A1, UDP-GT and plasma thyroid hormone levels measured to evaluate possible correlations among these parameters and resistance to PCBs.

Principle Findings and Significance

Our first principle finding was that PCBs are present and bioavailable in the TB/MR system[1]. We found resident species in the remediated area of the Town Branch/Mud River system have PCB body burdens that are similar to, and in some cases well above, those known to induce CYP1A1 in other fish species [1, 11]. Highly elevated levels of PCBs remain in the sediments from the remediated section of Town Branch (up to 45 ppm total Aroclor dry wt basis, [6] 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; [6]. 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 principle finding was that resident fish in PCB-impacted areas of the TB/MR system have developed resistance to at least some of the biological effects of PCBs. Based on a comparison of PCB body burdens and CYP1A1 levels in fresh-caught fish from reference and impacted sites, we initially characterized 6 populations (5 species) as likely to be PCB-resistant in the TB/MR system: longear sunfish and green sunfish from the TB remediated site, and yellow bullhead, spotted bass, rock bass, and longear sunfish from the unremediated MR site. These populations exhibited a lack of CYP1A1 induction, despite elevated body burdens of CYP1A1 inducing PCBs. The only species that appears not to have developed PCB-resistance in the PCB-impacted regions of the TB/MR system are creek chubs. In creek chubs from the unremediated TB site, elevated body burdens of PCBs were accompanied by elevated CYP1A1 expression, as would be expected in fish that retain responsiveness to the CYP1A1-inducing effects of PCBs and thus show the expected increase in CYP1A1 with increased PCB tissue levels .

[1] Some of these data were cited in our 2002/3 report.

To confirm our initial designations of PCB responsivity, and to identify two species for further enzyme and hormone analyses, we collected resident species from TB and MR and held them for 4 months in clean, dechlorinated water. This extensive depuration period was necessary to avoid confounding our challenge experiments by using fish with high PCB body burdens (half-life of PCBs in fish is 4 months [13]). For example, after an 18 week depuration period, PCB levels in edible flesh of yellow bullhead from the TB remediated site averaged 3.32 ± 0.92 (SE) ppm (n=5) compared to freshly caught longear sunfish, green sunfish and creek chub from this site whose body burdens ranged from 16 - 75 ppm. After the depuration period, we conducted laboratory-based PCB challenge experiments on TB yellow bullheads, TB green sunfish, TB creek chubs and MR spotted bass, and on corresponding species from reference sites. Because of the large numbers of fish involved (>100), experiments were conducted sequentially over several months.

As expected, yellow bullhead from reference, but not from the TB remediated site, responded to induction of CYP1A1 in the PCB laboratory challenge experiments. This confirmed our initial designations of responsive and resistant, respectively, for these resident populations, and is in keeping with the demonstrated ability of fish to develop resistance to halogenated hydrocarbons, including PCBs [9]. Green sunfish populations from both the reference and the unremediated TB sites failed to respond to laboratory PCB exposure. This finding was not unexpected as we had preliminary data on another *Lepomis* species, longear sunfish, that indicated that at least some members of this genus appear to have a natural, genetic resistance to CYP1A1 induction by PCBs [7] indicating that *Lepomis* species may not be useful biomonitoring organisms for PCBs.

Spotted bass proved difficult to work with in the laboratory, developing infections during the long-depuration period that resulted in high mortality rates. Seventy-five percent of the MR unremediated site fish, and 50% of the reference MR site fish died during depuration. After 14 weeks (rather than the full 16), we conducted PCB challenge experiments, but as expected, experimental mortality was high (33%) and the small replicate number for the depurated MR fish (n=2) made the results unreliable.

We are currently conducting PCB-challenge experiments to determine if creek chub, unlike yellow bullhead and green sunfish from the TB remediated site site, have failed to develop PCB resistance. Once we have confirmed the responsivity of the TB creek chub, we will commence the final biochemcial analyses for this project using the killifish, *Fundulus heteroclitus*. The purpose of this final experiment (conducted in Fall 2003) was to look at the relationship between CYP1A1, UDP-GT and thyroid hormone, and whether PCB resistance alters this relationship. The ability of halogenated aromatic hydrocarbons, including PCBs, to alter thyroid hormones, possibly by altering the activity of UDP-GT, has been reported in mammals [12, 16, 18]. We chose *Fundulus heteroclitus* for these experiments because PCB-resistant and -responsive populations have been well-characterized by our laboratory and others [2, 3, 10, 14, 15].

The current clean-up efforts in the TB/MR system provide an unparalleled opportunity to evaluate the effects of chronic chemical exposure and site remediation on local populations. The results of our present studies provide significant insights into current conditions in the TB/MR system and the response of resident fish to those conditions.

Specifically, the findings of our 2003/4 studies demonstrate that:

(1) it is likely that the duration of exposure, rather than the level of exposure, is what provokes the development of resistance to PCBs; MR fish developed resistance even though their PCB body burdens (1.2 - 7.4 ppm) were far lower than those of resistant TB fish (16 - 75 ppm).

