TFO-EC-05-01 Tennessee Field Office

AN ENVIRONMENTAL QUALITY ASSESSMENT
of
CLARKS RIVER NATIONAL WILDLIFE REFUGE
a component of the
TENNESSEE NATIONAL WILDLIFE REFUGE COMPLEX

U.S. Fish and Wildlife Service
Ecological Services
446 Neal Street
Cookeville, Tennessee 38501

July 2005

U.S. FISH and WILDLIFE SERVICE / SOUTHEAST REGION / ATLANTA, GEORGIA

U.S. Fish and Wildlife Service
Southeast Region

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by

Steven R. Alexander

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July 2005

July 20, 2005

Memorandum

To: Roxanna Hinzman, FWS (ES), Washington, DC
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Michael Johnson, FWS, Clarks River NWR, Benton, KY
John Taylor, FWS, Tennessee NWR, Paris, TN

From: Lee A. Barclay, Field Supervisor, FWS (ES), Cookeville, TN

Subject: An Environmental Quality Assessment of Clarks River National Wildlife Refuge a component of the Tennessee National Wildlife Refuge Complex (TFO-EC-05-01)

We are transmitting this Environmental Contaminants report which covers the investigation conducted at Clarks River National Wildlife Refuge from 2000 to 2003. Results from Fish and Wildlife Service investigation No. 2000-4N62 are included. This FFS number corresponds to Division of Environmental Quality (Contaminants) I.D. No.'s 200040006.1 through 200040006.3.

Should you have any questions regarding this report, please contact Steve Alexander of my staff at 931/528-6481 (ext. 210) or via e-mail at *steven_alexander@fws.gov*.

Enclosure

EXECUTIVE SUMMARY

This study, Project ID No. 4N62, was initiated in September 2000. Habitat and fish community assessments were completed at seven sites in the Clarks River watershed in 2000. Composite young-of-year and whole-body fish and sediment samples were collected and submitted (ECDMS Catalog No. 4050036) for analyses. Organochlorine analyses and a metals scan for the fish and sediment samples were performed by the Hand Chemical Laboratory at Mississippi State University and Laboratory & Environmental Testing, respectively. Organophospahte, aromatic hydrocarbon, and aliphatic hydrocarbon analyses were also conducted on the sediment samples. A quantitative mussel survey was performed at Bryants Ford in McCracken County in 2001.

Sediment samples were collected at an additional six sites in 2001. In 2002, our efforts included the collection of composite young-of-year, whole-body, and fillet fish samples from the West Fork Clarks River, Blizzard Pond acquisition area, and mainstem. Organochlorine analyses and a metals scan for the fish and sediment samples were performed by the Hand Chemical Laboratory at Mississippi State University, Research Triangle Institute, and Laboratory and Environmental Testing (ECDMS Catalog Nos. 4050057 and 4050058). Organophosphate, aromatic hydrocarbon, and aliphatic hydrocarbon analyses were also conducted on the sediment samples.

Although not part of this project, one surface water sample from an active agricultural production area was collected in 2002 and analyzed for triazine herbicides. This sample was intended to be representative of a worst-case scenario of atrazine run-off after a significant precipitation event. A malformed amphibian survey was also completed in 2002 and the results included in a separate report (Lienesch and Alexander 2002).

Spatial analysis of wetland habitats within the current refuge boundary was performed and acreages of broad wetland classes determined. Fish and sediment data were compared with current regulatory guidance and toxicity screening values. Biological metrics were also calculated and utilized to indicate the relative quality of the Clarks River watershed.

Low residual levels of several chlorinated hydrocarbon insecticides were detected in fish collected during this investigation. Mercury concentrations in higher-trophic level fish species (i.e., black bass) exceeded current risk-based levels for human consumption. Chromium, nickel, and arsenic concentrations in several sediment samples exceeded toxicity thresholds for macroinvertebrates.

ACKNOWLEDGMENTS

The assistance and support provided by the following people are appreciated and gratefully acknowledged: Dr. Lee Barclay, Mr. Doug Winford, Mr. Robert Bay and Ms. Laila Lienesch. Richard Huffines, refuge manager, provided logistical support and resource information and refuge maps. Mr. Ron Cicerello, Kentucky State Nature Preserves Commission, and Mr. Paul Rister, Kentucky Department of Fish and Wildlife Resources, provided sampling support in the field. Mr. Kurt Snider, Ms. Debbie Harris, and Ms. Tammy Bilbrey performed Geographic Information System (GIS) analyses for the project. Ms. Sandra Silvey provided assistance with word processing.

Funding for this work was provided by the Fish and Wildlife Service through the Environmental Contaminants Program. Results from Fish and Wildlife Service investigation No. 2000-4N62 are included. This FFS number corresponds to Division of Environmental Quality (Contaminants) I.D. No.'s 200040006.1 through 200040006.3.

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INTRODUCTION

The Clarks River is a low-gradient, riverine system with areas of contiguous palustrine forested, scrub-shrub, and emergent wetland complexes. It is the only current refuge established wholly within the Commonwealth of Kentucky (Figure 1). There are approximately 400 acres of cropland at Clarks River NWR currently enrolled in cooperative farming agreements. Region IV Integrated Pest Management (IPM) guidelines and procedures have been implemented, and are currently being coordinated with IPM staff at Tennessee National Wildlife Refuge, as well as staff from the Regional and Washington offices.

Within the previously assessed reaches of Clarks River, aquatic community structure is comprised primarily of pollution-tolerant species with low diversity. The reach from RM 59.2 to RM 48.4 was partially supporting the designated use of aquatic life and not supporting the designated use of primary contact recreation in 1998. More recent information indicates that the reach between RM 50.9 and RM 48.4 now fully supports the aquatic life and swimming uses. This reach has been de-listed (Van Arsdall 2004). Other reaches and tributaries of the Clarks River watershed in Calloway, Marshall, Graves, and McCracken Counties remain listed on the State's 2004 303(d) list of impaired waters, and have been given a high priority for total maximum daily load (TMDL) development. These reaches include: the mainstem from RM 59.9 to RM 50.9 in Calloway County; Clayton Creek from RM 7.1 to RM 3.3 in Calloway County; Damon Creek from RM 1.8 to RM 0.0 in Calloway County; Middle Fork Clarks River from RM 2.7 to RM 0.0 in Calloway County; Middle Fork Creek from RM 6.6 to RM 0.2 in Marshall County; an unnamed tributary to Old Beaverdam Slough from RM 0.5 to RM 0.0 in Marshall County; and the West Fork Clarks River from RM 16.8 to RM 12.8 in Graves County. Blizzard Pond of the West Fork Clarks River from RM 3.7 to RM 0.0 in McCracken County was also listed as impaired on the 2004 303(d) list. The cause of impairment is an unknown source of pathogens. In contrast, the downstream reach of the Clarks River from RM 31.1 to the confluence of the Tennessee River at Paducah is fully supporting all designated uses. Previous biological data collected by various agencies indicated a fair to poor fish and benthic community in this reach.

In FY 2002, a malformed amphibian survey was completed on the refuge. Results for that investigation were included in a separate investigative report (Lienesch and Alexander 2002). The refuge has initiated the biological review for the development of a Comprehensive Conservation Plan (CCP). A Contaminant Assessment Process (CAP) investigation for the refuge was funded in FY 2001 and completed in May 2002. Geographic Information System (GIS) databases have been developed with the proposed acquisition and current refuge boundaries digitized. Results from this investigation and the other efforts will aid in CCP development, as well as evaluating current IPM practices.

The Kentucky Division of Water, Kentucky Department of Fish and Wildlife Resources, Tennessee Valley Authority, Murray State University, and various other State and local agencies and volunteers are actively involved in the Cumberland/Tennessee/Mississippi Basin Management Unit monitoring activities (Colten 1996). Individuals from these agencies and groups assisted in our field work and sample collection both on and off the refuge.

FIGURE 1

WETLAND RESOURCES

Wetlands provide essential watershed functions related to floodwater storage, groundwater flow moderation, sediment removal, nutrient cycling, and water purification. They provide diverse habitats for wildlife foraging and reproduction, and essential refugia for a wide variety of mammals, reptiles, amphibians, and fish. Two broad categories of wetlands exist within the boundaries of Clarks River NWR: palustrine and riverine.

Palustrine systems include all wetlands dominated by trees, shrubs, persistent emergents, and emergent mosses or lichens. They also include: 1) wetlands lacking this type of vegetation, but less than 20 acres in size with active wave-formed or bedrock shorelines and a water depth less than 2 meters, and 2) small, shallow, permanent or intermittent water bodies (ponds). *Riverine* systems include all wetlands and deepwater habitats contained within a stream channel excluding those areas dominated by trees, shrubs, persistent emergents, emergent mosses, or lichens. These systems are further defined by water permanence, gradient, water velocity, substrate, extent of floodplain development, and vegetation type.

Our GIS analyses indicated that the following general wetland types and acreages are present on Clarks River NWR:

Palustrine	
Unconsolidated Bottom	20 acres
Emergent	64 acres
Scrub-Shrub	317 acres
Forested	6,169 acres
Riverine	300 acres

Field verification has not been performed in all of the mapped wetland areas. Field verification of NWI mapped wetland areas should be conducted as part of future biological and water quality monitoring efforts on Clarks River NWR.

METHODS AND MATERIALS

Prior to this investigation, the reach between RM 48.4 and RM 31.1 was not fully assessed and is within the current refuge boundary. Seven sampling stations (Figure 2) were identified during reconnaissance activities conducted on May 9, 2000, by the principal investigators, and through consultation with other members of the Cumberland/Tennessee/ Mississippi Basin Management Unit. Three of these stations are within the previously un-assessed reach. Fish and sediment samples were collected from immediately downstream of the Burkholder Deadening area (RM 14.3) to immediately upstream of Murray, Kentucky (RM 60.4).

Habitat evaluations (Plafkin et al.1989) and field physicochemical analyses (i.e., temperature, dissolved oxygen, pH, conductivity, total dissolved solids) were performed at seven sites on and off the refuge (Table 1) in 2000. Fish and sediment were collected at these sites. Fish collections were performed by backpack electroshocking simultaneously with seine hauls. Two riffle, run, and pool complexes were sampled by this method at each station except Site 4. Due to significant entrenchment at this site, gill nets were deployed across the stream channel. Approximately two to four hours were spent sampling fish at each station. Fish community structure collections (Appendix A) were preserved in the field with a 10% buffered formalin solution. A quantitative mussel survey was performed at Site 2 (Bryants Ford) in FY 2001 (Appendix B). One invertebrate, a threeridge mussel (*Amblema plicata*), was also collected from Site 1 and submitted for tissue analysis.

Whole-body and composite specimens that were submitted for contaminant analyses were placed in pre-cleaned aluminum foil and/or plastic ziplock bags, placed on ice, and transferred to the Tennessee Field Office. They were then frozen until shipment to the analytical laboratory. Larger specimens, which were not submitted for contaminant analyses, were identified in the field, lengths and weights recorded, and released. Easily identified fish that were collected in large numbers were also recorded in the field and released. Photographs of larger specimens and/or vouchers of all released specimens were made for verification. At least five specimens of each species that were released were kept as voucher specimens from each sample station. Other characteristics of fish communities that were evaluated in the field included the presence of hybrids, size/age distribution of populations, incidence of disease, and occurrence of parasites. Where appropriate, the age and size distribution of species populations was noted and assessed in relation to recruitment potential. Spawning and nursery area availability was also noted.

