# Biomonitoring of Environmental Status and Trends (BEST) Program: Contaminants and Related Effects in Fish from the Mississippi, Columbia, and Rio Grande Basins

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## ABSTRACT

The Biomonitoring of Environmental Status and Trends (BEST) Program was initiated, in part, as a revision and expansion of the National Contaminant Biomonitoring Program (NCBP). One aspect of the BEST program focuses on monitoring contaminants and effects across broad geographic areas. This approach is currently being tested in the Mississippi, Columbia, and Rio Grande basins. The overall objectives of this project are to describe the occurrence and distribution of contaminants and their effects on fish in the three basins; to quantitatively evaluate the performance of aquatic methods used by the BEST program; and to evaluate potential collaborations with the National Stream Quality Accounting Network (NASQAN-II) and the National Water Quality Assessment (NAWQA) programs of the USGS-Water Resources Division. Fish were collected from 46 sites in the Mississippi River basin (1995); 16 sites in the Columbia River basin (1997); 10 sites in the Rio Grande basin (1997); and from a reference site in West Virginia. Sites were located at the historic NCBP fish monitoring stations in all three basins; at NASQAN-II water quality sampling sites in the Columbia and Rio Grande basins; and at NAWQA sites in the Mississippi Embayment and Eastern Iowa Basins study units within the Mississippi River basin. The primary species targeted at each site were common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*); other species, mostly other black basses (*Micropterus* spp.), percids (*Stizostedion* spp.), salmonids, suckers (Catostomidae), and catfish (Ictaluridae) were collected as alternates, depending on habitat and location. Individual fish (about 40 per station) were analyzed for reproductive biomarkers (vitellogenin and sex steroid hormones), histopathological alterations, macrophage aggregates, EROD activity, lysozyme activity, and general fish health measures (organosomatic and ponderal indices, observations of grossly visible lesions, deformities, and parasites). Organochlorine (pesticides and total PCB's) and elemental (heavy metals and metalloids) contaminant analyses and the H4IIE bioassay for dioxin-like activity were performed on fish samples composited by species and sex. In the Mississippi basin, DDT (mostly as *p,p'*-DDE) residues in fish remained sufficiently high in the southern parts of the watershed to represent a hazard to sensitive species of fish-eating birds. Toxaphene residues also remained evident. The combined results of organochlorine chemical, H4IIE bioassay, and biomarker analyses also indicated the presence of other organic contaminants in the lower Mississippi valley. Cyclodiene pesticides (dieldrin, endrin, and chlordane) were present in many agricultural areas, especially in the Corn Belt. Concentrations of these pesticides were also elevated at Memphis, Tennessee, where there is a point-source. Selenium concentrations were sufficiently high to constitute a hazard to piscivorous fishes and wildlife in the upper Arkansas River, where levels have been increasing for approximately 10 years. As expected, mercury concentrations were higher in the predator species than in bottom fish. The occurrence of vitellogenin in plasma and of ovarian cells in the testes of male fish from several sites, along with abnormal ratios of sex steroids, suggest that fish from some sites are exposed to endocrine-modulating substances.

## BACKGROUND

Development of the Biomonitoring of Environmental Status and Trends (BEST) program was initiated in 1991 by the U.S. Fish and Wildlife Service (FWS) in part as a revision and expansion of the National Contaminant Biomonitoring Program (NCBP). The BEST program also sought to address contaminant issues on National Wildlife Refuges. The NCBP originated as part of the National Pesticide Monitoring Program (NPMP) --a multi-agency effort to document temporal and geographic trends in persistent contaminant concentrations through the collection and chemical analysis of environmental media (Johnson et al. 1967). As part of its NPMP activities, the FWS periodically collected and analyzed freshwater fish from a national network of stations that was last sampled completely in 1986 (Schmitt and Bunck 1995; Schmitt et al. in press). By the mid-1980's, the FWS had re-named its monitoring program NCBP in anticipation of adding biological monitoring and assessment components. The BEST program incorporates FWS planning for this expanded monitoring. In 1993, the BEST program, as well as curatorial responsibility for the historic NCBP data bases and sample archives, were transferred to the National Biological Service (NBS). The NBS became the Biological Resources Division (BRD) of the U. S. Geological Survey (USGS) in 1996.