(2) even under the same exposure regime, not all fish species appear to develop resistance. TB remediated site yellow bullhead, but apparently not creek chub (confirmation experiment in progress), have developed resistance to CYP1A1 induction by PCBs.

(3) the severity and extent of pollution problems in the TB/MR system, most significantly the presence of extraordinarily high levels of PCBs in the TB remediated site, suggest either insufficient remediation or continued input of PCBs to the system. We suggest that further remediation efforts be halted until the present source of PCBs to this system can be identified.

Chemical resistance in fish is a recently recognized phenomenon and almost nothing is known regarding the consequences in affected populations. Because resistance has 'costs', tolerant populations may demonstrate heightened susceptibility to further stressors (e.g. site remediation), resulting in unexpected population crashes during cleanup efforts. The final analyses of our 2003/4 study should reveal whether resident populations resistant to CYP1A1 induction are likely to be resistant to the hormone disrupting effects of PCBs and/or whether there is a tradeoff, whereby resistant fish lose the ability to retain normal hormone function.

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. Arzuaga X, Calcaño W and Elskus AA, The DNA de-methylating agent, 5-azacytidine, does not restore CYP1A induction in PCB resistant Newark Bay killifish *(Fundulus heteroclitus*). *Marine Environmental Research*, in press.
3. Arzuaga X and Elskus A, Evidence for resistance to benzo a pyrene and 3,4,3 ' 4 '- tetrachlorobiphenyl in a chronically polluted Fundulus heteroclitus population. *Marine Environmental Research* **54**(3-5)**:** 247-251, 2002.
4. Bello SM, Franks DG, Stegeman JJ and Hahn ME, Acquired resistance to Ah receptor agonists in a population of Atlantic killifish (Fundulus heteroclitus) inhabiting a marine superfund site: In vivo and in vitro studies on the inducibility of xenobiotic metabolizing enzymes. *Toxicological Sciences* **60**(1)**:** 77-91, 2001.
5. Water Science and Technology Board, Envisioning the agenda for water resources research in the 21st century. 49 pp. National Research Council, Washington, D.C., 2000/1.
6. Brammell BF, McClain JS, Oris JT, Price DJ, Birge WJ and Elskus AA, CYP1A1 expression in caged rainbow trout discriminates among sites with varying degrees of PCB contamination. submitted.
7. Brammell BF, Price DJ, Arzuaga X, Birge WJ and Elskus AA, CYP1A expression in longear sunfish as a biomarker of PCB exposure. In: *23rd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), P3382002*.
8. Brammell BF, Price DJ, Birge WJ and Elskus AA, Apparent lack of CYP1A response to high PCB body burdens in fish from a chronically contaminated PCB site. *Marine Environmental Research*, in press.
9. 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.
10. 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* **45**(2-3)**:** 99-113, 1999.
11. 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.
12. Kohn M, Sewall C, Lucier GW and Portier C, A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol and Applied Pharmacology* **165:** 29-48, 1996.
13. Niimi AJ and Oliver BG, Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci.* **40:** 1388-1394, 1983.
14. 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.

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: II. metabolic considerations. *Environ. Toxicol. Chem.* **14:** 589-595, 1995b.
16. Schuur A, Boekhorst F, Brower A and Visser T, Extrathyroidal effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on thyroid hormone turnover in male Sprague-Dawley rats. *Endocrinology* **138:** 3727-3734, 1997.
17. Weaver G, PCB contamination in and around New Bedford, Mass. *Environ. Sci. Technol.* **18:** 22A-27A, 1984.
18. Zhou T, Ross DG, DeVito MJ and Crofton KM, Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicological Sciences* **61**(1)**:** 76-82, 2001.
19. Zielinski JF, Responsiveness of Hudson River fish to Cytochrome P4501A inducers and its relationship to genotoxicity. In: *Marine Sciences Research Center*, pp. 100. State University of New York, Stony Brook, 2001.
20. Zielinski JF, McElroy AE and Elskus AA, Evaluating CYP1A resistance and DNA adduct formation in Hudson River fish. In: *Society of Environmental Toxicology and Chemistry 21st Annual Meeting., Nashville, TN November 12-16, 2000,2000*.

Occurrence and Distribution of Mercury in Mammoth Cave National Park - Phase II

Basic Information

Title:	Occurrence and Distribution of Mercury in Mammoth Cave National Park - Phase II
Project Number:	2003KY18B
Start Date:	3/1/2003
End Date:	9/30/2004
Funding Source:	104B
Congressional District:	Kentucky 2nd
Research Category:	Water Quality
Focus Category:	Groundwater, Sediments, Solute Transport
Descriptors:	karst, bioaccumulation, biomagnification
Principal Investigators:	Cathleen Joyce Webb

Publication

1. Berryman, Gretchen E., Sreedevi Dawadi, and Cathleen J. Webb, 2004, Occurrence and distribution of mercury in Mammoth Cave National Park, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 71-72.