The condition of the fish community was determined by calculating an Index of Biotic Integrity (IBI) for each site. Relative abundance, species composition and richness, the evaluation of species tolerances to environmental perturbations, and the condition of fishes are all criteria that were factored into the IBI score. Various richness indices and candidate metrics were calculated, however, to provide a preliminary assessment of fish community structure and general watershed health.

FIGURE 2

Table 1. Sampling Locations on or near Clarks River National Wildlife Refuge.

Site Identification	Location	River Mile	County	Latitude	Longitude
Site 1	Mainstem at KY 131 Bridge	14.3	McCracken	36.97194°	-88.51444°
Site 2	Mainstem at Bryants Ford	16.4	McCracken	36.96139°	-88.49444°
Site 3	Mainstem at Tucker Lane	31.7	Marshall	36.89861°	-88.38222°
Site 4	Mainstem at Tucker Lane	32.0	Marshall	36.89500°	-88.37778°
Site 5	Mainstem at Refuge Road off KY 1445	41.2	Marshall	36.82778°	-88.29694°
Site 6	Mainstem at Squier Holland Road	56.7	Calloway	36.65389°	-88.27917°
Site 7	Mainstem at Murray	60.4	Calloway	36.60639°	-88.29028°
CR01S1	Mainstem at KY 1346 Bridge	48.9	Calloway	36.74123°	-88.27358°
CR01S2	Squier Holland Road Drainage Ditch	NA	Calloway	36.65425°	-88.28703°
CR01S3	Sharp-Elva Road Wetland	NA	Marshall	36.92991°	-88.45494°
CR01S4	Dogtown Road Slough	NA	Marshall	36.81625°	-88.29964°
CR01S5	Watch Creek at KY 1445 Bridge	NA	Marshall	36.83234°	-88.32322°
CR01S6	Middle Fork Clarks River at Martins Chapel Road	NA	Calloway	36.57791°	-88.32777°
WF	West Fork Clarks River at KY 348 Bridge	7.7	Graves	36.93231°	-88.54415°
BP1	Blizzard Pond Drainage Canal	NA	McCracken	36.97627°	-88.56744°
BP2	Blizzard Pond Wetland	NA	McCracken	36.97501°	-88.58680°

Thirty-eight fish (Table 2) tissue samples were analyzed for percent moisture, percent lipid, twenty-two organochlorine compounds, and metals. One invertebrate sample was also analyzed for seventy-one PAH compounds and aliphatic hydrocarbons. Organic analytical quality control procedures utilized by the contract laboratory have been verified by the Patuxent Analytical Control Facility.

Duplicate sediment samples were collected in FY 2000 at each of the seven initial sites. Additional sediment samples were collected at six other sites (CR01S1 to CR01S6) in FY 2001 (Table 3). The sediment samples were removed from depositional areas with a stainless steel spoon. The sample was then transferred to a chemically pre-cleaned amber glass container and stored on ice for transport to the Tennessee Field Office. The samples were then refrigerated and held until shipment to the analytical laboratory. The seven sediment samples collected in FY 2000 were analyzed for percent moisture, twenty-two organochlorine compounds, seventeen organophosphate compounds, seventy-one PAH compounds and aliphatic hydrocarbons, and metals. The six sediment samples collected in FY 2001 were analyzed for percent moisture, total organic carbon, grain size, select organic parameters (see explanation below), and metals.

Site 4 was sampled again with a boat electroshocker in FY 2002. Additional fish and amphibian samples were also collected from the West Fork Clarks River at the KY 348 bridge and Blizzard Pond. These fillet, edible-size whole-body, and composite fish specimens were analyzed for select organic parameters and metals. Relative species abundance at Site 4 and the West Fork Clarks River and Blizzard Pond sites was also determined (Appendix C).

A Geographic Information System (GIS) evaluation of wetland habitats within the current refuge boundary and proposed expansion areas was also conducted in FY 2003. Although not part of this study, a malformed amphibian survey was completed on the refuge (Figure 3) in FY 2002. Atrazine monitoring was also conducted in an agricultural field on the refuge in FY 2002.

Sample Analysis. Fish samples were prepared for chlorinated hydrocarbon pesticides (Table 4), total PCBs, and PCB arochlor analyses by taking a five-gram (g) aliquot from a well-mixed composite sample and combining with up to 150 g of anhydrous sodium sulfate. These samples were then extracted with hexane for seven hours in a Soxhlet extractor and concentrated using a rotary evaporator. The extract was then air-dried to a constant weight (usually 4 days) for lipid determinations. After weighing, the lipid sample was dissolved in petroleum ether (12 ml) saturated with acetonitrile and extracted four times each with 30 ml of acetonitrile saturated with petroleum ether. Residues were partitioned into petroleum ether, washed, concentrated to 5 ml, and transferred to a glass elution column containing 20 g of Florisil.

The Florisil column was eluted with a 200 ml mixture of 6% diethyl ether and 94% petroleum ether (Fraction I) followed by 200 ml of 15% diethyl ether and 85% petroleum ether (Fraction II). Fraction II was then concentrated for quantification of chemical residues using capillary or megabore columns and electron capture gas chromatography. Fraction I was concentrated to 5 ml and transferred to a silicic acid chromatographic column for additional cleanup and separation of PCBs. Three elutriate fractions were obtained and concentrated to 10 ml for quantification using capillary or megabore columns and electron capture gas chromatography. Elution profiles for the separation columns are shown in Appendix D.

Sediment samples were prepared for analysis of insecticides and PCBs by taking a 20 g aliquot and mixing with sulfuric acid, acetone, and a 1:1 mixture of petroleum ether and ethyl ether. Detailed procedures are included in Appendix F.

Pesticide, total PCB, and PCB arochlor assays on fish and sediment samples were performed by the Hand Chemical Laboratory at Mississippi State University. Organophosphate, aromatic hydrocarbons, aliphatic hydrocarbons, and triazine pesticide analyses (Table 4) were also performed by the Hand Chemical Laboratory.

Fish samples were prepared for metal analyses (Table 5) by freeze-drying aliquots of well homogenized composite samples. After moisture content was determined, a 0.25 to 0.5 g aliquot of freeze-dried tissue was digested with nitric acid. Specific procedures are included in Appendix B. Fish and sediment were analyzed for metals by Laboratory and Environmental Testing and Research Triangle Institute. Arsenic and Se were analyzed using atomic absorption (AA) spectrophotometry. Mercury was analyzed using a standard cold vapor AA technique. All other metals were analyzed using inductively coupled plasma (ICP) emission spectrometry. Detailed information on tissue sample preparation for metal analyses is contained in Appendix E.

Results for tissue samples are given in wet weight values to provide comparisons with other studies. Percent moisture and lipid values are included in Table 6. Results for sediment samples are given in dry weight values. Conversion between wet weight and dry weight values was accomplished using the following formulas:

Dry weight concentration = wet weight concentration/(1-(%Moisture÷100))
Wet weight concentration = dry weight concentration*(1-(%Moisture÷100))

Both wet and dry weight values are provided to ease comparisons with data reported from previous or future studies by other investigators.

Figure 3

Table 2. Fish Samples Collected for Analysis on or near Clarks River NWR.

Site-Sample Number	Species	Sample Type	Weight (lbs)	Length (cm)
1-BG	Bluegill	Composite	NA	NA
1-M	Bluntnose minnow	Composite	NA	NA
1-SB	Spotted bass*	Whole-body	NA	NA
2-BG	Bluegill	Composite	NA	NA
2-SS*	Spotted sucker	Whole-body	NA	NA
2-SM	Stoneroller	Composite	NA	NA
3-BC*	Brown bullhead	Whole-body `	NA	NA
3-SM	Stoneroller	Composite	NA	NA
4-BB	Black buffalo	Whole-body	3 lbs. 4 oz.	46.5
4-BG1	Bluegill	Composite	NA	NA
4-CC1	Common carp	Whole-body	8 lbs. 0 oz.	64.5
4-CCF1	Channel catfish	Fillet	NA	NA
4-CCF2	Channel catfish	Fillet	NA	NA
4-CCWB1*	Channel catfish	Whole-body	NA	NA
4-CCWB2	Channel catfish	Whole-body	2 lbs. 1 oz.	41.9
4-Drum1	Drum	Whole-body	8 oz.	26.0
4-GR1	Golden redhorse	Whole-body	13 oz.	32.0
4-GS1	Gizzard shad	Whole-body	20 oz.	31.0
4-LMB1*	Largemouth bass	Whole-body	NA	NA
4-LMBF2	Largemouth bass	Fillet	NA	NA
4-LMBWB2	Largemouth bass	Whole-body	2 lbs. 15 oz.	43.0
4-SB1*	Spotted bass	Whole-body	NA	NA
4-SMB1	Smallmouth buffalo	Whole-body	4 lbs. 6 oz.	52.0
4-YB1*	Yellow bullhead	Whole-body	NA	NA

* Young-of-year or small specimen

Table 2 (cont.). Fish Samples Collected for Analysis on or near Clarks River NWR.

Site-Sample Number	Species	Sample Type	Weight (lbs)	Length (cm)
4-WC1*	White crappie	Whole-body	NA	NA
5-SS*	Spotted sucker	Whole-body	NA	NA
5-SM	Stoneroller	Composite	NA	NA
6-SB	Spotted bass	Composite	NA	NA
6-SM	Stoneroller	Composite	NA	NA
7-G	Green sunfish	Composite	NA	NA
7-SM	Stoneroller	Composite	NA	NA
WF-BCF1	Black crappie	Fillet	NA	NA
WF-BCWB1*	Black crappie	Whole-body	NA	NA
WF-LMB1	Largemouth bass	Composite	NA	NA
WF-P1*	Grass pickerel	Whole-body	NA	NA
WF-NH1	Northern hogsucker	Composite	NA	NA
BP-BG*	Bluegill	Composite	NA	NA
BP-PBB1	Brown bullhead	Composite	NA	NA

Table 3. Sediment Samples Collected for Analysis on or near Clarks River National Wildlife Refuge.

Sample Number	Location	Weight (g)	% Moisture	% TOC	% Sand	% Silt	% Clay
1SED	Site 1	*	29.0	NA	NA	NA	NA
2SED	Site 2	*	58.4	NA	NA	NA	NA
3SED	Site 3	*	25.6	NA	NA	NA	NA
4SED	Site 4	*	68.9	NA	NA	NA	NA
5SED	Site 5	*	27.3	NA	NA	NA	NA
6SED	Site 6	*	27.1	NA	NA	NA	NA
7SED	Site 7	*	38.5	NA	NA	NA	NA
CR01SED1	E. Fk. Clarks River @ KY 1346 Bridge	291.5	18.1	.350	65.2	29.9	.290
CR01SED2	Squier Holland Road Drainage Ditch	292.5	31.1	.550	43.9	54.2	2.01
CR01SED3	Sharp-Elva Road Wetland	285.9	60.6	1.22	89.9	8.91	1.79
CR01SED4	Dogtown Road Slough	331.7	35.1	.810	47.5	52.3	.590
CR01SED5	Watch Creek @ KY 145 Bridge	242.1	29.7	.370	46.7	52.9	.630
CR01SED6	Middle Fork Clarks River @ Martins Chapel Road	259.4	25.0	.550	65.5	34.0	.270

*****Sample weight not provided by laboratory

NA – Not Analyzed

Table 4. Chlorinated Hydrocarbon Insecticides, Organophosphate Insecticides, and Triazine Herbicides Analyzed in Samples from Clarks River National Wildlife Refuge.