The present goal of the BEST program is to monitor, identify, and understand the effects of contaminants on the Nation's biological resources, especially those managed by the Department of the Interior (DOI), and to provide scientific information for guiding management actions to DOI agencies. To achieve this goal, the BEST program employs a variety of approaches including assessment of existing information, development of biomonitoring methods, technical assistance, and monitoring a suite of contaminants and contaminant effects across broad geographic areas.

### Large Rivers Pilot Project

The pilot project described here evolved from two primary needs: (1) to field test the suite of monitoring methods selected for use in the BEST program (NBS 1996); and (2) to update information on the distribution and concentrations of persistent toxic contaminants in large rivers. The latter need arose because the NCBP fish network was last sampled in its entirety in 1986, prior to the extensive flooding of 1993 and 1995 in the Midwest and South. To meet these combined needs, BEST program methods that could be performed on fish collected to meet the NCBP collection goals and requirements were incorporated into the project.

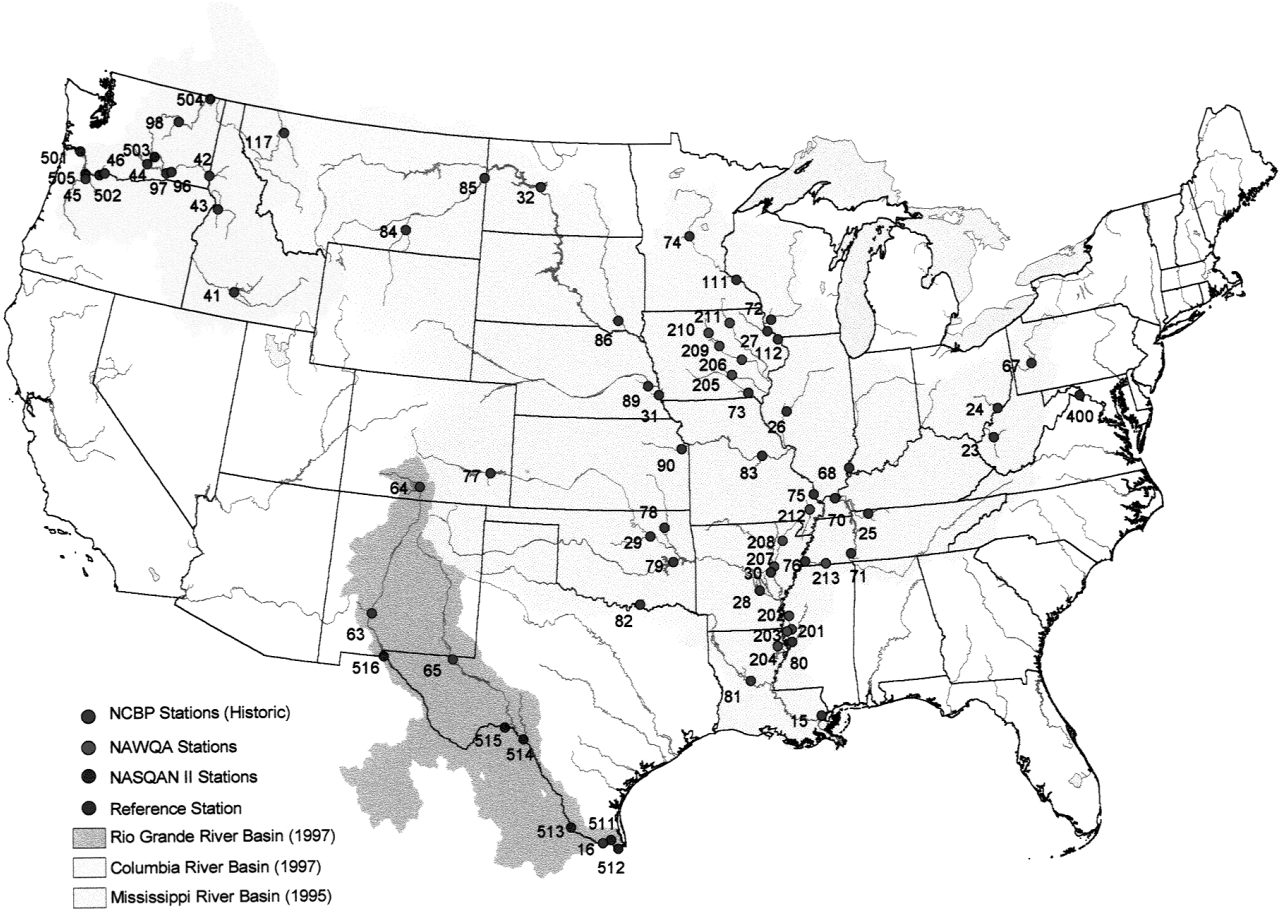
The NCBP fish network was designed to monitor spatial and temporal trends in the concentrations of bioaccumulable contaminants over large expanses of the U.S. The network was based on periodic sampling at "fixed" stations chosen to reflect the general concentrations of accumulative toxins in the environment. Fish were collected periodically from a national network of stations located at or near key points, such as at the confluences of major tributaries and below dams, in the larger rivers of the 50 states and in the open waters of the Great Lakes. The overriding assumption was that periodic collections and analyses of whole fish from key locales would integrate large expanses of time and space with respect to the concentrations of the measured contaminants. Results could then be generalized to taxa other than those collected and used to assess the risk to piscivorous fishes and wildlife represented by the contaminants measured in the fish.