Problem and Research Objectives

Atmospheric deposition is the largest single source of mercury (Hg) in a karst aquifer system. Coal naturally contains background levels of mercury, which if burned releases mercury into the atmosphere. The mercury emitted binds to particulate matter in the atmosphere and deposits in that form. Coal-fired power plants are the number one contributor to atmospheric deposition of mercury. As a result of increasing demand for power, over 20 applications for new coal-fired power plants are being considered in the Commonwealth of Kentucky in addition to the 18 power plants in operation. Karst systems such as the South-Central Kentucky karst (SCKK) ecosystem, are limestone based drainage systems that result form high degrees of rock fractures and solubility. As a result, transport of contaminants such as mercury in this type of system can be rapid. Mammoth Cave National Park (MCNP), part of this extensive karst terrain system is affected by the atmospheric deposition of mercury, contributed to by coal burning power plants. There is little current data that can be used to predict the potential impact on MCNP and the karst ecosystem. Determination of mercury levels in MCNP and the factors that affect mercury levels and distribution are important and are being examined.

The scope of this work is to understand the complex physical and geochemical processes that govern the fate and transport of mercury in a karst aquifer system. Mercury mobility in surface water and ground water are of great concern because of toxic effects on the environment. The impacts on aquatic species can be devastating. The Park and the surrounding watershed are home to over 150 species of fish. Mercury is converted by biota to a more toxic form called methyl mercury. Hg is a severe neurotoxin and can impair sensory and motor functions. This toxic metal is bioaccumulated in aquatic organisms and biomagnifies up the food chain when it is transferred from one organism to another. The extent of bioaccumulation in aquatic as well as other animals that make their home at Mammoth Cave is being investigated. Attempts will be made to correlate levels and distribution of mercury in biota, water, and sediment, with levels of mercury measured in the atmosphere.

The study site for this project is the Green River watershed which covers 71,000 km^2 and consists of upper and lower basins. It is part of the SCKK ecosystem. The specific objectives of this effort are to: (1) establish the occurrence and distribution of mercury in the ground water, surface water, and sediments of the study site, including during storm events, (2) examine the bioaccumulation of mercury in surface, subsurface, and aquatic organisms at the Park, (3) investigate the fate and transport of mercury in the SCKK ecosystem, and (4) determine the total maximum daily load of mercury at MCNP.

At this point, objectives 1 and 2 have been actively pursued over a two and half year time span. Experimental work for objective 3 has started, beginning with basic batch experiments. Objective 4 will be calculated when more data has been collected and analyzed for accurate results.

Methodology

Water and sediment samples were collected at the study site from 14 different locations. Water samples from 13 locations were collected monthly and sediment samples from 9 different sites were collected seasonally. The rainfall for each day of sampling and the monthly precipitation totals were also recorded. Mercury analysis was done using Leeman Hydra atomic absorption (Hydra AA) spectrometry. The samples were digested using Leeman Hydra Prep to convert all forms of mercury into elemental mercury.

Procedure for sample digestion:

1) Sample mass determined and placed into a sample cup.
2) 2.25 mL concentrated AquaRegia (3:1 HCl:HNO_3) was added.
3) The sample cup was heated at 95ºC for 2 minutes in a water bath.
4) After the sample cooled, 6.75 mL 5% $KmnO_4$ was added.
5) 4.5 mL deionized (DI) water was added.
6) A second aliquot of DI water (4.5 mL) water was added.
7) The sample was heated in the water bath at 95ºC for 30 minutes.
8) After the sample cooled, 3.6 mL 12%:12% NaCl:hydroxylamine sulfate was added.

Procedure for sample analysis:

The concentration of mercury for water samples (ppt) was determined by fluorescence spectrometry (PSA). The concentrations of mercury for sediment (ppb) and biological (ppm) samples were determined by Leeman Hydra Atomic Absorption. Both methods are similar to the following procedure:

1) The spectrometer was calibrated with standard solutions.
2) The sample was withdrawn by a pump and mixed with the 10% $SnCl_2$:10% HCl solution, which ensures Hg^{2+} was reduced to Hg^o, a volatile species.
3) In a liquid/gas separator, the mixed solution was bubbled by ultra high purity nitrogen and the mercury vapor was carried to pass through a dryer to dehumidify the gaseous mixture. The dry mercury vapor then entered a dual beam optical cell. A mercury lamp controlled by the error signal of the reference beam delivered a stable source of emission at 254 nm. Absorbance by the mercury cold vapor was measured using a solid state detector with a wide dynamic range.

The Hg results for the water and sediment samples were then organized based on sample site location, mercury concentration, and the sampling month. The biological samples included freshwater drum, large mouth bass, *M. Salmoides,* Asiatic clam, *Corbicula fluminea*, and mayflies. These samples were homogenized and analyzed using the same methods as for the sediment samples.

