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The Oak Ridge Y-12 Plant Biological Monitoring and Abatement Program for East Fork Poplar Creek

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J. M. Loar
S. M. Adams
L. J. Allison
J. M. Giddings
J. F. McCarthy
G. R. Southworth
J. G. Smith
A. J. Stewart

Environmental Sciences Division
Publication No. 3348

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ENVIRONMENTAL SCIENCES DIVISION

THE OAK RIDGE Y-12 PLANT BIOLOGICAL MONITORING AND ABATEMENT PROGRAM FOR EAST FORK POPLAR CREEK

J. M. Loar, S. M. Adams, L. J. Allison,[1] J. M. Giddings,[2]
J. F. McCarthy, G. R. Southworth, J. G. Smith,[1]
and A. J. Stewart

[1]Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831.
[2]Springborn Bionomics, Inc., Wareham, Massachusetts 02571.

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Prepared for
C. C. Hill, Manager
Department of Environmental Management
Health, Safety, Environment, and Accountability Division
The Oak Ridge Y-12 Plant
Oak Ridge, Tennessee 37831

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LIST OF ACRONYMS

BCF	Bioconcentration factors
BMAP	Biological Monitoring and Abatement Program
CFS	Cubic feet per second
DOE	U.S. Department of Energy
DOM	Dissolved organic matter
EC50	Median effective concentration
EFPC	East Fork Poplar Creek
EFPCK	East Fork Poplar Creek Kilometer
EPA	U.S. Environmental Protection Agency
ERDA	Energy Research and Development Administration
FDA	Food and Drug Administration
LC50	Median lethal concentration
LOEC	Lowest-observed-effect concentration
NHP	New Hope Pond
NOEC	No-observed-effect concentration
NPDES	National Pollutant Discharge Elimination System
ORNL	Oak Ridge National Laboratory
PAHs	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyls
PCP	Sodium pentachlorophenate
TDHE	State of Tennessee Department of Health and Environment
TDPH	Tennessee Department of Public Health
TVA	Tennessee Valley Authority
USGS	U.S. Geological Survey
WPCP	Water Pollution Control Program

PREFACE

The proposed Biological Monitoring and Abatement Program (BMAP) for East Fork Poplar Creek as outlined in this document will be conducted for the duration of the National Pollutant Discharge Elimination System (NPDES) permit issued for the Oak Ridge Y-12 Plant on May 24, 1985. The basic approach to biological monitoring utilized in this Program was developed by staff in the Environmental Sciences Division at Oak Ridge National Laboratory (ORNL) at the request of the Oak Ridge Y-12 Plant. The proposed BMAP is based on preliminary discussions held on October 25, 1984, and April 4, 1985, between staff of Martin Marietta Energy Systems, Inc. (ORNL, the Oak Ridge Y-12 Plant, and Central Management), the U.S. Department of Energy, the U.S. Environmental Protection Agency, and the State of Tennessee Department of Health and Environment (TDHE). The plan also reflects the results obtained from a reconnaissance of the proposed sampling sites on East Fork Poplar Creek conducted on May 9, 1985, by staff from ORNL, the Oak Ridge Y-12 Plant, and TDHE. Because the composition of existing effluent streams entering New Hope Pond will be altered shortly after the NPDES permit is issued, baseline (pre-remediation) conditions in East Fork Poplar Creek will exist only for the next few months. Consequently, preliminary studies of the fish population, as required by Tasks 2, 3, and 4 (see Sections 2.2 through 2.4, respectively), were initiated on May 14, 1985.

The approach to biological monitoring described in this BMAP represents a combination of established monitoring protocols with more innovative state-of-the-art techniques to determine regulatory compliance. Although details are provided of the specific procedures to be utilized in the initial stages of the Program, experimental designs associated with studies that will be implemented after the first year are described in less detail. The overall strategy will be to utilize the results obtained in the initial characterization studies to define the scope of future monitoring efforts. Such efforts may require more intensive sampling than initially proposed in some areas (e.g., additional toxicity testing if initial results indicate poor survival or growth) and a reduction in sampling intensity in others (e.g., reduction

in benthic invertebrate sampling frequency from monthly to bimonthly or quarterly after the first year). By using the results of previous monitoring efforts to define the needs and short-term goals of future studies, an effective, integrated monitoring program can be developed to protect the ecological integrity of East Fork Poplar Creek.