During the period spanned by the NPMP-NCBP, the use of bioaccumulable pesticides and other contaminants in the U.S. was regulated, and concentrations in biota and other media declined (e.g., Schmitt et al. in press). Concomitantly, concerns about new pesticides and the cumulative effects of exposure to multiple contaminants and to other environmental stresses increased. Traditional contaminant monitoring programs, including the NCBP, relied almost exclusively on chemical analyses of selected environmental media. Although such programs provide useful information on the distribution and magnitude of the chemicals, they provide little insight regarding the effects of the measured contaminants on biota. In addition, chemical analyses alone provide no information about contaminants or their metabolites and degradation

**Figure 1.** Locations sampled in 1995 and 1997.

products that either do not persist or accumulate

## Project Objectives and General




sufficiently to measure; for which there are no methods or analytical standards; or about the cumulative effects of multiple contaminants or contaminants and other environmental factors.

For the above-stated reasons, the BEST program incorporates a weight-of-evidence approach that includes bioassays and biomarkers (biological responses to contaminants at the sub-organism level) in addition to chemical analyses to monitor and assess the effects of contaminants on biota. The suite of methods, including the subset selected for evaluation in this project, was based on maximum sensitivity, cost-effectiveness, and the ability of the methods to detect a wide variety of contaminants and their effects in freshwater fish (NBS 1996). In this paper we present an overview of the large rivers pilot project and some preliminary findings.

## Approach

The specific objectives of the project are to: (1) document the occurrence and distribution of contaminants and their effects on fish in the Mississippi, Columbia, and Rio Grande basins; (2) quantitatively evaluate the performance of aquatic methods used by the BEST program. (3) evaluate the logistic and administrative feasibility of implementing a large-scale monitoring program through partnerships with other bureaus, USGS divisions and programs, universities, and BRD research centers; and (4) evaluate opportunities for collaboration with the National Stream Quantity Accounting Network (NASQAN-II--Hooper et al. 1996) and National Water Quality Assessment (NAWQA--Hirsch et al. 1988) programs of the USGS-Water Resources Division.

## METHODS OF STUDY

### Sampling Locations

A total of 71 sites in the three basins were sampled, as was a reference site located at the BRD research center in Leetown, WV. (Figure 1, Table 1). The study sites included NCBP stations in each basin (34 of the 38 NCBP stations in the Mississippi River basin; 10 stations in the Columbia River basin; and four stations in the Rio Grande basin). In the Rio Grande and Columbia River basins, fish were also collected at 12 NASQAN-II sites (Bartish et al. 1997). The NASQAN-II program, which presently operates in four major U.S. river systems, collects water on a regular basis for analysis of hydrophilic pesticides, nutrients, and elemental contaminants (including metals). Sediments and additional water quality data are also collected at NASQAN II sites (Hooper et al. 1996). The NCBP and NASQAN II sites are typically located in large rivers, often at or near confluences or other key features such as dams. In the Mississippi River basin (Schmitt et al. 1995), fish were also collected in two NAWQA study units (13 sites)--four in the Eastern Iowa Basins Study Unit and nine in the Mississippi Embayment Study Unit (Figure 1, Table 1). The NAWQA sites are