Batch experiments were performed to test the fate of mercury in a limestone system. Two sizes of Kentucky limestone (<0.5 mm and 1-2 mm) were examined to see if limestone is efficient in removing Hg. Round bottom flasks were filled with 170 ppt Hg solution with pH 8 and mixed with varying amounts of limestone. The flasks shook for 48 hours and were analyzed for remaining amounts of mercury.

Principal Findings and Significance

The water, sediment, and biological samples of the park indicate detectable levels of mercury are present in the surface and ground water of the aquifer system as well as in the subsurface organisms that make their home at MCNP. As expected, mercury levels in the water are quite low (0-25 ppt) since mercury preferentially binds to sediments and organic material. Big Spring and Buffalo Creek Spring sampling locations show abundant levels of mercury on average. The higher levels of Hg in the water seem to be evenly dispersed throughout the upstream and downstream parts of the river. The monthly water samples will continue to be collected, analyzed, and interpreted.

Seasonal impacts on mercury levels are observed. In 2003, the Hg levels were higher in the sediment during the summer and higher in the water during the remaining seasons. Precipitation levels may also affect the concentration of mercury that was detected. While there is direct correlation between the amount of mercury and the level of the water, the variation is too great to conclude the exact relationship between precipitation levels and mercury concentration levels. This correlation will continue to be studied as well as a possible link between water temperatures and Hg concentration.

The sediments collected contain mercury levels ranging from 0-3000 ppb. Big Spring and Buffalo Creek Spring consistently show higher levels of mercury in the sediment than they do in the water. There is a general correlation between Hg in the water and Hg in the sediment. Seasonal collection of the sediment samples and their analysis will continue.

Land along the Green River study site is used for agriculture, oil and gas, urban development, and as part of the Mammoth Cave National Park. The water sample locations that contain the highest Hg are found near the Park lands.

The 0.6000 ppm Hg level found in the muscle of the Large Mouth Bass illustrates the potential threat of bioaccumulation of mercury in the food chain. The 0.2330 ppm Hg concentration in the muscle of the freshwater Drum demonstrates biomagnification because these fish are long-lived bottom feeders. The Hg levels in fish in MCNP are comparable to those seen in other species that are considered within the safety limits of the U.S. Food and Drug Administration. Collection of fish will continue, and the biological samples will be expanded using other organisms such as bats and mussels.

The fate and transport of Hg in a karst system can be explored by testing the removal efficiency of limestone on this neurotoxin. Results from batch experiments using fines size (<0.5mm) and 1-2 mm size Kentucky limestone show that limestone is

very efficient in removing inorganic Hg from the water (over 90%). Additional batch experiments will be performed in the future, as well as limestone column studies to further investigate the mobility characteristics of both inorganic and organic Hg.

The levels of Hg found in MCNP demonstrate the potential threat Hg emissions from coal-fired power plants pose on the South-Central Kentucky Karst Ecosystem. Therefore, it is important to continue the study of the fate and transport of Hg in MCNP. As the project continues, methods to prevent the threat of Hg to the Park will be explored.

Does straight pipes removal improve water quality in eastern Kentucky?

Basic Information

Title:	Does straight pipes removal improve water quality in eastern Kentucky?
Project Number:	2003KY19B
Start Date:	3/1/2003
End Date:	2/29/2004
Funding Source:	104B
Congressional District:	Kentucky 6th
Research Category:	Water Quality
Focus Category:	Water Quality, Treatment, Waste Water
Descriptors:	pathogens, septic systems, nutrients
Principal Investigators:	Mark Steven Coyne

Publication

1. Coyne, Mark, George Hofius, Ann Freytag, and Siva Gandhapudi, 2004, Survey of water quality in several Kentucky PRIDE counties impacted by straightpipes, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 43-44.
2. Ritchey, Sloan, and Mark Coyne, 2004, Antibiotic resistance patterns of fecal streptococcus and fecal coliforms as water quality indicators, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 45-56.

Problem and Research Objectives

Straight pipes and other inadequate on-site waste water disposal systems contaminate surface and groundwater resources with various potentially pathogenic microorganisms, reactive organic carbon, and readily available nutrients. These contaminants contribute to making over 1/3 of Kentucky's surface waters unfit for swimming and primary contact. Attempts have been made to reduce watershed contamination in eastern Kentucky by providing financial assistance to individuals, groups, and communities to install effective on-site waste water treatment systems or hook into existing community sewerage systems. These actions should improve water quality in the affected streams, but there is little proof that they do, and little follow-up on the performance of new on-site systems. The objectives of this project were: 1) identify streams affected by straight pipes and other failing on-site systems and characterize the number and composition of fecal bacteria and other water quality parameters; 2) follow changes in water quality parameters after existing systems are removed and replaced by new on-site waste-treatment facilities; and 3) evaluate the performance of new onsite systems.