ACKNOWLEDGMENTS

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ABSTRACT

LOAR, J. M., S. M. ADAMS, L. J. ALLISON, J. M. GIDDINGS, J. F. MCCARTHY, G. R. SOUTHWORTH, J. G. SMITH, and A. J. STEWART. 1989. The Oak Ridge Y-12 Plant Biological Monitoring and Abatement Program for East Fork Poplar Creek. ORNL/TM-10265. Oak Ridge National Laboratory, Oak Ridge, Tennessee. 89 pp.

In May 1985, a National Pollutant Discharge Elimination System permit was issued for the Oak Ridge Y-12 Plant, a nuclear weapons components production facility located in Oak Ridge, Tennessee, and operated by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy. As a condition of the permit, a Biological Monitoring and Abatement Program (BMAP) was developed to demonstrate that the effluent limitations established for the Oak Ridge Y-12 Plant protect the classified uses of the receiving stream (East Fork Poplar Creek), in particular, the growth and propagation of fish and aquatic life, as designated by the Tennessee Department of Health and Environment. A second purpose for the BMAP is to document the ecological effects resulting from implementation of a water pollution control program that will include construction of nine new wastewater treatment facilities over the next 4 years. Because of the complex nature of the effluent discharged to East Fork Poplar Creek and the temporal and spatial variability in the composition of the effluent (i.e., temporal variability related to various pollution abatement measures that will be implemented over the next several years and spatial variability caused by pollutant inputs downstream of the Oak Ridge Y-12 Plant), a comprehensive, integrated approach to biological monitoring was developed for the BMAP.

The proposed BMAP consists of four major tasks that reflect different but complementary approaches to evaluating the effects of the Oak Ridge Y-12 Plant effluents on the biotic integrity of East Fork Poplar Creek. These tasks include (1) ambient toxicity testing, (2) bioaccumulation studies, (3) biological indicator studies, and (4) ecological surveys of the benthic invertebrate and fish communities.

Ecological effects will be examined at different levels of organization, from individual tissues to populations and communities. A variety of approaches, including laboratory studies, manipulative field experiments, and direct instream sampling of biota, will be used to identify casual mechanisms underlying the observed ecological effects. The overall biomonitoring plan combines established monitoring protocols with innovative, state-of-the-art techniques to document regulatory compliance and to ensure environmental protection and restoration.

1. INTRODUCTION

On May 24, 1985, a National Pollutant Discharge Elimination System (NPDES) permit was issued for the Oak Ridge Y-12 Plant, a nuclear weapons production facility operated by Martin Marietta Energy Systems, Inc. for the U.S. Department of Energy (DOE). As specified in Part III(C): Special Condition No. 7 of the permit, a plan for the biological monitoring of the East Fork Poplar Creek (EFPC) shall be submitted to the U.S. Environmental Protection Agency (EPA) and the State of Tennessee Department of Health and Environment (TDHE) within 90 days of the effective date of the permit. The Biological Monitoring and Abatement Program (BMAP), as outlined in Sect. 2, has been developed to meet this requirement.

1.1 OBJECTIVES

The proposed BMAP was developed to meet two major objectives. First, studies were designed to provide sufficient data to demonstrate that the effluent limitations established for the Oak Ridge Y-12 Plant protect and maintain the classified uses of EFPC, as identified in the State of Tennessee Water Quality Management Plan for the Clinch River Basin (TDPH 1978). The two most significant uses of EFPC are (1) growth and propagation of fish and aquatic life and (2) recreation, including fishing and swimming. Primarily because of elevated levels of mercury in fish, fishing and swimming have been prohibited by the TDHE since November 1982. Adequate data are not available to determine whether or not the use of the stream for fish and aquatic life is presently being met. Consequently, the major emphasis of the BMAP will be directed toward this use.