**Table 1.** Locations sampled by the BEST program in 1995 and 1997. Station 400 was a reference site.

| Sta. No. | River | Nearest city or feature | State |
|---|---|---|---|
| 15 | Mississippi | Luling | LA |
| 23 | Kanawha | Winfield | WV |
| 24 | Ohio | Marietta | OH |
| 25 | Cumberland | Clarksville | TN |
| 26 | Illinois | Beardstown | IL |
| 27 | Mississippi | Guttenburg | IA |
| 28 | Arkansas | Pine Bluff | AR |
| 29 | Arkansas | Keystone Reservoir | OK |
| 30 | White | Devall's Bluff | AR |
| 31 | Missouri | Nebraska City | NE |
| 32 | Missouri | Garrison Dam | ND |
| 33 | Missouri | Great Falls | MT |
| 67 | Allegheny | Natrona | PA |
| 68 | Wabash | New Harmony | IN |
| 69 | Ohio | Cincinnati | OH |
| 70 | Ohio | Metropolis | IL |
| 71 | Tennessee | Savannah | TN |
| 72 | Wisconsin | Woodman | WI |
| 73 | DesMoines | Keosauqua | IA |
| 74 | Mississippi | Little Falls | MN |
| 75 | Mississippi | Cape Girardeau | MO |
| 76 | Mississippi | Memphis | TN |
| 77 | Arkansas | John Martin Res. | CO |
| 78 | Verdigris | Oologah | OK |
| 79 | Canadian | Eufaula | OK |
| 80 | Yazoo | Redwood | MS |
| 81 | Red | Alexandria | LA |
| 82 | Red | Lake Texoma | TX |
| 83 | Missouri | Hermann | MO |
| 84 | Big Horn | Hardin | MT |
| 85 | Yellowstone | Sidney | MT |
| 86 | James | Olivet | SD |
| 89 | Platte | Louisville | NE |
| 90 | Kansas | Bonner Springs | KS |
| 111 | Mississippi | Lake City | MN |
| 112 | Mississippi | Dubuque | IA |
| 201 | Big Sunflower | Anguilla | MS |
| 202 | Bogue Phalia | Leland | MS |
| 203 | Steele Bayou | Rolling Fork | MS |
| 204 | Tensas | Tendal | LA |
| 205 | S. Skunk | Oskaloosa | IA |
| 206 | Iowa | Morengo | IA |
| 207 | Cache | Cotton Plant | AR |
| 208 | Cache | Egypt | AR |
| 209 | S. Fork Iowa | New Providence | IA |
| 210 | Iowa | Rowan | IA |
| 211 | Cedar | St. Charles City | IA |
| 212 | Lt. River Ditch | Moorehouse | MO |
| 213 | Wolf | LaGrange | TN |
| 16 | Rio Grande | Mission | TX |
| 63 | Rio Grande | Elephant Butte Res. | NM |
| 64 | Rio Grande | Alamosa | CO |
| 65 | Pecos | Red Bluff Lake | TX |
| 511 | Rio Grande | Harlingen | TX |
| 512 | Rio Grande | Brownsville | TX |
| 513 | Rio Grande | Falcon Dam | TX |
| 514 | Rio Grande | Amistad Res. | TX |
| 515 | Rio Grande | Langtry | TX |
| 516 | Rio Grande | El Paso | TX |
| 41 | Snake | Hagerman | ID |
| 42 | Snake | Lewiston | ID |
| 43 | Salmon | Riggins | ID |
| 44 | Yakima | Granger | WA |
| 45 | Willamette | Oregon City | OR |
| 46 | Columbia | Cascade Locks | OR |
| 96 | Snake | Ice Harbor Dam | WA |
| 97 | Columbia | Pasco | WA |
| 98 | Columbia | Grand Coulee | WA |
| 117 | Flathead | Creston | MT |
| 501 | Columbia | B. A. Terminal | OR |
| 502 | Columbia | Warrendale | OR |
| 503 | Columbia | Vernita Bridge | WA |
| 504 | Columbia | Northport | WA |
| 505 | Willamette | Portland | OR |
| 506 | Columbia | Vancouver | WA |
| 400 | Leetown Res. | Leetown | WV |

typically located in lower-order streams; as such, their inclusion allows for an examination of the issue of scale relative to biomonitoring results. Extensive additional information on land use, land cover, and the condition of the aquatic community is also collected at NAWQA sites.