Due to time constraints and the inability to identify new onsite systems, we did not carry out objective 3 – evaluation of new systems. We were also unable to get cooperation from the KY PRIDE project to facilitate identifying locations that were about to be converted from straight pipes to onsite systems as specified in objective 2. Therefore, we opted to work with our alternative plan for this objective, which was to conduct a pair-wise comparison of streams that were mapped as having numerous straight pipes versus those that were not.

Methodology

There were no essential changes in methodology in the proposed study. Four sites with high and four sites with low straight pipe impact were identified in four counties (Green, Lee, Wolf, and Breathitt). A control watershed in Robinson Forest in which there is no influence of human septage was also identified with cooperation from the Department of Forestry. The control site was sampled with the same frequency as the research sites. The paired stream sites (impacted or unimpacted by straight pipes) were sampled on a weekly basis from October to December 2003. Water and sediment samples were collected and processed within 8 hours for fecal coliforms and fecal streptococci by membrane filtration, biological oxygen demand (BOD), electrical conductivity (EC), pH, inorganic N, TN, and TP. The 'pristine' site in Breathitt Co. (Robinson Forest) was used as a standard against which other sites were compared. Antibiotic resistance profiles were created for a subset of enterococi, fecal coliforms, and indigenous gram-negative bacteria for later use in assessing whether changes in septage load affected stream microbial ecology. Ecological profiles of species and antibiotic resistance patterns in impacted waters were assessed by discriminant analysis. Other statistical analyses will be performed as appropriate.

Principle Findings and Significance

Weekly sampling at each site gave consistent results, and was sensitive to environmental influences that may have affected the measured water quality parameters. No stream met primary contact water standards for fecal bacteria (except for the pristine site) regardless of

whether it was identified as impacted or unimpacted by straight pipes. Most samples in most streams met recreational water standards during the sample period. The data did not allow us to distinguish between the two types of streams without prior knowledge of straight pipe density.

The lack of distinction could have been because of the season of sampling, or mis-classification of streams in terms of pollution level. It may also simply reflect that dilution in these sites and native water properties were a more significant effect on microbial population than incident pollution.

In terms of antibiotic resistance patterns, fecal coliforms and fecal streptococci were comparable as water quality indicators. Preliminary data indicated that the antibiotic resistance analysis method did not separate location information based on the antibiotic resistance pattern (impacted vs. non-impacted). However, there was apparent separation based on the host source. Mis-classification was greatest with human impacted streams and least in environments where the known pollution source was poultry. The larger the library of isolates from a particular host origin, the lower the misclassification.

Effects of a waterborne herbicide, Atrazine, on the auditory physiology of fish

Basic Information

Title:	Effects of a waterborne herbicide, Atrazine, on the auditory physiology of fish
Project Number:	2003KY21B
Start Date:	3/1/2003
End Date:	8/31/2004
Funding Source:	104B
Congressional District:	Kentucky 6th
Research Category:	Biological Sciences
Focus Category:	Agriculture, Toxic Substances, Water Quality
Descriptors:	herbicides, agricultural runoff, neurotoxicity
Principal Investigators:	Hong Y. Yan

Publication

1. Anraku, Kazuhiko, N.V. Lintecum, and H.Y. Yan, 2004, Effects of water acidification on chemoreception abiltiy of fathead minnow (Pimephales Promelas), in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 47-48.
2. Lintecum, N.V., and H.Y. Yan, 2004, Effect of acidification on fish olfactory response to alarm substance, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentukcy, 49-50.

Problems and Research Objectives

Atrazine (2-chloro-4-ethylamino-6-isopropylamine-1,3,5-triazine) is a major agricultural herbicide that has been in use worldwide for more than 40 years. It is the primary pesticide used on corn. In Kentucky, about 423,000 kg of atrazine was used on 1,562,000 acres of farmland in 1999. Concentrations of atrazine in runoff from more than 1,543,000 acres exceeded the 3 ppb maximum contaminant level set by United States Environmental Protection Agency. Recent studies raise concern for all aquatic life in atrazine-contaminated field runoff.

This project investigates the neurotoxicological effects of atrazine exposure on the auditory physiology of a cyprinid fish, the fathead minnow (*Pimephales promelas*), that is widely distributed in the waterways of Kentucky. Atrazine can alter thyroid function of animals. Animals with compromised thyroid glands may suffer great loss of auditory function through altered calcium channels which play an important role in auditory physiology. Hearing is a major sensory modality used by fish for survival in the wild (predator evasion, prey detection, mate recognition, conspecific and heterospecific communication). A compromised mechanosensory system may render fish unable to survive in the wild even though their reproductive system may not be affected by atrazine. Since fathead minnow is a cosmopolitan species, it can serve as a biological indicator and the results of neurotoxicological assays can be applied to the majority of fish fauna in Kentucky.