Chlorinated Hydrocarbon Insecticides
HCB (hexachlorobezene) *aka* perchlorobenzene
BHC (benzene hexachloride) *aka* hexachlorocyclohexane
alpha, beta, gamma, delta isomers

Oxychlordane
Chlordane (alpha, gamma)
Nonachlor (trans, cis)
Heptachlor epoxide

DDT (*o,p'* and *p,p'*)
DDE (*o,p'* and *p,p'*)
DDD (*o,p'* and *p,p'*)

Endrin
Dieldrin
Mirex
Toxaphene

Organophosphate Insecticides
Azinphos-methyl
Chlorpyrifos
Coumaphos
Diazinon
Dibenzothiophene
Dichlorvos
Dimethoate
Ethoprop
Famphur
Fensulfothion
Fenthion
Malathion
Methyl parathion
Parathion
Phorate
Terbufos

Triazine Herbicides
Atrazine
Simazine
Propazine

Table 5. Metals Analyzed in Samples from Clarks River National Wildlife Refuge.

Aluminum	(Al)	Lead	(Pb)
Arsenic	(As)	Magnesium	(Mg)
Barium	(Ba)	Manganese	(Mn)
Beryllium	(Be)	Mercury	(Hg)
Boron	(B)	Molybdenum	(Mo)
Cadmium	(Cd)	Nickel	(Ni)
Chromium	(Cr)	Selenium	(Se)
Copper	(Cu)	Strontium	(Sr)
Iron	(Fe)	Vanadium	(Vn)
		Zinc	(Zn)

Table 6. Information for Organic Analyses of Fish Samples Collected at Clarks River NWR.

Sample Number	Weight (g)	% Moisture	% Lipids
1BG	30.4	76.2	3.52
1SB	72.7	78.9	.872
2BG	81.1	73.2	4.26
2SM	83.9	76.4	6.06
2SS	125	81.6	.452
3BC	23.2	80.9	2.27
3SM	51.8	73.1	9.43
4BB	1820	76.5	3.55
4BG1	70.3	76.7	2.94
4Drum1	183	76.9	4.09
4GR1	364	75.5	5.57
4GS1	425	60.6	22.6
5SM	56.9	76.6	3.72
5SS	34.1	75.8	1.73
6SB	157	73.9	5.36
6SM	154	76.9	3.52
7G	25.8	73.2	6.00
7SM	54.8	78.3	2.91

Table 6 (con't). Information for Organic Analyses of Fish Samples Collected at Clarks River NWR.

Sample Number	Weight (g)	% Moisture	% Lipids
CCF1	*	68.8	13.1
CCF2	*	73.1	14.7
LMBF2	*	75.7	.330
WFBCF1	*	74.8	.947
BPBG	*	77.4	1.54
BPPBB1	*	82.4	1.59
BPA	*	84.4	2.09
CC1	*	69.3	8.17
CCWB1	*	63.6	16.1
CCWB2	*	57.7	20.1
LMB1	*	75.2	.534
LMBWB2	*	69.9	4.27
SB1	*	75.9	1.14
SMB1	*	78.4	6.40
WC1	*	69.3	.827
WFBCWB1	*	72.9	2.27
WFLMB1	*	75.2	.440
WFNH1	*	76.3	1.05
YB1	*	74.1	.294

*Weight not provided by laboratory

QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

Laboratory quality control was verified by the Patuxent Analytical Control Facility at the Patuxent National Wildlife Research Center in Laurel, Maryland. The precision and accuracy of the analytical results were confirmed with matrix and reagent blanks, duplicate analysis of randomly selected samples, recoveries of spiked analytes, and analysis of samples from the National Institute of Standards and Technology, the U.S. Environmental Protection Agency (EPA), and the use of FWS reference materials. With only a few exceptions, all analytical results were considered to be acceptable.

The laboratories used in these investigations completed a considerable amount of QA/QC analyses. This included analyzing several samples in duplicate. The analytical results for these laboratory duplicates and other detailed QA/QC information are not included in this report, but are available upon request.

RESULTS

Our habitat evaluations indicated that a diversity of aquatic habitats were present with relatively good water quality. Numerous riffle, run, and pool complexes were at most of our sampling stations. These riffle components are generally not associated with low gradient streams in the Mississippi Valley Loess Plains Ecoregion. With the exception of Stations 1 (RM 14.3) and 4 (RM 32.0), our selected sampling station habitats ranked in the sub-optimal to optimal categories for all parameters evaluated. Channel alteration in the form of recent dredging or channelization was essentially absent with the exception of occasional gravel and sediment removal from the bridge abutments at Sites 1, 5, CR01S5, and WF. A portion of the mainstem reach at Site 1 is severely entrenched and fish collection efforts were difficult. The entrenchment observed at Site 4 is the result of the presence of an oxbow meander with recent evidence of beaver (*Castor canadensis*) activity. These two sites had many parameters within the marginal category. There was little or no evidence of agricultural or silvicultural activity encroachment on the riparian zones at the mainstem sites evaluated.

Epifaunal substrate/available cover (i.e., snags, submerged logs, undercut streambanks) exhibited slight variability with a 30-50% or slightly greater than 50% mix of stable habitat present. Well developed riffles with a length extending two times the width of the stream and as wide as the stream with an abundance of cobble and gravel was prevalent at all sites sampled. Moderate distances between riffle/run complexes were observed. These patterns are subject to periodic change based on the dynamics of the fine and coarse particles in the stream bed, the frequency of high flow events, and the occurrence of natural stream obstructions. Embeddedness was constant and averaged 25-50% coarse material (gravel, cobble and boulders) surrounded by fine sediment. Although there was some evidence indicating recent deposition of coarser materials near islands and point bars, no significant sedimentation from erosion or poor agricultural practices was observed. Pool substrate (i.e., gravel and firms, root mats, submerged vegetation) and variability (i.e., large-shallow, large-deep, small-shallow, small-deep) was considered optimal at all stations except Sites 1 and 4. These sites exhibited moderate deposition of sand and fine sediment at obstructions and constrictions in the stream channel. Channel flow at all sites was generally considered to be optimal with water reaching both lower banks and a minimal amount of channel substrate exposed. Channel alteration and channel sinuosity at all sites were considered sub-optimal to optimal. Minimal areas of bank scour and failure were observed, and the average width of the riparian zones was estimated at 12 meters to greater than 18 meters, with a predominance of native vegetation.

There was no evidence of disease or parasites on the larger fish specimens collected. The initial sampling of Site 4 by the use of gill nets indicated poor fish community diversity. Excellent fish diversity and abundance was observed at Site 3. Fish community diversity and abundance observed at Sites 1, 2, 5, 6, and 7 would be considered fair to good.

Analytical results indicate that PCBs and most other organochlorines were below the limits of detection for a majority of the fish tissue samples (Table 7). With the exception of p,p'-DDE, no fish samples from the West Fork Clarks River contained any of the other chlorinated hydrocarbon pesticide residues.

Alpha chlordane was detected in channel catfish (*Ictalurus punctatus*) fillet (0.007 mg/kg ww) and whole-body samples (0.010 and 0.024 mg/kg ww) from Site 4, a largemouth bass (*Micropterus salmoides*) whole-body sample (0.005 mg/kg ww) from Site 4, a composite whole-body spotted bass (*Micropterus punctulatus)* from Site 6 (0.057 mg/kg wet weight (ww)), and a composite largescale stoneroller *(Campostoma oligolepis)* from Site 7 (0.015 mg/kg ww). Gamma chlordane residues were present in a channel catfish fillet (0.005 mg/kg ww) from Site 4, whole-body channel catfish (0.007 and 0.012 mg/kg ww) from Site 4, and the composite whole-body spotted bass (0.013 mg/kg ww) from Site 6. Oxychlordane was detected in whole-body channel catfish (0.010 mg/kg ww) and largemouth bass (*Micropterus salmoides*) (0.008 mg/kg ww) from Site 4, as well as the composite whole-body spotted bass (0.025 mg/kg ww) from Site 6. Heptachlor epoxide residues were also detected in a channel catfish fillet (0.006 mg/kg ww) from Site 4, whole-body channel catfish (0.006 and 0.015 mg/kg ww) from Site 4, and the composite whole-body spotted bass (0.033 mg/kg ww) sample from Site 6.

Cis-nonachlor was detected in a whole-body channel catfish (0.006 mg/kg ww) from Site 4 and the composite whole-body spotted bass (0.010 mg/kg ww) from Site 6. Trans-nonachlor was detected in both channel catfish fillet samples at 0.008 mg/kg ww, whole-body common carp (*Cyprinus carpio*) (0.010 mg/kg ww), whole-body channel catfish (0.014 and 0.019 mg/kg ww), and whole-body smallmouth buffalo (*Ictiobus bubalus*) (0.006 mg/kg ww) from Site 4. Trans-nonachlor was also detected in the composite whole-body spotted bass from Site 6 (0.048 mg/kg ww), a composite largescale stoneroller sample from Site 6 (0.012 mg/kg ww), a whole-body green sunfish *(Lepomis cyanellus)* from Site 7 (0.012 mg/kg ww), and the composite largescale stoneroller sample from Site 7 (0.013 mg/kg ww).

Dieldrin was detected in a whole-body gizzard shad *(Dorosoma cepedianum)* (0.012 mg/kg ww), both channel catfish fillet samples (0.026 and 0.021 mg/kg ww) and whole-body largemouth bass (0.009 mg/kg ww), and a smallmouth buffalo (0.010 mg/kg ww) from Site 4. Dieldrin residues were also present in the composite whole-body spotted bass from Site 6 (0.120 mg/kg ww), the composite largescale stoneroller sample from Site 6 (0.026 mg/kg ww), and the composite whole-body green sunfish sample from Site 7 (0.040 mg/kg ww).