## Specific Methods Included in the Pilot Study

The methods selected for the pilot were those BEST program methods for aquatic habitats (NBS 1996) that could be applied to fish (Table 2). The fish carcasses were analyzed for elemental and organic chemical contaimnants following NCBP protocol (Schmitt et al. in press). Extracts of the carcass samples were tested with the H4IIE rat hepatoma cell bioassay, a sensitive *in vitro* method for detecting and quantifying planar halogenated hydrocabons such as the polychlorinated dioxins and biphenyls (Tillitt et al. 1991). Liver tissue was assayed for ethoxyresorufin-O-deethylase (EROD) activity, an indicator of exposure to polycyclic and polyhalogenated hydrocarbons (Pohl and Fouts 1990). Fish were examined and scored using a quantitative health assessment based on the methods of Goede (1989), Adams (1990), and Adams et al. (1993). Selected tissues and organs were also examined histopathologically for evidence of parasites, tumors, and other lesions potentially indicative of chemical exposure (Hinton et al. 1992).

Several markers of endocrine modulation or disruption in fish that had been identified as promising by the BEST program (NBS 1996) and which were being field tested by the NAWQA program (Goodbred et al. 1994; 1996) were also incorporated into the suite of fish health indicators. These included plasma concentrations of reproductive hormones (Guillette et al. 1994) and the protein vitellogenin, an egg yolk precursor (Folmar et al 1996). In addition, the composite fish carcass samples were analyzed for stable isotopes of nitrogen ($\delta^{15}N$), a potential indicator of trophic position and nitrogen source (Cabana and Rasmussen 1996), as a corollary variable and tracer of sewage inputs.

Many contaminants are also known or suspected to suppress immune system function in animals (e.g., Matthews et al. 1990; Hutchinson and Simmonds 1994), and immune system indicators were consequently among the methods included (NBS 1996) The immune system indicators are splenic macrophage aggregates and plaxma lysozyme activity (Blazer et al. 1994).

## Field Procedures

Sampling was conducted in the fall of 1995 in the Mississippi River basin and in the fall of 1997 in the Columbia and Rio Grande basins. The preferred collection method was D.C. electrofishing from a boat. Seining or hook-and-line capture was also permitted. In keeping with NCBP protocol, one piscivorous and one bottom-dwelling species was sought at each site. The primary species targeted at each site were common carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) as the bottom-dweller and piscivore, respectively. Other fishes, mostly other black basses (*Micropterus* spp.), percids (*Stizostedion* spp.), salmonids, suckers (Catostomidae), and catfishes (Ictaluridae), were collected as alternates depending on habitat and location. These fishes were selected because the responses of the biological markers have been documented in them and because they were expected to be the most widespread in their distributions. In addition, these taxa had been collected historically at many NCBP sites (Schmitt et al. in press) and are on the list of NAWQA target taxa (Crawford et al. xx). At NCBP, NASQAN-II, and reference sites, the collection target was 40 fish (10 of each sex of each of two species). At the NAWQA sites, only one species was normally collected (common carp at 11 sites, largemouth bass at one site, and both species at one site).

Following capture, fish were held alive until processed for sample collection (generally < 4 h). Fish were weighed and measured, and samples of blood and liver (the latter cryogenically frozen for the EROD assay, preserved for histopathology); and of gill, kidney, spleen, and gonads (preserved for histopathology) were collected. Weights of the liver (most species), spleen, and gondads were also

determined as part of the necropsy-based health assessment. Plasma was obtained from the blood samples by centrifugation and cryogenically frozen for analysis of lysozyme activity, sex steroid hormone concentrations (i.e., testosterone, 11-ketotestosterone, and 17β-estradiol), and vitellogenin. Individual fish were then composited by species and gender for chemical extraction and analysis, including the H4IIE bioassay. Details of the laboratory procedures are given elsewhere (see references listed in Table 2).