Methodology

A total of 160 young fathead minnows were used in the experiment. Each 10 fish were randomly selected as a group and housed in individual 10-gallon glass tanks (with aeration but no filtration—to avoid atrazine being filtration by the system). Each tank received one of the following combinations: exposure concentration: 3 ppb, 0.3 ppb, 0.03 ppb or 0 ppb (the control); exposure duration: 2, 4, 6 or 8 weeks. Such a 4x 4 design allowed detailed investigation on how atrazine exposure concentration and duration impact auditory responses of fish. At the end of each exposure interval, fish were subjected to auditory brainstem response (ABR) recording to document changes of hearing threshold over the experimental period (and compared to the baseline group). At the end of each ABR recording, select fish from each exposure regimen were perfused with physiological saline followed with histological fixative and their lower jaws were processed for histological examination of changes in their thyroid gland follicles.

Principal findings and Significance

The hearing thresholds for the 3 ppb group started showing significant elevation by 4 weeks of exposure. However, no significant increase in mortality was observed (as compared to the control group). For the 0.3 ppb group, significant changes only showed after 8 weeks of exposure. Less than 8 weeks exposure at 0.3 ppb apparently did not exert any significant physiological impacts to the endocrine systems that regulate the hearing ability of these fish. For the 0.03 ppb exposure group, no hearing threshold

changes could be observed in any exposure duration group. We are currently conducting a long term (6 months) low concentration (0.03 ppb) exposure experiment to delineate possible chronic exposure effects.

We are still in the process of analyzing the histological sections of thyroid glands of all exposed groups. Based on the data so far collected, exposure to the highest concentration (3 ppb) is associated with signs of hypothyroidism (i.e., reduction of secretion of thyroxin as indicated by shrinkage of follicle cells from the sections). The analysis of histological sections is a rather tedious and time consuming process. However, the completed histological analysis will add great power for the explanation of the hearing threshold data collected in this investigation.

Evaluation of ground water sustainability in the Ohio River alluvial aquifer near Westport, Oldham County, Kentucky

Basic Information

Title:	Evaluation of ground water sustainability in the Ohio River alluvial aquifer near Westport, Oldham County, Kentucky
Project Number:	2003KY24B
Start Date:	3/1/2003
End Date:	4/30/2004
Funding Source:	104B
Congressional District:	Kentucky 4th
Research Category:	Ground-water Flow and Transport
Focus Category:	Groundwater, Water Supply, Models
Descriptors:	alluvial aquifer, flow models, management and planning
Principal Investigators:	Samuel Boateng

Publication

1. Boateng, Samuel, 2004, Evaluation of ground water sustainability in the Ohio River Alluvial Aquifer near Westport, Oldham County, Kentucky, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 11-12.
2. Boateng, Samuel, and Matt Suedkanmp, 2004, Managing ground water resources to meet future water supply demands in a rural setting in northwestern Kentucky, in GSA Abstracts with Programs, Geological Society of America, 36(3).

Problem and Research Objectives

Ground water is an important source of drinking water in some regions of the State of Kentucky, including Oldham County where alluvium and glacial deposits along the Ohio River valley are the main source of ground water. The aquifer in this area is made up of sand and gravel. Generally, the aquifer can produce water to supply 50 to 250 homes per well (Carey and Stickney, 2001). There is a projected increase in population to about 53,000 residents in the county by the year 2020. Cities such as La Grange, Westport, and Buckner are growing rapidly. Future development of new subdivisions for single and multi-family homes is becoming almost inevitable. Some of these subdivisions may be developed in areas that contribute water to the existing ground water production wells of the Oldham County Water Division. The increase in population means more demand for water. The associated increase in human activities that generate contaminants may also pose serious problems to the quality of the drinking water source.

Objectives of this study include answering the following questions:

1. Can ground water supply in Oldham County be augmented by installing additional high-yield wells near Westport, Kentucky?
2. Would these new wells have any adverse impacts on the total aquifer drawdown?
3. What would be the potential impact of expansion of residential development be on the quality of ground water?

Objective question (1) is slightly different from the original proposal. After consulting the Oldham County Water Division, it became clear that the existing wells already are pumping at their maximum design capacity.

Methodology

Flow simulations and particle tracking were used to answer the objective questions. Flow simulations were performed by using the computer program MODFLOW and the subsequent particle tracking was completed with the MODPATH computer program. The simulated area encompassed all of the existing Oldham County Water Division production wells near Westport, Kentucky. This area has a transverse width that ranges from 600 m to1,800 m, and a longitudinal dimension of about 4,900m. From well log data and studies in the vicinity of the area, a mean hydraulic conductivity value of about 90 m/day was used (ranges between 30 to 180 m/day). Two conceptual flow models were proposed: a one-layer unconfined model and a two-layer semi-confined/confined model. In both cases, the western boundary was modeled as a river and the eastern boundary was a general head boundary. The models were calibrated by using water levels measured in observation wells. After the calibration, the total drawdown of the aquifer was noted pumping all five of the current production wells at their maximum capacities. The pumping rates range from 830 m^3/day to 4870 m^3/day. The capture zones were also determined.