The whole-body gizzard shad from Site 4, whole-body spotted bass from Site 6, and the composite largescale stoneroller sample from Site 6 contained p,p'-DDD residues of 0.010 mg/kg ww, 0.016 mg/kg ww, and 0.010 mg/kg ww, respectively. A channel catfish fillet sample from Site 4 also had 0.007 mg/kg ww p,p'-DDD. Residues of p,p'-DDD were also detected in whole-body channel catfish and common carp from this site. Residues of p,p'-DDE were also detected in a whole-body largemouth bass (0.020 mg/kg ww), whole-body black buffalo *(Ictiobus niger)* (0.013 mg/kg ww), whole-body smallmouth buffalo (0.016 mg/kg ww), whole-body freshwater drum *(Aplodinotus grunniens)* (0.013 mg/kg ww), whole-body golden redhorse *(Moxostoma erythrurum)* (0.011 mg/kg ww), whole-body gizzard shad (0.012 mg/kg ww), and whole-body white crappie (*Pomoxis annularis*) from Site 4. Residues of p,p'-DDE were also detected in the composite whole-body spotted bass from Site 6 (0.036 mg/kg ww), composite largescale stoneroller sample from Site 6 (0.017 mg/kg ww), and the composite largescale stoneroller sample from Site 7 (0.012 mg/kg ww).

The only fish samples from the West Fork Clarks River to contain detectable residues of p,p'-DDE were a composite whole-body bluegill (*Lepomis macrochirus*) from the Blizzard Pond drainage canal (Site BP1) and a whole-body largemouth bass from Site WF. These residues were 0.006 mg/kg ww and 0.005 mg/kg ww, respectively.

Organochlorine and organophosphate residues were below the limits of detection in the thirteen sediment samples collected. Analytical results for aliphatic hydrocarbons and PAHs in the sediment samples were variable, with the highest individual PAH concentrations recorded at Sites 1,4, 6, and 7. No PAHs were detected in sediment samples collected in tributaries, drainage ditches, or other off-channel sites.

Analytical results for fish tissue indicate that concentrations of most metals in the 2000 fish samples (Table 8) and 2002 fish samples (Table 9) were above the limits of detection and within expected ranges typical for most species. Beryllium and molybdenum concentrations, however, were not detected in any fish sample. Mercury residues in fillet and whole-body largemouth bass, spotted bass, and smallmouth buffalo ranged from 0.300 to 0.910 mg/kg ww. Mercury concentrations in other species were lower.

Metal concentrations in the 2000 sediment samples (Table 10) collected in the mainstem Clarks River were typically higher than the 2001 sediment samples (Table 11), which were collected from tributaries, drainage ditches, and wetland habitats. Arsenic concentrations in sediment ranged from 0.56 mg/kg dry weight (dw) at Site CR01 to 7.50 mg/kg dw at Site 2. Nickel concentrations were below the limits of detection (4.94 mg/kg dw) at Sites CR01 and CR02. The highest nickel concentration (22.0 mg/kg dw) was recorded at Site 2. Chromium levels (36.0 mg/kg dw) were highest at Site 4, while selenium was only detected (0.500 mg/kg dw) in sediment from Site 2. Sediment concentrations for the other metals were generally at or below background levels.

Table 7. Organochlorine Constituents Found in Fish Samples from Clarks River NWR and Tennessee NWR.

	Oxy-chlordane	alpha chlordane	gamma chlordane	p,p' DDD	p,p' DDE	DDT	Dieldrin	trans Nonachlor	Cis Nonachlor	PCBs	Toxaphene	Heptachlor Epoxide
Clarks River NWR												
Bluegill					x							
Green sunfish							x	x				
Longear sunfish												
Largemouth bass	x			x	x		x	x				
Spotted bass		x	x	x	x		x	x	x			x
White crappie					x							
Black crappie												
Black bullhead					x							
Yellow bullhead												
Black buffalo												
Smallmouth buffalo					x			x				
Channel catfish	x		x	x	x		x	x				x
Common carp												
Gizzard shad				x	x		x					
Drum					x							
Northern hogsucker												
Spotted sucker												
Golden redhorse					x							
Stoneroller		x		x	x		x	x				

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	Oxy-chlordane	alpha chlordane	gamma chlordane	p,p' DDD	p,p' DDE	DDT	Dieldrin	trans Nonachlor	Cis Nonachlor	PCBs	Toxaphene	Heptachlor Epoxide
Tennessee NWR												
Duck River Unit (1996)												
Bluegill					x							
Gizzard shad					x							
Busseltown Unit (1997)												
Bigmouth Buffalo				x	x							
Bowfin					x							
Bluegill					x							
Gizzard shad					x							
Big Sandy Unit (1998)												
Bluegill					x							
Emerald shiner				x	x							
Longear sunfish					x							
Largemouth Bass (2)					x							
Smallmouth Bass					x							
Spotted Sucker					x							

Table 8. Metal Concentrations in 2000 Fish Samples Collected at Clarks River NWR (mg/kg, ww).

Sample Number	1BG	1M	1SB	2BG	2SM	2SS	3BC	3SM	4BB	4BG1
Al	73.2	144	27.0	29.0	94.6	8.90	65.0	216	8.20	42.0
As	.100	.150	.090	.100	.200	.100	.100	.350	.100	<.100
B	.700	.500	<.600	<.600	<.600	.700	1.60	<.700	<.500	<1.00
Ba	7.75	10.8	7.88	8.26	20.7	4.80	4.00	13.1	2.00	16.5
Be	<.030	<.030	<.030	<.030	<.030	<.030	<.030	<.040	<.030	<.050
Cd	.060	.040	.030	.040	.040	.030	.030	.050	.060	.060
Cr	<.200	.620	<1.00	.200	.100	<.100	.200	.300	<.100	.720
Cu	.480	.710	1.10	.600	1.90	.740	.550	2.70	.830	.970
Fe	63.6	131	30.0	33.0	82.4	14.0	72.8	170	14.0	25.0
Hg	.090	.090	.170	.080	.030	.110	.110	.040	.350	.100
Mg	696	455	702	513	460	647	454	567	367	959
Mn	64.9	35.6	13.4	80.4	73.1	26.2	36.0	91.7	6.30	75.5
Ni	<.200	.300	<.100	<.100	<.100	<.100	<.100	.200	.200	.300
Pb	.100	.100	<.060	<.060	<.060	<.050	.070	.200	<.050	.200
Se	.500	.470	.510	.280	.200	.570	.440	.310	.620	.490
Sr	47.5	21.5	50.9	32.0	25.7	35.7	24.4	33.8	8.29	65.5
V	.400	.330	.200	.200	.300	<.100	.530	.500	<.100	<.200
Zn	39.3	35.5	31.4	28.0	27.2	26.1	22.7	37.6	11.4	49.5

Table 8 (con't). Metal Concentrations in 2000 Fish Samples Collected at Clarks River NWR (mg/kg,ww)

Sample Number	4Drum1	4GR1	4GS1	5SM	5SS	6SB	6SM	7G	7SM
Al	18.0	9.10	28.0	595	91.5	6.10	328	11.0	389
As	<.050	<.060	<.080	.440	<.060	<.060	.100	<.060	.180
B	<.500	<.600	<.800	1.00	1.00	<.600	.600	<.600	.600
Ba	7.78	2.70	1.40	16.3	7.42	.980	12.7	7.40	29.4
Be	<.030	<.030	<.040	<.030	<.030	<.030	<.030	<.030	<.030
Cd	.040	.040	<.040	.050	.030	.030	.040	.030	.050
Cr	.420	<.100	<.200	2.90	.380	.200	1.50	<.100	1.20
Cu	.460	.590	.740	2.00	1.20	.400	3.00	.570	2.90
Fe	21.0	18.0	39.0	488	40.0	12.0	222	20.0	304
Hg	<.030	.092	<.040	.050	.040	.100	<.030	.110	.050
Mg	427	423	304	414	745	470	490	532	422
Mn	26.6	21.3	12.0	168	44.4	1.50	80.1	29.5	159
Ni	.200	<.100	<.200	1.30	.200	<.100	.670	<.100	.630
Pb	.090	<.060	<.080	.370	.100	<.060	.300	.080	.620
Se	.500	.530	.200	.340	.410	.270	.270	.320	.310
Sr	22.5	14.9	6.20	18.1	43.1	16.2	24.3	26.8	19.3
V	.200	<.100	<.200	1.10	.200	<.100	.570	<.100	.760
Zn	14.1	17.0	11.0	29.0	35.7	23.3	32.2	24.7	35.4

Table 9. **Metal Concentrations in 2002 Fish Samples Collected at Clarks River NWR (mg/kg, ww).**

Sample Number	CCF1	CCF2	LMBF2	WFBCF1	BPBG	BPPBB1	BPA*	CC1	CCWB1	CCWB2
Al	2.20	1.00	12.0	2.60	29.0	101	1080	15.0	25.0	2.90
As	.070	<.070	.080	.050	.060	.100	.420	.070	.080	<.080
B	<.700	<.700	<.400	<.500	<.500	.400	.830	<.600	<.700	<.800
Ba	.100	<.070	.320	2.80	2.60	9.73	16.4	4.50	.970	1.00
Be	<.030	<.030	<.020	<.020	<.020	<.020	.034	<.030	<.030	<.040
Cd	<.030	<.030	<.020	<.020	<.020	<.020	.030	.100	.110	.130
Cr	.300	.200	.600	.200	.200	.300	2.00	.600	<.200	.200
Cu	<.100	<.100	.200	.460	.470	.630	1.00	1.20	1.10	.360
Fe	6.60	6.00	13.0	8.30	31.0	108	774	45.0	36.0	34.0
Hg	.120	.150	**.910**	.280	.140	.110	.020	.160	.120	.100
Mg	243	234	308	403	510	302	201	350	272	305
Mn	.300	.200	1.10	7.30	19.0	27.0	113	11.8	4.50	4.20
Ni	<.200	<.200	.200	.300	<.100	<.090	.600	<.100	<.200	<.200
Pb	<.070	<.070	<.040	<.050	.050	.090	.480	<.060	<.070	<.080
Se	.200	.220	.420	.340	.470	.230	.230	.550	.200	.320
Sr	.270	.460	3.60	21.1	27.5	17.7	2.04	14.9	9.61	8.32
V	<.200	<.200	<.100	<.100	.200	.200	1.60	<.100	<.200	<.200
Zn	8.40	8.70	6.90	10.1	27.5	19.9	5.70	32.6	20.3	17.0

*** Composite Amphibian Sample (*Rana catesbeiana*)**

Table 9 (con't.). Metal Concentrations in 2002 Fish Samples Collected at Clarks River NWR (mg/kg, ww).

Sample Number	LMB1	LMBWB2	SB1	SMB1	WC1	WFBCWB1	WFLMB1	WFNH1	WFP1	YB1
Al	6.80	6.10	20.0	37.0	19.0	8.90	28.0	36.0	9.10	20.0
As	.050	.100	.070	.080	.050	.070	.040	.090	.270	.050
B	<.400	<.600	<.400	<.500	<.400	<.500	<.400	<.400	<.400	.400
Ba	1.70	1.50	3.90	5.62	1.30	2.40	1.70	4.00	4.69	2.70
Be	<.020	<.030	<.020	<.030	<.020	<.020	<.020	<.020	<.020	<.020
Cd	<.020	<.030	.030	.080	<.020	<.020	<.020	<.020	<.020	.061
Cr	1.00	.380	.200	.540	1.60	.200	.820	.670	<.100	1.40
Cu	.310	.360	.760	.880	.600	.260	.440	.590	.460	.930
Fe	18.0	27.0	38.0	50.0	27.0	12.0	30.0	34.0	20.0	50.2
Hg	**.320**	**.550**	**.320**	**.430**	.150	.290	**.300**	.120	.250	.150
Mg	442	470	307	421	285	332	342	325	357	272
Mn	6.70	4.60	12.1	35.0	12.6	7.50	5.90	22.5	35.9	24.1
Ni	.200	<.100	<.100	<.100	.100	<.100	.100	<.100	.100	.200
Pb	<.040	<.060	<.040	.060	<.040	<.050	.050	.100	<.040	.040
Se	.420	.530	.420	.370	.370	.390	.400	.500	.510	.320
Sr	15.8	21.5	10.4	25.0	7.90	15.4	13.3	12.7	14.2	9.68
V	<.100	<.100	<.100	.100	<.100	<.100	<.090	.100	<.100	.200
Zn	17.9	14.3	15.5	15.5	18.2	14.8	20.0	18.6	46.0	11.6

Table 10. Metal Concentrations (mg/kg, dw) in FY 2000 Sediment Samples from Clarks River National Wildlife Refuge.