**Table 2.** Methods incorporated into the large rivers pilot project.

| Method | Description | Tissue(s) examined | Sensitivity | Primary reference(s) |
|---|---|---|---|---|
| Histopathology | Microscopic examination for the presence of lesions; can provide early indication of chemical exposure | Liver, gill, gonads, spleen, and kidney | Overall organism health and contaminants | Hinton et al. (1992) |
| Ethoxyresorufin - O-deethylase (EROD) activity | Enzyme induction by planar hydrocarbons | Liver | PCBs, PAHs, dioxins, and furans | Pohl and Fouts (1980); Kennedy and Jones (1994) |
| Lysozyme activity | A disease resistance factor that can be suppressed in the presence of contaminants | Blood plasma | Overall organism health | Blazer et al. (1994) |
| Macrophage aggregate analysis | Macrophages are important in the immune system, serving as a first line of defense for the organism and as an antigen processing cell | Spleen, hemopoetic kidney, and liver | Multiple contaminants including PAHs and metals | Blazer et al. (1994) |
| H4IIE bioassay | A screening tool to determine the presence of certain classes of planar halogenated compounds | Whole fish (composites) | PCBs, dioxins and furans; and PAHs | Tillitt et al. (1991) |
| Vitellogenin | A precursor of egg yolk, normally synthesized in the liver of female fish | Blood plasma | Endocrine modulating compounds | Folmar et al. (1996) |
| Chemical analyses | Organochlorine chemical residues and elemental contaminants | Whole fish (composites) | Specific analytes | Schmitt et al. (in press) |
| Somatic indices | The relative mass of some organs is often indicative of chemical exposure | Gonads, spleen, liver | Overall organism health | Grady et al. (1993) |
| Stable N isotopes ($^{14}N$ and $^{15}N$) | The ratio of $^{15}N$ to $^{14}N$ ($\delta^{15}N$) increases with trophic position and sewage pollution | Whole fish (composites) | Trophic position, nitrogen sources | Cabana and Rassmussen (1996) |
| Necropsy-based fish health assessment | Visual assessment of external/internal anomalies (e.g., lesions, parasites, tumors), which may indicate contaminant-related stress | All | Overall organism health | Goede (1996); Adams et al. (1993) |

## PRELIMINARY RESULTS

A total of 2,323 fish were collected in the three basins during 1995 and 1997. Of these, 72% were common carp and largemouth bass. Common carp were collected at 63 sites and largemouth bass at 35 sites. The predominant alternative species in the Mississippi River basin were smallmouth bass (*Micropterus*

*dolomieu*), spotted bass (*M. punctulatus*), goldeye (*Amphiodon alosoides*) and sauger (*Stizostedion canadense*). In the Columbia River basin the predominant alternative species were largescale sucker (*Catostomus macrocheilus*), smallmouth bass and northern squawfish (*Ptychocheilus oregonensis*), and in the Rio Grande basin they were channel catfish (*Ictalurus punctatus*), and white bass (*Morone chrysops*).

Concentrations of organochlorine chemicals at NCBP sites in the Mississippi River basin were generally lower in 1995 than when last sampled in 1986 (Schmitt et al. in press); almost no unmetabolized *p,p'*-DDT (the parent insecticide) was present, and concentrations of many pesticides were below detection levels. DDT-derived residues (mostly *p,p'*-DDE) in fish from historic use on cotton remained evident in the southern parts of the watershed; concentrations were highest at the NAWQA sites in the Mississippi Embayment Study Unit. Concentrations of toxaphene, which was also used extensively and almost exclusively on cotton, followed a similar pattern. Total DDT concentrations in fish from these sites, although much lower than historically reported values, nevertheless remained sufficiently high to represent a hazard to sensitive species of fish-eating birds (Anderson 1975; USEPA 1980). Because toxaphene is a complex mixture of chemicals that weather at different rates, it is not possible to assess the ecological risk of weathered mixtures based on total concentrations.