A second major objective of the biological monitoring that will be conducted on EFPC is to document the effects on stream biota resulting from implementation of a Water Pollution Control Program (WPCP) at the Oak Ridge Y-12 Plant (Y-12 1985). The Program consists of strategies to (1) eliminate direct discharges of wastewaters to EFPC and (2) minimize the inadvertent release of pollutants to the environment. Significant elements of the WPCP include (1) construction of numerous collection and

storage facilities; (2) construction of nine major new wastewater treatment facilities; and (3) development of numerous countermeasures to reduce or preclude the release of pollutants to EFPC: a Best Management Practices Plan (Y-12 1985, Sect. 4.5.1); Area-Source Pollution Control Management (Y-12 1985, Sect. 4.5.2); a Spill Prevention, Control, Countermeasures, and Contingency Plan (Pritz and Sanders 1982; Y-12 1985, Sect. 4.5.3); and various spill prevention projects (Y-12 1985, Sects. 4.5.4 and 4.5.5). Unlike the first objective in which monitoring will be utilized to determine compliance, the second objective is directed toward evaluating the effects of specific pollution control measures, also utilizing biological criteria. New wastewater treatment facilities, for example, may either reduce or, in some cases, increase the levels of contaminants presently discharged to EFPC. Construction of these facilities constitutes major remedial actions when viewed within the context of past waste management practices. Organic contaminants will no longer be disposed of in the burial grounds in Bear Creek Valley west of the Oak Ridge Y-12 Plant but instead will be treated and discharged to EFPC. Incorporation of waste treatment in the overall waste management operation at the Oak Ridge Y-12 Plant will reduce the adverse effects on Bear Creek watershed but may alter the existing environment in EFPC. To understand the resultant ecological effects (i.e., identify causal relationships) of effluent discharges that are complex and will be changing over time requires a multitiered yet integrated approach to biological monitoring.

The proposed BMAP consists of several tasks that examine environmental effects at different levels of organization ranging from individual tissues to populations and communities. A variety of approaches, including diagnostic laboratory bioassays, bioindicator studies, manipulative field experiments, and direct field sampling of stream invertebrates and fish, will be utilized to identify causal mechanisms underlying observed effects. Although instream sampling of biota is often used to determine the overall ecological health of a stream, the application of bioassays and bioindicators (see Sects. 2.1 and 2.3, respectively) are especially useful as short-term indicators of potential adverse effects on stream communities. Documentation of the

effects and also of the causes of the observed effects is especially important in monitoring new wastewater treatment facilities during initial operation. Potential adverse effects must be recognized quickly so the information can be used to identify corrective measures and evaluate alternatives for plant operation. A single approach utilizing only instream biotic surveys (Sect. 2.4) would be inadequate for this purpose because of the long period of time (months to years) required to identify chronic effects on the population or community. Moreover, effects due to toxicant stress may be masked by the inherent variability of ecological systems or of the physical environment.

Several new wastewater treatment facilities will be placed on-line over the next few years. While individual facilities may immediately alter the toxicity of effluent discharges to EFPC, their effect on contaminant levels in biota may only be evident after a much longer period of time. Development of a predictive model to evaluate the time course of contaminant levels in biota following changes in effluent chemistry (Sect. 2.2) will ensure that appropriate actions are taken to reduce biotic contamination.

To understand the nature and complexity of the existing environment, a brief description of the EFPC watershed is given below. A detailed description of each of the four tasks included in the BMAP is given in Sects. 2.1 through 2.4.

1.2 DESCRIPTION OF STUDY AREA

The EFPC drainage basin is located near the northern boundary of the DOE Oak Ridge Reservation and has an area of 77.2 km^2 from its headwaters to its mouth near Poplar Creek Kilometer 8.8.* The Oak Ridge Y-12 Plant is located on the headwaters of the creek, which originates on the north slope of Chestnut Ridge. Discharges from the plant average 9-12 cfs and constitute the majority of the flow in the upper reaches. Just east of the plant boundary, the creek flows into New Hope Pond

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*Poplar Creek Kilometer 0.0 is located at the confluence of Poplar Creek with the Clinch River.