Sample Number	1SED	2SED	3SED	4SED	5SED	6SED	7SED
Al	10700	26500	20600	24500	7290	12000	19400
As	3..50	7.50	5.30	5.30	1.60	3.00	2.30
B	10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
Ba	81.2	205.0	174.0	179.0	56.5	96.5	167.0
Be	.500	.990	.780	.830	.300	.500	.820
Cd	<.200	<.200	<.200	<.200	<.200	<.200	<.200
Cr	16.0	30.0	22.0	36.0	11.0	15.0	20.0
Cu	5.20	15.0	10.0	12.0	6.70	6.80	8.70
Fe	11100	18900	17200	18800	6600	9430	6750
Hg	<.100	<.100	<.100	<.100	<.100	<.100	.100
Mg	849	2220	1730	1830	500	928	1570
Mn	362	685	801	852	170	637	83
Mo	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Ni	8.00	22.0	16.0	20.00	5.00	10.0	17.0
Pb	9.00	27.0	21.0	27.0	7.00	16.0	10.0
Se	<.500	.500	<.500	<.500	<.500	<.500	<.500
Sr	9.60	19.0	15.0	20.0	5.40	11.0	21.0
V	22.0	50.0	36.0	43.0	16.0	22.0	30.0
Zn	26.0	65.0	39.0	57.0	20.0	33.0	42.0

Table 11. Metal Concentrations (mg/kg, dw) in FY 2001 Sediment Samples from Clarks River National Wildlife Refuge.

Sample Number	CR01SED1	CR01SED2	CR01SED3	CR01SED4	CR01SED5	CR01SED6
Al	4271	4855	11000	7211	4312	3692
As	0.56	1.99	3.26	1.61	2.19	1.22
B	5.00	8.00	12.10	7.10	6.80	3.10
Ba	54.80	48.70	164.00	70.20	66.90	39.50
Be	.337	.305	.732	.422	.366	.236
Cd	<.198	<.198	.370	<.197	<.194	<.195
Cr	7.29	7.44	12.80	8.55	10.80	5.10
Cu	<4.94	<4.95	11.80	6.74	<4.85	<4.87
Fe	5637	9132	20830	10860	10590	5333
Hg	.0208	<.0198	.0532	.0259	<.0194	.0198
Mg	336	511	1175	694	370	270
Mn	176	212	693	393	126	150
Mo	<4.94	<4.95	<4.85	<4.93	<4.85	<4.87
Ni	<4.94	<4.95	10.40	7.69	<4.85	<4.87
Pb	6.09	6.14	14.60	9.76	<4.85	6.31
Se	<.494	<.495	<.485	<.493	<.485	<.487
Sr	3.94	6.91	16.40	5.82	4.30	3.06
V	11.2	12.8	24.8	15.9	27.5	8.85
Zn	14.0	20.5	54.4	19.1	8.50	10.8

DISCUSSION

In this study, dieldrin, cis- and trans-nonachlor, oxychlordane, alpha chlordane, gamma chlordane, and heptachlor epoxide residues were detected at low levels in seven of the thirty-eight fish samples analyzed from Clarks River NWR. One specimen in particular, a composite whole-body spotted bass collected from Site 6, contained low levels of each of these compounds. None of these chlorinated hydrocarbons were detected in fish samples collected at Tennessee NWR (Alexander *et al*. 2003). This may be reflective of historical pesticide use patterns or disposal practices in the Clarks River watershed.

DDT isomers were detected at low levels (0.007 and 0.014 mg/kg ww) in two of the four fillet samples collected for this study. Similar levels of p,p'-DDE and p,p'-DDD were detected in twelve whole-body and composite fish samples. These results suggest a continuation of the declining trends reported in our Tennessee NWR investigation (Alexander *et al*. 2003) and the 1995 Biomonitoring of Environmental Status and Trends (BEST) program effort (USGS 2002). This is likely indicative of the continued weathering of these compounds in sediments on and off the refuge.

None of the organochlorine results observed in this study exceeded the maximum values reported by Schmitt *et al.* (1985, 1990) and the 1995 BEST program (USGS 2002), and most concentrations were below the geometric means calculated for previous National Contaminant Biomonitoring Program (NCBP) data collection efforts. No organochlorine or organophosphate compounds were detected in any of the sediment samples collected at Clarks River NWR.

No PCBs were detected in fish or sediment samples collected from Clarks River NWR. On average, PCBs results (Alexander *et al*. 2003) from Tennessee NWR were lower than those reported by Robison *et al*. (2000) and Winger *et al.* (1988) for refuges in Tennessee.

Although most of the Hg results observed for our samples were considered low, two of 38 samples (5.3%) fell between maximum values for 1984 NCBP samples (0.37 mg/kg dw) and the 1980-81 NCBP samples (0.77 mg/kg dw) reported by Schmitt and Brumbaugh (1990). One sample, a largemouth bass fillet taken from a specimen at Site 4, contained 0.910 mg/kg ww mercury. This level approaches the current FDA action-level of 1.00 mg/kg. Average Hg values at Tennessee NWR did not exceed mean values reported by Winger *et al.* (1988) for NWRs in Tennessee and the 1995 BEST samples (<0.15 μg/g ww) collected in the Tennessee River at Savannah, Tennessee, and the Cumberland River in Clarksville, Tennessee (USGS 2002).

None of the other metal concentrations in fish samples from the present study exceeded the respective mean values reported from the 1995 BEST effort (USGS 2002) or published action levels for fish tissue. Although there is not enough data to perform a statistically significant trend analysis of the Hg concentrations within similar species for the samples we collected during this investigation, our empirical observations indicate that mercury concentrations may be increasing in higher-trophic level organisms on the refuge. Geometric mean Hg concentrations at the NCBP stations sampled in 1995 were also generally higher than when those sites were last sampled in the mid-1980s. The maximum Hg concentration found (0.45 μg/g ww) in the 1995 BEST effort was in a largemouth bass collected from the Mississippi River at Memphis, Tennessee.

Based on the Illinois stream sediment classification reported by Kelly and Hite (1984), chromium would be considered elevated ($\geq$23 mg/kg dw) at Site 4. In this investigation, nickel (22.0 mg/kg dw) was also elevated in the sediment sample from Site 2. In contrast to sediment values for Tennessee NWR (Alexander *et al*. 2003), molybdenum was not detected in any sediment sample collected on or near Clarks River NWR.

Although the United States has not promulgated formal sediment quality guidance, Canadian sediment quality guidelines published by Persaud *et al.* (1989) and Jaagumaji (1992) and other sediment quality guidance may be useful in assessing potential toxicity of the sediment samples collected on Clarks River NWR. Toxicity screening levels were determined from biological metrics and data generated from numerous invertebrate toxicity tests. Lowest effect levels (LELs) published by Persaud *et al.* (1989) and Jaagumaji (1992) were exceeded for Cr (26 mg/kg dw) and Ni (16 mg/kg dw) in the sediment sample collected from Site 2. These values (Table 12) were similar to sediment concentrations found on Tennessee NWR (Alexander *et al*. 2003). Average Hg concentrations did not exceed the Canadian tolerance values, although maximum values (0.10 mg/kg dw) approached the LEL (0.12 mg/kgdw) reported by Persaud *et al.* (1989).

The Threshold Effects Level (TEL) is calculated as the geometric mean of the 15th percentile concentration of the toxic effects data set and the median of the no-effect data set. TELs represent the concentration below which adverse effects are expected to occur only rarely. The Probable Effects Level (PEL) is calculated as the geometric mean of the 50th percentile concentration, and is the level above which adverse effects are frequently expected. The chromium concentration of 36.0 mg/kg dw in the sediment sample from Site 4 was near the TEL of 36.286 mg/kg dw for *Hyalella azteca*. The nickel concentration of 22.0 mg/kg dw in the sediment sample from Site 2 exceeded the TEL of 18.00 mg/kg dw. Arsenic values at Sites 2, 3, and 4 exceeded the TEL (5.90 mg/kg dw) reported by Smith *et al*. (1996).

Table 12. Comparison of Maximum Sediment Metal Concentrations (mg/kg, dw) at Tennessee NWR Complex with Soil Values (geometric means) Reported by Shacklette and Boerngen (1984).

		TENNESSEE NWR COMPLEX			
Metals	**Eastern United States**	**Clarks River NWR**	**Duck River**	**Busselltown**	**Big Sandy**
Al	33000	26500	27293	16410	5813
As	4.80	**7.50**	3.88	**12.40**	3.07
B	31.0	12.10	4.47	15.00	5.42
Ba	290	205	209	132.0	46.4
Be	0.55	0.99	1.42	1.17	0.41
Cr	33.0	**36.0**	24.0	31.9	9.5
Cu	13.0	15.0	16.1	16.10	6.3
Fe	14000	20830	29621	26610	8729
Hg	0.08	**0.10**	**0.11**	0.09	0.05
Mg	2100	2220	2170	2563	526
Mn	300	852	**2590**	1036	388
Mo	0.32	<0.50	2.21	**19.4**	ND
Ni	11.0	**22.0**	**25.4**	20.0	7.9
Pb	14.0	27.0	22.0	16.4	7.03
Se	0.30	0.50	0.72	0.66	ND
Sr	53.0	20.0	46.9	11.2	6.2
V	43.0	50.0	37.9	44.1	13.6
Zn	40.0	65.0	85.3	79.9	25.3

CONCLUSIONS AND RECOMMENDATIONS

Whole-body results are considered applicable to estimating potential ecological risk. Since organic mercury partitions throughout the flesh of the fish, they may also be useful as a screening tool in evaluating potential human health concerns. Based on the whole-body fish sample results, none would be expected to exceed any applicable action levels established by the Food and Drug Administration (FDA 1990). The fillet value for one largemouth bass collected at Site 4 (0.91 mg/kg ww) was near the FDA action level of 1.0 mg/kg for Hg.

The Commonwealth of Kentucky currently utilizes a risk-based screening approach that resulted in the issuance of a state-wide fish consumption advisory for Hg in 2000. Precautionary fish consumption advisories, particularly those which involve mercury, place recommended limits on the number of meals of fish that special populations should consume on a weekly, monthly, and/or yearly basis. Populations of concern would include women of child-bearing age and children under the age of 6 years old.