The effect of potential future increases in production rate was simulated by including three wells to the north of the existing well field with each new well pumping

at a rate of 4,200 m^3/day. The maximum drawdown within the aquifer was noted for different scenarios representing normal, drought, and high (flood) Ohio River flow conditions. Again, the capture zones were determined.

This methodology is slightly different from the original proposal because of the change in objective question 1. Thus, the evaluation of flow by progressive increase in pumping rates was not practical. Also, a distribution of septic systems was not used to evaluate the potential for contamination as proposed. The capture zone areas combined with the thickness of the overlying silt and clay were used to evaluate the potential for septic system contamination.

Principal Findings and Significance

1. From the calibration data, the semi-confined/confined simulation represented the flow regime better.
2. Maximum drawdown occurring when all of the wells (including potential future wells) are pumping in a drought condition is about 7 m. The aquifer has a thickness of 22 m and this may not have a significant effect on the aquifer yield and could sustain the potential increase in water demand.
3. Aquifer is less vulnerable to contamination along the Ohio River but may be vulnerable in the capture zone areas where silt and clay cover is thin.
4. The capture zones cover about a third of the study area (mainly in the north central part) and any residential development within this zone may pose a potential threat to ground water quality. About half of this area has very thin silt and clay overlying the aquifer, although the water level is about 12 m below the ground surface. At high river stage (flooding scenario), the water level is within about 3 m of the ground surface.

Information Transfer Program

The Information Transfer Program of KWRRI has numerous components including lectures, seminars, symposia, and publications.

04/01/2003 National Ground Water Association Darcy Lecture: Richelle Allen-King, Ground Water and Surface Water Contributions to Chemical Mass Discharge: Considering the Problem at Field and Basin Scales

The Environmental Systems Seminar (ES600) is managed with assistance from the Institute. Students working toward the Graduate Certificate in Environmental Systems are required to participate in the seminar for two semesters. The presentations are also open to the general public. The topic for the fall 2003 semester was montaintop mining and valley fills. A compact disk was created by the course coordinator (Tom Greider) Mountaintop Mining and Valley Fills: Laws, Regulations, Court Decisions, Impacts, and a Virtual Fieldtrip.

08/19-20/2003 Solving the Watershed Puzzle: Kentucky Watershed Roundtable. Roundtable sponsors included the Kentucky Waterways Alliance, Inc., the Kentucky Division of Water, US EPA Region 4, the Tennessee Valley Authority, the US Geological Survey, and the Kentucky Water Resources Research Institute. Conference objectives included raising awareness of watershed issues and resources, building trust and forging links among partners, and providing tools to support watershed and community development. A total of 228 people attended some portion of the day and a half event. A final report was prepared and distributed after the conference.

Final Report - Solving the Watershed Puzzle: Kentucky Watershed Roundtable, November 2003, Kentucky Waterways Alliance, Inc., Munfordville, Kentucky, 53 p.

10/15/2003 - Earth Science Week Open House in conjunction with the Kentucky Geological Survey, the University of Kentucky Department of Geological Sciences, and the Tracy Farmer Center for the Environment.

Cyberseminars provided through the Consortium for the Advancement of Hydrologic Sciences, Inc. (CUAHSI) were made available by the KWRRI in the Health Sciences Learning Center on the campus of the University of Kentucky

10/21/2003 Ken Reckhow, Development of a Prototype Hydrologic Observatory of the Neuse River Basin

10/24/2003 George Hornberger, Concentration-Discharge Relationships in Headwaters Streams: Models and Muddles

11/14/2004 Laura Toran, Stormwater Sampling in Karst: Implications for Contaminant Transport

01/23/2004 Jeffrey McDonnell, The Old Water Paradox: A Grand Challenge for Catchment Hydrology

2/27/2004 Dave Tuck, Time for a New Dimension in Capillary Pressure-Saturation Space

The Kentucky Water Resources Annual Symposium was held February 19, 2004. This one-day symposium allowed individuals from universities, government agencies, and the private sector to present information on completed and ongoing research and management activities. An opening plenary session was followed by two concurrent sessions. Twenty-six platform presentations and nine poster presentations were included in the program. There were 107 registrants for the conference. Abstracts were printed as a proceedings volume and distributed to all participants.

Proceedings Kentucky Water Resources Annual Symposium, 2004, Kentucky Water Resources Research Institute, February 19, 2004, Lexington, Kentucky, 72 p.