Point sources of contaminants (i.e. pesticide manufacturing or formulating facilities) remained evident at some large river NCBP sites in the Mississippi basin; conversely, concentrations of some agricultural chemicals were higher at NAWQA sites, which are typically located on lower order streams. As noted above, concentrations of toxaphene and DDT (as *p,p'*-DDT) remained evident at many sites in the south, especially in the Mississippi Embayment Study Unit. Cyclodiene pesticides (dieldrin, endrin, and chlordanes, including heptachlor epoxide) were present in many agricultural areas, especially in the Corn Belt; concentrations were elevated relative to other areas of the Mississippi River system in the Eastern Iowa Basins Study Unit. These pesticides were also present in high concentrations in fish from the Mississippi River at Memphis, Tennessee, where there is a history of cyclodiene pesticide contamination from a site of synthesis (Schmitt et al. 1990; in press). Concentrations of cyclodiene pesticides approached levels of concern for fish-eating wildlife at Memphis (Eisler 1990).

In contrast to concentrations of organochlorine chemicals, which generally decreased since the NCBP stations were last sampled in 1986, concentrations of mercury increased at some sites in the Mississippi River basin. As expected, mercury concentrations were higher in the predator species (e.g., largemouth and smallmouth bass, walleye) than in bottom fish (common carp, suckers). Greatest concentrations were detected in largemouth bass from the Mississippi River at Memphis. Opinions on the toxicity of mercury to fish-eating wildlife vary; according to some (Yeardley et al. 1998), concentrations at some sites exceeded toxicity thresholds.

Selenium concentrations in the Mississippi basin were generally low except for one site on the upper Arkansas River (John Martin Reservoir). At that site, concentrations in common carp were sufficiently high to constitute a risk to the fish themselves as well as to fish-eating wildlife (Lemly 1996). Selenium concentrations in common carp from John Martin Reservoir have been increasing for approximately 10 y (Schmitt et al. in press).

Greatest dioxin-like activity, as measured by the H4IIE bioassay, occurred in fish from NAWQA sites in the Mississippi Embayment Study Unit. Combined results of organochlorine pesticide analyses and those from the H4IIE bioassay and EROD measurement suggest the presence of other organic contaminants in the lower Mississippi River valley.

Histopathological analysis revealed the presence of ovarian cells (oocytes) in male bass from five sites. At one site (Mississippi River at Lake Pepin, MN), oocytes were present in 8 of the 11 male smallmouth bass examined. Vitellogenin was present in the serum of male

largemouth bass from at least 9 sites. Seemingly abnormal ratios of sex steroid hormones were also found in common carp and bass from several sites. Collectively, these findings suggest that fish from some sites are exposed to endocrine-modulating substances.

## RELATED ACTIVITIES OF THE BEST PROGRAM

In addition to the large rivers pilot study described here, the BEST program is conducting and supporting a number of related activities. These include in-depth reviews and syntheses of extant information on the biomarkers being evaluated in this project alsong with the development of supporting data bases and bibliographies. Additional projects include: (1) An assessment of contaminant information from Atlantic Coast estuaries to determine suitable avian wildlife species for environmental monitoring. (2) Examination of fish and wildife mortality incidence reports as a means for identifying new contaminant problem areas. (3) Providing technical assistance to the FWS in the conduct of standardized, comprehensive assessments of contaminant threats to National Wildlife Refuges. And (4) Conducting pilot projects with the National Park Service to further evaluate and refine the contaminant assessment process developed for FWS refuges.


### Acknowledgments

Most of the field portions of this study were conducted by FWS Environmental Contaminants Specialists duty-stationed at Ecological Services Field Offices throughout the Mississippi River, Columbia River, and Rio Grande basins, and by NAWQA biologist at the WRD District offices in Mississippi, Illinois, and Texas. T.J. Kubiak of the FWS was a co-investigator in the 1995 portion of the study and coordinated much of the early FWS support. M. Wilson and S. Zylstra of the FWS were co-investigators and coordinated 1997 field activities. N.D. Denslow, of the University of Florida-Gainesville, supervised the vitellogenin analyses.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