This approach adheres to the 2004 national advisory issued by EPA, however, it is currently under revision. Based on mean mercury concentrations in fish within the state, the Commonwealth of Kentucky recommends that this population consume no more than one meal of fish per week. This advisory is based on a reference dose of $1X10^{-4}$ mg/kg-d of mercury and a concentration range in fish fillets of >0.12 mg/kg to 0.47 mg/kg. Current EPA and FDA guidance (2000) for fish consumption suggests that the mercury concentration observed in the largemouth bass fillet from Site 4 would warrant limiting consumption to no more than one meal per month. This is based on a concentration range of >0.47 mg/kg to 0.94 mg/kg. The mercury concentration observed in this sample, however, is very near the recommended 0.5 meal per month threshold of >0.94 mg/kg.

Additional guidance on evaluating mercury concentrations in fish tissue is currently being developed by EPA, FDA, and the World Health Organization. The FWS has not formally adopted a similar risk-based approach or issued specific guidance on National Wildlife Refuges in the United States. Subsequent investigation and evaluation of mercury in fish fillet samples may be warranted on Clarks River National Wildlife Refuge.

Concentrations of p,p'-DDE and Hg were sufficient at several locations to warrant concern for fish-eating birds, particularly when cumulative exposures and effects are considered. Concentrations of As and Se were also elevated in a small percentage of the samples collected. Depending upon the foraging and feeding habits of specific species, elevated levels of these and other contaminants in tissue and internal organs of fish may pose additional un-quantified risks to avian and other piscivorous species. Avian receptors should be evaluated for potential exposure to these contaminants. Future biota sampling activities should also include reptiles to provide a more complete evaluation of contaminant exposure pathways.

Overall, there does not appear to be any immediate need for mitigation or clean-up of environmental contamination at Clarks River National Wildlife Refuge sites sampled in this study. Due to the current non-attainment of the aquatic life designated use in specific stream reaches within the watershed, an evaluation of current National Pollution Discharge Elimination System (NPDES) wastewater discharges in the watershed is warranted. This is especially important since aquatic-dependent species are currently not fully evaluated when developing water quality criteria or establishing NPDES permit limits. Establishing site-specific water quality criteria or designating the Clarks River within the current refuge boundary as an Outstanding National Resource Water would also provide a higher level of protection than that currently afforded.

Refuge managers can best enhance the overall environmental quality on Clarks River NWR by:

1) improving cooperative farming practices on the refuge to reduce soil erosion and the associated transport of environmental contaminants to aquatic systems;

2) continue the implementation of the integrated pest management program on the refuge that couples the proper use of appropriate pesticides with other techniques;

3) installing and protecting vegetative buffer strips along stream channels, ditches, swales, and other water-conveyance conduits on the refuge; and

4) working actively with private landowners, other Federal and State agencies, and non-governmental organizations in the refuge watershed to improve land use practices.

REFERENCES

Alexander, S.R., L.Lienesch and R.Wheat. 2003. An Environmental Quality Assessment of Tennessee National Wildlife Refuge. U.S. Fish and Wildlife Service, Atlanta, Georgia. 60 pp.

Colburn, T., F.S. vom Seal, and A.M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspec. 101:378-384.

Colten, L. 1996. Kentucky Watershed Management Framework. Kentucky Natural Resources and Environmental Protection Cabinet, Department for Environmental Protection, Division of Water, Frankfort, Kentucky. 18pp.

Eisler, R. 1987. Mercury Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. Biological Report 85(1.10). U.S. Fish and Wildlife Service. 90 pp.

Environment Canada (EC). 1999. Canadian Tissue Residue Guidelines for the Protection of Wildlife Consumers of Aquatic Biota. Canadian Environmental Quality Guidelines.

Goede, R.W. and B.A. Barton. 1990. Organismic indices and autopsy-based assessment as indicators of health and condition of fish. American Fisheries Society Symposium 8:93-108.

Hinton, D.E., P.C. Baumann, G.R. Gardner, W.E. Hawkins, J.D. Hendricks, R.A. Murchelano, and M.S. Okihiro. 1992. Histopathological biomarkers. *In*: Huggett, R.J., R.A. Kimerle, P.M. Mehrle, and H.L. Bergman, eds. Biomarkers. Lewis Publishers, Chelsea, Michigan.

Hunn, J.B. 1988. Field Assessment of the Effects of Contaminants on Fishes. Biological Report 88(19). U.S. Fish and Wildlife Service, Washington, D.C. 25pp.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing Biological Integrity in Running Waters: A Method and Its Rationale. Special Publication 5. Illinois Natural History Survey.

Jaagumagi, R. 1992. Development of the Ontario Provincial Sediment Quality Guidelines for Arsenic, Cadmium, Chromium, Copper, Iron, Lead, Manganese, Mercury, Nickel, and Zinc. Water Resources Branch, Ontario Ministry of the Environment, Ontario, Canada. 10pp.

Kelly, M.H. and R.L. Hite. 1984. Evaluation of Illinois Stream Sediment Data: 1974-80. Report No. IEPA/WPC/84-004. Illinois Environmental Protection Agency, Springfield, Illinois. 103 pp.

Lemly, A.D. 1996. Selenium in Aquatic Organisms. Pp. 427-445 *In:* Beyer, W.N., Heinz, G.H., and Redmon- Norwood, A.W., eds, Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations in Wildlife. Lewis Publishers, Boca Raton, Florida.

Lienesch, L. and S.R. Alexander. 2002. A Malformed Amphibian Survey of Clarks River National Wildlife Refuge. U.S. Fish and Wildlife Service, Atlanta, Georgia. 23 pp.

Lowe T.P., T.W. May, W.G. Brumbaugh and D.A. Kane. 1985. National contaminant biomonitoring program: concentrations of seven elements in freshwater fish, 1978-1981. Arch. Environ. Contam. Toxicol. 14:363-388.

May, T.W. and G.L. McKinney. 1981. Cadmium, lead, mercury, arsenic and selenium concentrations in freshwater fish, 1976-1977, National Pesticide Monitoring Program. Pesticides Monitoring Journal 15:14-38.

National Academy of Sciences and National Academy of Engineering (NAS). 1973. Water Quality Criteria, 1972. U.S. Environmental Protection Agency, Washington, D.C., EPA R3-73-003, 594p.

Newell, A.J., D.W. Johnson and L.K. Allen. 1987. Niagra River Biota Contamination Project, Fish Flesh Criteria for Piscivorous Wildlife. New York State Department of Environmental Conservation, Division of Fish and Wildlife, Bureau of Environmental Protection. Technical Report 87-3, 180 pp.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Ann. Assoc. Am. Geograph. 77(1):118-125.

Persaud, D., R. Jaagumari, and A. Hayton. 1989. Development of provincial sediment quality guidelines. Ontario Ministry of the Environment, Water Resources Branch, Toronto, Ontario.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Cross, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. EPA/444/4-89-001. USEPA, Office of Water, Washington, DC. (Numbered by chapters).

Robison, W.A., S.R. Alexander, J.Wilson and M.Wilson. 2000. Environmental Contaminant Surveys at National Wildlife Refuges in West Tennessee. U.S. Fish and Wildlife Service, Atlanta, Georgia. 98 pp.

Rosgen, D. 1996. Applied River Morphology. Wildland Hydrology, Pagosa Springs, Colorado. (numbered by chapter).

Schmitt, C.J and W.G. Brumbaugh. 1990. National contaminant biomonitoring program: concentrations of arsenic, cadmium, copper, lead, mercury, selenium, and zinc in U.S. freshwater fish, 1976-1984. Arch. Environ. Contam. Toxicol. 19:731-747.

Schmitt, C.J., J.L. Zajicek and P.H. Peterman. 1990. National contaminant biomonitoring program: residues of organochlorine chemicals in U.S. freshwater fish, 1976-1984. Arch. Environ. Contam. Toxicol. 19:748-782.

Schmitt, C.J., J.L. Zajicek and M.A. Ribick. 1985. National pesticide monitoring program: residues of organochlorine chemicals in freshwater fish, 1980-1981. Arch. Environ. Contam. Toxicol. 14: 225-260.

Shacklette, H.T. and J.G. Boerngen. 1984. Element Concentrations in Soils and Other Surficial Materials of the Conterminous United States. U.S. Geological Survey Professional Paper 1270. U.S. Government Printing Office, Washington, D.C. 104 pp.

Smith, S.L., D.D. MacDonald, K.A. Keenleyside, C.G. Ingersoll, and J. Field. 1996. A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. Journal of Great Lakes Research 22:624-638.

U.S. Environmental Protection Agency (EPA). 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 2: Risk Assessment and Fish Consumption Limits, 3rd Edition. Office of Water, Washington, D.C., EPA-823-B-00-008.

_____. 1992. National Study of Chemical Residues in Fish. EPA, Office of Science and Technology, Standards and Applied Science Division, Washington, D.C., EPA/823-R-92-008a (166 pp) and b (263 pp.).

_____. 1984. Ambient Water Quality Criteria for Mercury. EPA 440/5-80-058. National Technical Information Service, Springfield, Virginia. 136 pp.

_____. 1980. Ambient Water Quality Criteria for DDT. EPA, Office of Water Regulations and Standards, EPA/440/5-80/038, 173 pp.

U.S. Fish and Wildlife Service (FWS). 1995. Ecosystem Vision Statement and Charter Document. FWS, Southeast Region, Atlanta, Georgia. 7pp.

U.S. Fish and Wildlife Service (FWS). 1996. Draft Environmental Assessment and Land Protection Plan for the Proposed Establishment of Clarks River National Wildlife Refuge. FWS, Southeast Region, Atlanta, Georgia. 36pp.

U.S. Food and Drug Administration (FDA). 1990. Action levels for residues of certain pesticides in food or feed: Federal Register, v. 55, p. 14,359-14,363.

U.S. Geological Survey (USGS). 2002. Biomonitoring of Environmental Status and Trends (BEST) Program: Environmental Contaminants and their Effects on Fish in the Mississippi River Basin. Biological Science Report USGS/BRD/BSR–2002-0004.

Van Arsdall, T. 1998. The Status of Water Quality in Kentucky: 1998 305(b) Report. Kentucky Natural Resources and Environmental Protection Cabinet, Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Van Arsdall, T. 2002. The Status of Water Quality in Kentucky: 2002 305(b) Report. Kentucky Natural Resources and Environmental Protection Cabinet, Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Van Arsdall, T. 2003. 2002 303(d) List of Waters for Kentucky. Kentucky Natural Resources and Environmental Protection Cabinet, Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Van Arsdall, T. 2004. 2004 303(d) List of Waters for Kentucky. Kentucky Natural Resources and Environmental Protection Cabinet, Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Winger, P.V., D.P. Schultz and W.W. Johnson. 1988. Contaminant Residues in Fish From National Wildlife Refuges in the Southeast. Pp. 38-46 *In*: P.J. Stuber, ed. Proceedings of the National Symposium on Protection of Wetlands from Agricultural Impacts. Biological Report 88(16). U.S. Fish and Wildlife Service, Washington, D.C.