(NHP), a 2.2-ha impoundment constructed in 1963 to equalize the pH of the effluent from the Oak Ridge Y-12 Plant (Pritz and Sanders 1982). The pond is also used for neutralization, sediment retention, and spill control (including provision for oil recovery by means of skimmers or chemical treatment). Recent construction of a bypass channel around NHP permits long-term retention of hazardous chemical spills within the pond and thus provides a "last line of defense." Above NHP, the creek (approximately 1.5 km) has been channelized and receives discharges from more than 200 individual outfalls. The stream flows for a distance of 22.9 km from NHP to the confluence with Poplar Creek, a tributary of the Clinch River.

1.2.1 Geohydrology

The principal groundwater-bearing formation in the Oak Ridge area is the Knox Dolomite, which comprises 25% of the surface area of the EFPC drainage basin; another 32% of the area consists of Chickamauga limestone (McMaster 1967). One of the largest springs in the basin [Crystal Spring located near East Fork Poplar Creek Kilometer (EFPCK) 10.1*] is at the contact between the Knox and the Chickamauga limestone and has an average flow of 1.7 cfs (range 1.0-3.2 cfs, based on monthly measurements over a 2-year period) (McMaster 1967).

Mean annual flow at the U.S. Geological Survey's (USGS's) gaging station at EFPCK 5.3 is 51.9 cfs (1960-1984); maximum and minimum daily flows over the same period were 4100 and 12 cfs, respectively (Lowery et al. 1985). The maximum flow occurred on November 28, 1973; this storm resulted in 22 cm of rainfall in 48 hours (a once-in-25 year flood), based on studies conducted by Edgar (1978) in nearby White Oak Creek watershed. The lowest mean discharge for seven consecutive days with a recurrence interval of 10 years (7Q10 flow) and for three consecutive days with a recurrence interval of 20 years (3Q20) are estimated to be 12.1 and 11.5 cfs, respectively, at the outfall of NHP;

*EFPCK 0.0 is located at the confluence of East Fork Poplar Creek with Poplar Creek.

at the mouth of EFPC, these values increase to 20.1 (7Q10) and 18.9 (3Q20) cfs (R. H. Kingrea, Department of Environmental Technology, Oak Ridge Y-12 Plant, personal communication).

1.2.2 Land Use

A large portion of the EFPC watershed consists of residential and commercial developments in the city of Oak Ridge. Approximately 14 km of the stream are located on privately owned property, while the remaining 9 km are within the boundaries of the DOE Oak Ridge Reservation, including the first kilometer immediately below NHP and approximately 8 km from the mouth upstream. Much of the floodplain of the creek in the vicinity of Illinois Avenue (Fig. 1) has been developed. Farther downstream, agricultural and residential uses predominate. About 40% of the watershed is wooded (McMaster 1967).

1.2.3 Ecology

Very limited information is available from previous studies of the ecology of EFPC. The first studies were conducted in the early 1970's and were qualitative surveys of the benthic invertebrates (McClain 1972, Reece 1973). Both noted (1) low levels of dissolved oxygen below the City of Oak Ridge Wastewater Treatment Facility and (2) significant silt deposits in the creek (up to 60 cm at some locations), especially at EFPCK 16.0 near the tennis courts on Route 95 (or approximately 2 km east of Wiltshire Drive; see Fig. 1)(Reece 1973). The primary source of this siltation was most likely the water treatment plant constructed in 1943 and operated by Rust Engineering Company. The facility is situated on Pine Ridge near the intersection of Bear Creek Road and Scarboro Road (Fig. 1). Sand filters at the facility were backwashed daily and the sludge in the sedimentation basins was removed quarterly; the effluent from both processes was discharged to EFPC at two locations downstream of the Bear Creek Road bridge (McClain 1972). Estimates of the annual loading to the creek ranged from 330 to 1025 tonnes (McClain 1972, ERDA 1975). This practice was terminated in late 1978 when zero discharge was implemented (J. Hall, Rust Engineering Company, personal