A half-time Information Specialist Sr. was employed by the Kentucky Water Resources Research Institute in 2003. Publication of the quarterly newsletter WATERWORKS was subsequently resumed fall 2003. The Information Specialist is also responsible for creating and maintaining the Institute web site: www.uky.edu/WaterResorces/

The Association of State Dam Safety Officials (ASDSO) is a national, non-profit association dedicated to the improvement of dam safety through research, education and communications. The national office is located in Lexington, Kentucky and the unit is affiliated administratively with the University of Kentucky through the Water Resources Research Institute. In addition to regional technical seminars held throughout the country, ASDSO also maintains a clearinghouse of books, videos, articles, and CD-ROMs on subjects related to dam safety. The association web site is: http://www.damsafety.org

The Ohio River Basin Commission seeks to improve the water resources programs and land programs of its member states. The commission endeavors to contribute to the formulation of a comprehensive, coherent, and coordinated national water policy that recognizes interstate water issues and the primary role of the states in water resources planning and management. The ORBC cosponsors the Institutes Annual Symposium. Its office is located in Lexington, Kentucky and the unit is affiliated administratively with the University of Kentucky through the Water Resources Research Institute.

Student Support

Student Support					
Category	**Section 104 Base Grant**	**Section 104 RCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	6	0	0	0	6
Masters	5	0	0	0	5
Ph.D.	1	0	0	0	1
Post-Doc.	2	0	0	0	2
Total	14	0	0	0	14

Notable Awards and Achievements

Gretchen Berryman (2003KY18B) was one of six undergraduate students who were invited to speak in the Rotunda of the Kentucky State Capitol at the 2004 Posters-at-the-Capitol Symposium in Frankfort. Of these students, Gretchen was also selected for formal recognition on the Senate floor.

Follow-on funding for project 2003KY18B (Webb) has been obtained from a number of sources. A closely related proposal entitled: Evaluation of Mercury Bioaccumulation in the Green River Ecosystem was funded by the National Park Service for $236,105 under a joint program with the USGS. This 3-year project will run from October 1, 2004 through September 30, 2007. Dr. Sreedevi Dawadi is being supported under a separate $60,000 grant from the Kentucky Science and Engineering Foundation to continue his work on the project. A small bridging grant ($7,000) from the Mammoth Cave National Park was also received to support efforts in summer 2003. Finally, Dr. Webb received a $262,000 grant from the National Science Foundation for a Research Experience for Undergraduates Site at Mammoth Cave National Park. The projects funded through the Institutes Program initiated efforts that were instrumental in obtaining this substantial follow-on funding.

Publications from Prior Projects

1. 2000KY4B ("Measurement and prediction of solute transport parameters for Kentucky soils") - Articles in Refereed Scientific Journals - D'Angelo, E.M., M. Vandiviere, W.O. Thom, and F. Sikora, 2003, Estimating soil phosphorus requirements and limits from oxalate extract data, Journal of Environmental Quality 32: 1082-1088.
2. 2000KY2B ("Using neural networks to identify and quantify significant sources of encysted protozoa in watersheds") - Water Resources Research Institute Reports - Brion, G.M., Srinivasa Lingireddy, and T.R. Neelakantan, 2003, Using neural networks to identify and quantify significant sources of encysted protozoa in watersheds, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, Kentucky, 65.
3. 2001KY2801B ("Does waste amendment affect abiotic N cycling in soils by naturally occurring reactive Fe(II)?") - Conference Proceedings - Matocha, Christopher, Gerald Haszler, and Mark

Coyne, 2003, Impact of iron on the reduction of nitrate in water, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 37-38.

4. 2002KY2B ("Environmentally-induced genes and mechanisms of inheritance: How are the effects of contaminant exposure transferred from one generation to the next?") - Articles in Refereed Scientific Journals - Johnson, J., J. Silverstein, B. Small, W.R. Wolters, and B.S. Shepherd, 2003, Disparate regulation of the insulin-like growth factor binding proteins in an ictalurid teleost (Ictaluras punctatus), General and Comparative Endocrinology, 134(2003): 122-130.
5. 2002KY2B ("Environmentally-induced genes and mechanisms of inheritance: How are the effects of contaminant exposure transferred from one generation to the next?") - Articles in Refereed Scientific Journals - Drennon, K., S. Moriyana, H. Kawauchi, B. Small, J. Silverstein, I. Parhar, and B. Shepherd, 2003, Development of an enzyme-linked immunosorbent assay (ELISA) for the measurement of plasma growth hormone (GH) levels in channel catfish (Ictalurus punctatus): Assessment of environmental salinity and GH-secretagogues on plasma GH levels, General and Comparative Endocrinology, 133(2003): 314-322.
6. 2002KY6B ("Impacts of surface mine valley fills on downstream peak flows in eastern Kentucky") - Articles in Refereed Scientific Journals - Phillips, Jonathan D., 2003, Impacts of surface mine valley fills on headwater floods in eastern Kentucky, Environmental Geology, 2004 (45): 367-380.
7. 2002KY7B ("Linking land use to water quality in the Muddy Creek subbasin, Kentucky River Watershed") - Conference Proceedings - Jones, Alice, Danita LaSage, Mark Wiljanen, Tom Edwards, 2004, Linking land use to water quality and linking research to the college classroom: The case of the Muddy Creek watershed, Madison County, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, 67-68.