APPENDICES

Appendix A. Sampling Stations, Fish Collections, and Diversity Indices.

Site#1:	**Highway 131 Bridge**	**36.97194^0**	**RM 14.3**	**9/21/00**
	McCracken Co.	**88.51444^0**		

Species	Common Name	# Collected	Tissue Sample
1. *Percina maculata*	blackside darter	4	
2. *Etheostoma histrio*	harlequin darter	2	
3. *Micropterus punctulatus*	spotted bass	1	**WB**
4. *Lepomis macrochirus*	bluegill	4	**C**
5. *Lepomis megalotis*	longear sunfish	3	
5. *Pimephales notatus*	bluntnose minnow	45	
6. *Lythrurus fumeus*	ribbon shiner	1	
7. *Campostoma oligolepis*	largescale stoneroller	1	

Darter Richness: 2	Taxa Richness: 8
Sunfish Richness: 2	Total No. of Individuals: 61
Sucker Richness: NA	Percent Omnivores: 73.77
Intolerant Richness: 2	Percent Insectivores: 22.95
Top Carnivore Richness: 1	Percent Tolerants: 75.40
Simple Lithophil Richness: 2	Percent Pioneers: 73.77
Minnow Richness: 3	Percent Green Sunfish: NA
Headwater Richness: NA	Darter + Sculpin Richness: 2

C- Composite Sample
WB- Whole Body Sample

Site #2: Bryants Ford Road **36.96139^{0}** **RM 16.4** **9/21/00**
McCracken Co. **88.49444^{0}**

Species	Common Name	# Collected	Tissue Sample
1. *Noturus miurus*	brindled madtom	3	
2. *Noturus nocturnus*	freckled madtom	1	
3. *Percina shumardi*	river darter	40	
4. *Lepomis megalotis*	longear sunfish	4	
5. *Lepomis macrochirus*	bluegill	3	**C**
6. *Lepomis* sp.	juvenile sunfish	1	
7. *Cyprinella whipplei*	steelcolor shiner	2	
8. *Hybognathus nuchalis*	silvery minnow	3	
9. *Phenocobius mirabilis*	suckermouth minnow	1	
10. *Opsopedeus emiliae*	pugnose minnow	1	
11. *Pimephales notatus*	bluntnose minnow	3	
12. *Lythrurus fumeus*	ribbon shiner	30	
13. *Campostoma oligolepis*	largescale stoneroller	11	**C**
14. *Gambusia affinis*	western mosquitofish	4	
15. *Hypentelium nigricans*	Northern hogsucker	2	
16. *Minytrema melanops*	spotted sucker	1	**WB**
17. *Ictalurus punctatus*	channel catfish	2	

Darter Richness: 1	Taxa Richness: 17
Sunfish Richness: 3	Total No. of Individuals: 112
Sucker Richness: 2	Percent Omnivores: 4.46
Intolerant Richness: 3	Percent Insectivores: 83.03
Top Carnivore Richness: NA	Percent Tolerants: 33.03
Simple Lithophil Richness: 4	Percent Pioneers: 2.68
Minnow Richness: 7	Percent Green Sunfish: NA
Headwater Richness: NA	Darter + Sculpin Richness: 1

Site#3: Tucker Lane **36.89861^0** **RM 31.7** **9/21/00**
Marshall Co. **88.38222^0**

Species	Common Name	# Collected	Tissue Sample
1. *Noturus miurus*	brindled madtom	3	
2. *Noturus nocturnus*	freckled madtom	3	
3. *Etheostoma proeliare*	cypress darter	1	
4. *Etheostoma stigmaeum*	speckled darter	44	
5. *Etheostoma zonistium*.	bandfin darter	7	
6. *Etheostoma gracile*	slough darter	3	
7. *Percina maculata*	blackside darter	1	
8. *Percina shumardi*	river darter	13	
9. *Centrarchis macropterus*	flyer	2	
10. *Lepomis humilis*	orange spotted sunfish	4	
11. *Lepomis megalotis*	longear sunfish	7	
12. *Lepomis macrochirus*	bluegill	2	
13. *Fundulus notatus*	blackstripe topminnow	2	
14. *Pimephales notatus*	bluntnose minnow	15	
15. *Hybognathus nuchalis*	silvery minnow	2	
16. *Cyprinella whipplei*	steelcolor shiner	36	
17. *Lythrurus fumeus*	ribbon shiner	85	
18. *Lythrurus umbratilis*	redfin shiner	1	
19. *Notemigonus crysoleucas*	golden shiner	1	
20. *Notropis atherinoides*	emerald shiner	3	
21. *Campostoma oligolepis*	largescale stoneroller	22	**C**
22. *Ameiurus natalis*	yellow bullhead	1	**WB**
23. *Ameiurus nebulosus*	brown bullhead	1	
24. *Gambusia affinis*	western mosquitofish	8	
25. *Hypentelium nigricans*	northern hogsucker	1	

Darter Richness: 6
Sunfish Richness: 4
Sucker Richness: 1
Intolerant Richness: 6
Top Carnivore Richness: NA
Simple Lithophil Richness: 7
Minnow Richness: 8
Headwater Richness: NA

Taxa Richness: 25
Total No. of Individuals: 268
Percent Omnivores: 6.72
Percent Insectivores: 84.70
Percent Tolerants: 41.41
Percent Pioneers: 5.60
Percent Green Sunfish: NA
Darter + Sculpin Richness: 6

Site#4: Tucker Lane (Gill Nets) 36.89500^0 RM 32.0 10/10/00
Marshall County 88.37778^0

Species	**Common Name**	**#Collected**	**Tissue Sample**
1. *Lepomis macrochirus*	bluegill	3	**C**
2. *Moxostoma erythrurum*	golden redhorse	TNC	**WB**
3. *Ictiobus niger*	black buffalo	2	**WB**
4. *Ictiobus bubalus*	smallmouth buffalo	1	
5. *Dorosoma cepedianum*	gizzard shad	2	**WB**
6. *Aplodinotus grunniens*	freshwater drum	4	**WB**

Darter Richness: NA	Taxa Richness: 5
Sunfish Richness: NA	Total No. of Individuals: TNC
Sucker Richness: 3	Percent Omnivores: 50.00
Intolerant Richness: NA	Percent Insectivores: 10.00
Top Carnivore Richness: NA	Percent Tolerants: NA
Simple Lithophil Richness: 1	Percent Pioneers: NA
Minnow Richness: NA	Percent Green Sunfish: NA
Headwater Richness: NA	Darter + Sculpin Richness: NA

TNC - Too Numerous to Count

Site#5: Refuge Road off KY 1445 36.82778^0 RM 41.2 10/11/00
Marshall Co. 88.29694^0

Species	**Common Name**	**# Collected**	**Tissue Sample**
1. *Noturus nocturnus*	freckled madtom	4	
2. *Etheostoma oophylax*	guardian darter	1	
3. *Etheostoma stigmaeum*	speckled darter	9	
4. *Etheostoma zonistium*.	bandfin darter	20	
5. *Percina vigil*	saddleback darter	5	
6. *Percina sciera*	dusky darter	1	
7. *Aphredoderus sayanus*	pirate perch	2	
8. *Lepomis megalotis*	longear sunfish	60	
9. *Lepomis macrochirus*	bluegill	2	
10. *Lepomis cyanellus*	green sunfish	4	
11. *Micropterus punctulatus*	spotted bass	5	
12. *Pimephales notatus*	bluntnose minnow	11	
13. *Fundulus notatus*	blackstripe topminnow	2	
14.*Cyprinella whipplei*	steelcolor shiner	77	
15.*Notropis boops*	big eye shiner	3	
16. *Campostoma oligolepis*	largescale stoneroller	22	**C**
17. *Hypentelium nigricans*	northern hogsucker	1	
18. *Minytrema melanops*	spotted sucker	1	**WB**
19. *Gambusia affinis*	western mosquitofish	11	

Darter Richness: 5
Sunfish Richness: 3
Sucker Richness: 2
Intolerant Richness: 6
Top Carnivore Richness: 1
Simple Lithophil Richness: 7
Minnow Richness: 4
Headwater Richness: 1

Taxa Richness: 19
Total No. of Individuals: 241
Percent Omnivores: 4.56
Percent Insectivores: 84.23
Percent Tolerants: 10.78
Percent Pioneers: 6.22
Percent Green Sunfish: 1.66
Darter + Sculpin Richness: 5

Site #6: Squier Holland Road Bridge **36.65389^0** **RM 56.7** **10/11/00**
Calloway Co. **88.27917^0**

Species	Common Name	# Collected	Tissue Sample
1. *Etheostoma flabellare*	fantail darter	7	
2. *Etheostoma parvipinne*	goldstripe darter	5	
3. *Micropterus punctulatus*	spotted bass	6	**WB**
4. *Lepomis macrochirus*	bluegill	1	
5. *Lepomis cyanellus*	green sunfish	2	
6. *Lepomis megalotis*	longear sunfish	18	
7. *Notropis boops*	big eye shiner	16	
8. *Notropis atherinoides*	emerald shiner	2	
9. *Lythrurus fumeus*	ribbon shiner	4	
10. *Pimephales notatus*	bluntnose minnow	3	
11. *Cyprinella whipplei*	steelcolor shiner	78	
12. *Campostoma oligolepis*	largescale stoneroller	121	**C**
13. *Hypentelium nigricans*	northern hogsucker	1	

Darter Richness: 2
Sunfish Richness: 3
Sucker Richness: 1
Intolerant Richness: 1
Top Carnivore Richness: 1
Simple Lithophil Richness: 3
Minnow Richness: 6
Headwater Richness: 2

Taxa Richness: 13
Total No. of Individuals: 264
Percent Omnivores: 1.14
Percent Insectivores: 50.75
Percent Tolerants: 3.41
Percent Pioneers: 1.89
Percent Green Sunfish: 0.76
Darter + Sculpin Richness: 2

Site #7: "Rattlin" Bridge at Murray 36.60639^0 88.29028^0 RM 60.4 10/18/00

Species	Common Name	# Collected	Tissue Sample
1. *Noturus nocturnus*	Freckled madtom	1	
2. *Etheostoma flabellare*	fantail darter	2	
3. *Etheostoma parvipinne*	goldstripe darter	3	
4. *Micropterus salmoides*	largemouth bass	2	
5. *Lepomis cyanellus*	green sunfish	3	**WB**
6. *Lepomis megalotis*	longear sunfish	33	
7. *Lepomis macrochirus*	bluegill	1	
8. *Fundulus notatus*	blackstripe topminnow	4	
9. *Pimephales notatus*	bluntnose minnow	18	
10. *Notropis boops*	big eye shiner	40	
11. *Cyprinella whipplei*	steelcolor shiner	13	
12. *Campostoma oligolepis*	largescale stoneroller	38	**C**
13. *Labidesthes sicculus*	brook silverside	7	
14. *Ameiurus natalis*	yellow bullhead	1	
15. *Hypentelium nigricans*	northern hogsucker	1	

Darter Richness: 2	Taxa Richness: 15
Sunfish Richness: 3	Total No. of Individuals: 167
Sucker Richness: 1	Percent Omnivores: 11.37
Intolerant Richness: 2	Percent Insectivores: 64.67
Top Carnivore Richness: 1	Percent Tolerants: 13.17
Simple Lithophil Richness: 2	Percent Pioneers: 12.57
Minnow Richness: 4	Percent Green Sunfish: 1.80
Headwater Richness: 2	Darter + Sculpin Richness: 2

Appendix B. Clarks River Mussel Collections.