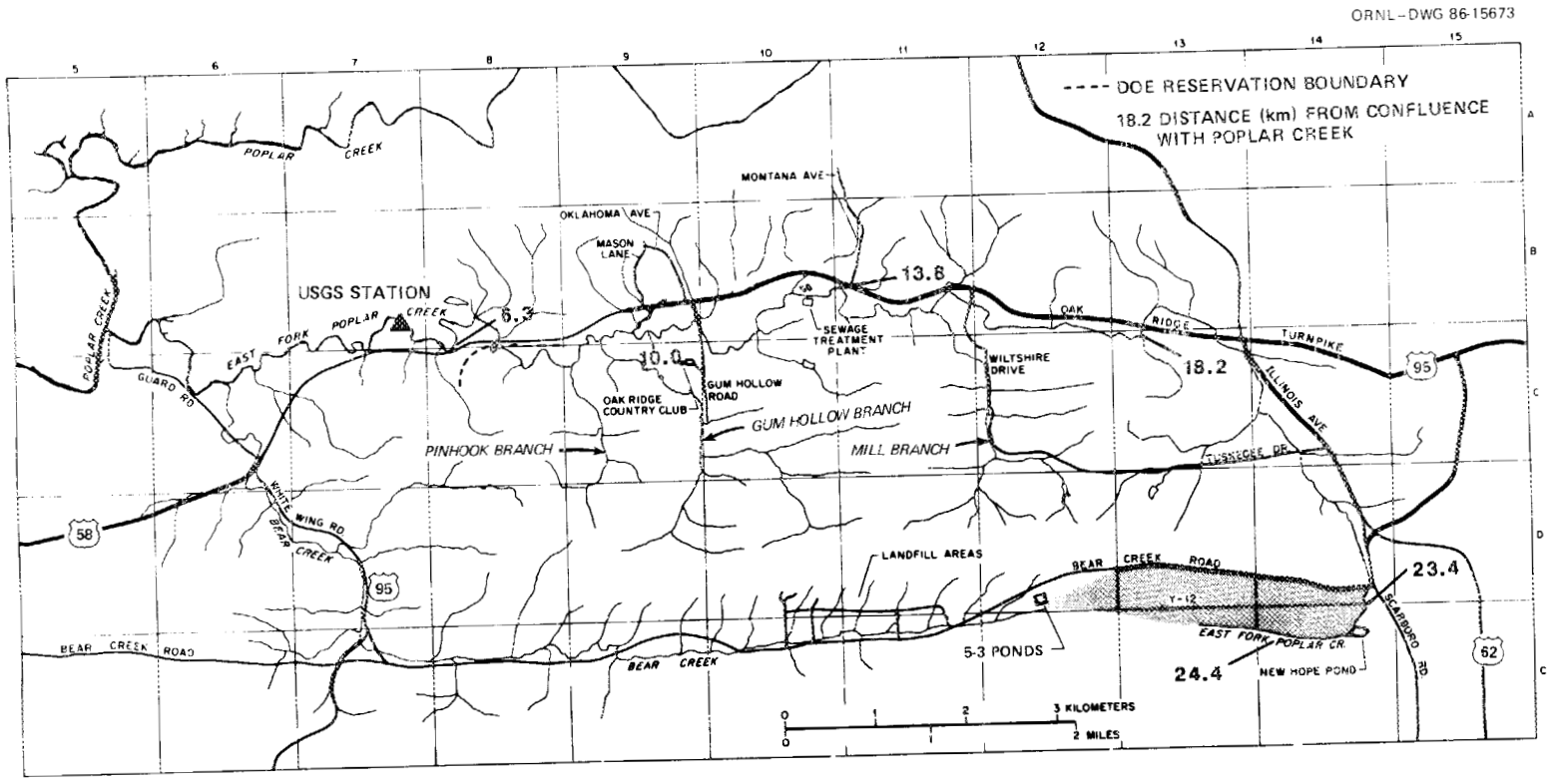

Fig. 1. East Fork Poplar Creek Study area showing location of the biological monitoring sites (•).

communication to R. R. Turner, Environmental Sciences Division, Oak Ridge National Laboratory, March 12, 1985).