Site #2: Bryants Ford Road — McCracken Co. — 36.96139^0 / 88.49444^0 — RM 16.4 — 7/18/01

Species	**Common Name**	**# Collected**	**Size**
1. *Quadrula pustulosa*	pimpleback	59	1.1"-2.9"
2. *Truncilla truncata*	deertoe	8	0.9"-1.3"
3. *Amblema plicata*	threeridge	5	1.4"-4.6"
4. *Leptodea fragilis*	fragile papershell	2	1.7"-2.2"
5. *Fusconaia flava*	Wabash pigtoe	2	3.5"
6. *Lampsilis cardium*	plain pocketbook	1	3.0"
7. *Lampsilis teres*	yellow sandshell	1	1.1"
8. *Megalonaias nervosa*	washboard	1	4.5"

- Fresh dead and relict shells of the white heelsplitter (*Lasmigona complanata*) and pink heelsplitter (*Potamilus alatus*) were also located approximately 200 meters downstream from the qualitative transects in a muskrat midden. No live mussels or relict shells were found at Site 3 (Tucker Lane) and Site 5(off KY 1445).

Appendix C. Relative Species Abundance of Fish Collected from the West Fork and Site 4 by Electrofishing in 2002.

		Inch-Class																							
Common Name	**Scientific Name**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Channel catfish	*Ictalurus punctatus*													1				1							
Common carp	*Cyprinus carpio*																							1	1
Smallmouth Buffalo	*Ictiobus bubalus*														1						1				
River carpsucker	*Carpiodes cyprinus*																1								
Northern hogsucker	*Hypentelium nigricans*										1														
Redhorse	*Moxostoma* spp.					4	5						2	1		1									
Spotted sucker	*Minytrema melanops*					2		1	5	1	4														
Shortnose gar	*Lepisosteus platostomus*																							1	
Gizzard shad	*Dorosoma cepedianum*							3	1	1	1	1	1												
Freshwater drum	*Aplodinotus grunniens*							1		2		2		1		1				1					
White crappie	*Pomoxis annularis*					1																			
Largemouth bass	*Micropterus salmoides*								1									1							
Spotted bass	*Micropterus punctulatus*			2	2		2		2																
Yellow bullhead	*Ameiurus natalis*								1																
Longear	*Lepomis megalotis*		2	12	13	8																			
Topminnow	*Fundulus* spp.			4																					
Redfin shiner	*Notropis umbratilis*				1																				
Bluegill	*Lepomis macrochirus*		2	3	1	4		1																	
Steel Color shiner	*Notropis whipplei*		1	6																					
Grass pickerel	*Esox americanus*			1																					
River shiner	*Notropis blennious*				2																				
Warmouth	*Lepomis gulosus*				1																				
Bluntnose minnow	*Pimephales notatus*		1																						

Appendix D

Elution Profiled For Florisil, Silica Gel and Silicic Acid Column Separations

Florisil Column (used for tissue samples)

Fraction I (6% ethyl ether containing 2% ethanol, and 94% petroleum ether): HCB, alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-nonachlor, toxaphene, PCBs, o,p'-DDE, alpha-chlordane, p,p'-DDE, p,p'-DDT, cis-nonachlor, o,p'-DDT, p,p'-DDD, mirex, dicofol, endosulfan I (split with F-II).

Fraction II (15% ethyl ether containing 2% ethanol, and 85% petroleum ether): dieldrin, endrin, dacthal, endosulfan I (split with F-I), endosulfan II (split with F-III), endosulfan sulfate (split with F-III).

Fraction III (50% ethyl ether containing 2% ethanol, and 50% petroleum ether): endosulfan II (split with F-II), endosulfan sulfate (split with F-II), malathion.

Florisil Mini-Column (used for soils)

Fraction I (12 ml of hexane, followed by 12 ml of 1% methanol in hexane): HCB, gamma-BHC (25%), alpha-BHC (splits with F-II), trans-nonachlor, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD (splits with F-II), o,p'-DDT, p,p'-DDT, cis-nonachlor, cis-chlordane, trans-chlordane, PCBs, mirex, photomirex and derivatives.

Fraction II (24 ml of 1% methanol in hexane): g-BHC (75%), b-BHC, a-BHC (splits with F-I), delta-BHC, oxychlordane, heptachlor epoxide, toxaphene, dicofol, dacthal, endosulfan I, endosulfan II, endosulfan sulfate, octachlorostyrene, kepone (with additional 12 ml 1% methanol in hexane).

Silicic Acid

SA Fraction I (20 ml petroleum ether): HCB, mirex

SA Fraction II (100 ml petroleum ether): PCBs, p,p'-DDE (splits with SA-III)

SA Fraction III (20 ml of 1% acetonitrile, 80% methylene chloride, 19% hexane): alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, oxychlordane, heptachlor epoxide, gamma-chlordane, trans-chlordane, toxaphene, o,p'-DDE, alpha-chlordane, p,p'-DDE (splits with SA-II), cis-nonachlor, o,p'-DDT, p,p'-DDD, p,p'-DDT, dicofol.

Appendix E

Tissue Sample Preparation For Metal Analyses

Tissue Preparation

Samples are homogenized using a Kitchen Aid food processor. Portions are then freeze dried for determination of moisture content and subsequent acid digestions.

Digestion for Inductively Coupled Plasma Emission (ICP) measurement. About 0.25 to 0.5 grams of freeze-dried tissue are placed in a 120 ml teflon microwave vessel and five ml of Baker Instra-Analyzed HNO_3 are added to the vessel. The vessel is then capped according to the manufacturer's instructions and heated in a CEM microwave oven for 3 minutes at 120 watts, 3 minutes at 300 watts, and 15 minutes at 450 watts. The resulting residue is diluted to 50 ml with 5% HCl.

Digestion for Graphite Furnace Atomic Absorption (GFAA) measurement. Using a CEM microwave oven, 0.25 to 0.5 grams of freeze-dried tissue are heated in a capped 120 ml teflon vessel in the presence of 5 ml of Baker Instra-Analyzed HNO_3 for 3 minutes at 120 watts, 3 minutes at 300 watts, and 15 minutes at 450 watts. The residue was is then diluted to 50 ml with laboratory pure water.

Digestion for mercury measurement by Cold Vapor Atomic Absorption (CVAA). About 0.25 to 0.5 grams of sample are refluxed for 2 hours in 10 ml HNO_3 (Baker Instr-Analyzed) and diluted to 50 ml with 1% HCl.

Metal Analyses

ICP measurements were made using a Leeman Labs Plasma Spec I sequential spectrometer. GFAA measurements were made using a Perkin-Elmer Zeeman 3030 AA spectrophotometer with an HGA-600 graphite furnace and an AS-60 autosampler. Mercury measurements were conducted using $SNCL_4$ (as the reducing agent) and an Instrumentation Laboratories Model 251 AA spectrophotometer.

Appendix F

Sediment Sample Preparation for Chlorinated Hydrocarbon Pesticides, Polychlorinated Biphenyls, and Chlorophenoxy Acid Herbicide Analyses

Sample Preparation

Twenty grams of soil are weighed into a pesticide residue quality (PRQ)* centrifuge bottle and 10 ml of PRQ H_2O are added to the dry samples. The pH is adjusted to $\leq$ 2 using PRQ 12N sulfuric acid (usually about 1 ml). Fifty ml of acetone are added and the sample is shaken well six times over a 90 minute period (about every 15 minutes). Fifty ml of a 1:1 petroleum ether/ethyl ether (PE:EtoEt) mixture are added and the shaking is repeated. The sample is then centrifuged and the liquid decanted into a 500 ml separatory funnel containing 200 ml of PRQ water. The soil is re-extracted by shaking one minute with 50 ml 1:1 PE:EtoEt (10 ml H_2O may need to be added and the pH re-adjusted to $\leq$ 2), centrifuging again, and decanting the liquid into a separatory funnel.

Using PRQ 6N KOH (5 ml), the contents of separatory funnel are adjusted to pH $\geq$ 12, shaken vigorously for two minutes, and then allowed to stand for 30 minutes with intermittent shaking. The H_2O layer is separated and re-extracted with 100 ml 1:1 PE:EtoEt. The two petroleum ether extracts are then combined, capped, and reserved for analysis (this contains the chlorohydrocarbon pesticides, aliphatic and polynuclear aromatic hydrocarbons).

The aqueous layer is adjusted to pH $\leq$ 2 using three ml of PRQ 12N sulfuric acid and extracted with 100 ml 1:1 PE:EtoEt. The H_2O layer is separated and re-extracted with 100 ml 1:1 PE:EtoEt. The two petroleum ether extracts are then combined, capped, and reserved for analysis (this contains the chlorophenoxy acid herbicides).

Both the acid and the basic extracts are concentrated with Kuderna-Danish evaporators and their volumes reduced to adequate size for column clean-up.

Column Cleanup

Neutral Fraction (N/P and chlorohydrocarbon pesticides, aliphatic and polynuclear aromatic hydrocarbons). The sample extract is adjusted to exact volume and an appropriate aliquot removed for column clean-up techniques specific to analyte. For pesticides, a mini-florisil column is used, and for hydrocarbons a 1% deactivated silica gel column is used (Appendix A and B).

Acid Fraction (Chlorophenoxy acid herbicides)

Derivitization. The sample volume is reduced to approximately 0.5 ml and ethylated using diazoethane (15 minutes). The sample is exchanged to hexane (N-EVAP) and the volume reduced to 0.2 ml.

Column Clean-Up. Two grams of 1% deactivated silica gel are placed in a 7 mm i.d. chromatography column (#22 Kontes). This is topped with one cm of Na_2SO_4 and the column pre-wetted with 10 ml of hexane. The sample is then divided into three fractions as follows:

Fraction A: The sample is added and the container rinsed with two 0.5 ml washes of 20 % benzene in hexane. The column is then eluted with nine ml of the same solution (this fraction contains PCP).

Fraction B: Ten ml of 40% benzene in hexane are added. This is followed by ten ml of 60% benzene in hexane (this fraction contains Dalapon, PNP, Silvex, Dinoseb and a portion of Dicamba).

Fraction C: Ten ml of 80% benzene in hexane are added and followed by ten ml of 100% benzene (this fraction contains Dichlorprop, 2,4-D, 2,4,5-T, 2,4-DB, Bentazon, Blazer, and the remaining Dicamba).

Reference for column clean-up for chlorophenoxy acid herbicides:

Shafik, T. A., H.C. Sullivan and H.R. Enos. 1973. Multi-residue procedure for halo and nitrophenols: measurement of exposure to biodegradable pesticides yielding these compounds as metabolites. J. Agr. Food Chemistry 21:295-298.

* PRQ glassware and other equipment is obtained by rinsing 3 times with acetone followed by 3 rinses with petroleum ether.