Very high sediment loading could account for the low number of benthic invertebrate species that was observed in a quantitative survey conducted 1 year after the Reece (1973) survey (ERDA 1975)(Table 1). The results of this limited sampling effort suggest the community that existed 10 km downstream was more significantly impacted than the community just below the NHP outfall, even though the minimum concentration of dissolved oxygen at the outfall was consistently below 5 mg/L at the time of the survey (Table 2). Because zero discharge was implemented at the water treatment facility more than 6 years ago, a substantial recovery of the benthic communities in the middle reaches of the creek may be found when the proposed BMAP is initiated. Likewise, earlier data on the benthic community in the upper reaches below NHP may not be representative of the community that currently inhabits this area. A significant improvement in the level of dissolved oxygen has occurred since 1976 (Table 2) with the addition of an aerator near the outfall of the pond. Although a more diverse benthic taxa from that observed 10 years ago could be expected, such predictions are tenuous because dissolved oxygen may not be the only limiting factor in a complex dynamic effluent, such as that being discharged from the Oak Ridge Y-12 Plant.

No adequate historical data exist on the fish communities in EFPC. A single survey conducted on September 24, 1974, reported five species near the NHP outfall and only three species (all minnows) from a site above the City of Oak Ridge Wastewater Treatment Facility (ERDA 1975, Vol VI, Table 2.Q.6). More extensive sampling conducted by the Tennessee Valley Authority (TVA) in late spring 1984 reported 11 and 13 species from sites located in the same general area as those sampled in 1974. Although the results of the two surveys suggest that a substantial change has occurred in the EFPC fish community over the past 10 years, the results may also reflect, in part, differences in sampling intensity and methodology between the two surveys.

Table 1. Mean densities (numbers/m^2) of benthic macroinvertebrates in East Fork Poplar Creek, 1974-1975 [Three Surber samples (253-μm-mesh net) were collected in riffle areas at each site in September and November 1974 and March 1975]

Taxon	Sampling site (EFPCK)[a]		
	22.8	13.0	10.9
Coleoptera			
Optioservus sp.	--	3.6	--
Decapoda			
Cambarus sp.	1.2	2.4	--
Diptera			
Chironomidae	1666.1	357.6	3589.3
Dicranota sp.	9.6	--	--
Simulium sp.	--	2.4	--
Ephemeroptera			
Baetis sp.	1.2	--	--
Gastropoda			
Physa sp.	35.8	--	--
Nematoda			
Lumbriculidae	--	3.6	--
Oligochaeta	--	2.4	--
Tubificidae	78.9	--	159.1
Odonata			
Enallagma sp.	20.3	--	--
Ischnura sp.	16.7	--	--
Tricoptera			
Cheumatopsyche sp.	3.6	--	--
Number of taxa	9	6	2
Total density	1833.4	372.0	3748.4

[a]EFPCK = East Fork Poplar Creek Kilometer with EFPCK 0.0 located at the mouth of the creek; EFPCK 22.8 is located 50 m below New Hope Pond; EFPCK 13.0 is located 100 m above the City of Oak Ridge Wastewater Treatment Facility; EFPCK 10.9 is located 25 m below Gum Hollow Road (see Fig. 1).

Source: ERDA (1975), Table 2.Q.5.

Table 2. Concentration of dissolved oxygen in the outfall of New Hope Pond, 1971-1983

	Monitoring frequency[a]	Concentration (mg/L)			Percent compliance with standard[b]
		Mean	Maximum	Minimum	
1971	NR (n = 49)[c]	7.6	10.8	5.7	100[c]
1972	NR (n = 19)	4.3	8.4	3.0	NR
1973	weekly	NR	8.5	2.5	58
1974	weekly	NR	8.5	3.4	60
1975	weekly	NR	9.5	2.9	58
1976-1983	daily	NR	NR	NR	96-100[d]

[a]n = number of samples analyzed, NR = not reported.

[b]Minimum of 5.0 mg/l, as stated in TDPH (1971) and the NPDES permit issued in 1975.

[c]All samples were collected between October and December 1971.

[d]Compliance was 100% in each of the 8 years, except 1980 (96%) and 1982 (99%).

Source: Union Carbide Corporation 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982, 1983; Martin Marietta Energy Systems, Inc., 1984.

1.2.4 Sampling Sites

Six permanent BMAP sampling sites were selected on EFPC (Fig. 1 and Table 3). Criteria used in the selection of these locations included: (1) location of sampling sites utilized in other studies, (2) known or suspected sources of downstream pollution, (3) proximity to DOE Oak Ridge Reservation boundaries, (4) concentration of mercury in the adjacent floodplain, and (5) accessibility. In general, although previously used sampling sites below potentially contaminated tributaries outside the Reservation and those near highly contaminated floodplains were avoided or relocated upstream, six of the sites utilized in previous studies were included in the BMAP. These six sites will be routinely sampled as part of the instream monitoring task (Sect. 2.4). Additional sites may be included based on the objectives of the other tasks. For example, the tissue analyses described in Sect. 2.2 will be conducted on fish collected near the mouth of the creek because this site was included in a previous study of contaminant levels in fish (Van Winkle et al. 1984). Because of the distinctly lentic (nonflowing) environment in the lower reaches of the creek, due to the presence of dams on the Clinch and Tennessee rivers, this site was not included in the instream monitoring studies. The need for this and possibly other sites on Poplar Creek below the confluence with EFPC will be based on the results of the ambient bioassays at these locations. Finally, multiple reference (control) sites, as appropriate, will be selected for each of the four tasks. Such sites will not be restricted to small, undisturbed streams on the Reservation but will also include larger watersheds that are outside the Reservation boundaries.

1.2.5 Other Characteristics of EFPC that Influence Biological Monitoring

As a study area for addressing questions related to compliance and biological monitoring in general, the EFPC watershed presents some unique problems. Because the Oak Ridge Y-12 Plant is located on the headwaters of the creek, no upstream reference site exists. Relatively undisturbed watersheds, even those like Mill Branch and Gum Hollow

Table 3. Location and description of the six biological monitoring sites on East Fork Poplar Creek

Distance below New Hope Pond (NHP) (km)	Description
-0.1	Above NHP
0.2	100 m below the NHP bypass channel
5.0	25 m below the bridge-crossing on Route 95 at Jefferson Avenue
9.5	500 m above the City of Oak Ridge Wastewater Treatment Plant
12.5	200 m below the bridge-crossing at Gum Hollow Road (adjacent to Oak Ridge Country Club)
16.5	1000 m below the bridge-crossing on Route 95

Branch that are within the EFPC drainage basin, may have different geological characteristics (McMaster 1967) that would influence hydrology, especially groundwater-surface water relationships.

Another important consequence of siting a large facility like the Oak Ridge Y-12 Plant in the headwaters is the minimal dilution of the effluent discharged via NHP. Plant discharges account for as much as 90% of the stream flow below NHP (T. Butz, Environmental Coordinator, Oak Ridge Y-12 Plant, personal communication to F. Munger, staff writer, Knoxville News Sentinel, July 1984). Even near the mouth of the creek, only a fivefold dilution can be expected during average stream flow conditions. On the other hand, the large contribution of plant discharges to stream flow enhances flow stability and minimizes streambed dewatering. The minimum daily flow recorded at EFPCK 5.3 was 12 cfs, whereas the minimum flow recorded at the USGS gaging station on Poplar Creek (approximately 13.5 km above the confluence with EFPC) was 4.0 cfs for a drainage basin that is more than four times as large as the EFPC basin (Lowery et al. 1985).

Two other characteristics of the study area are probably not unique but may occur in other streams that receive industrial or municipal wastes. First, the composition of the effluent discharged to EFPC will change over the next several years. As discussed previously, some contaminant concentrations will decrease (e.g., mercury) while others may increase (e.g., some organics). Second, any ecological effects that are observed several kilometers below NHP could, in part, be related to other pollution sources downstream. Although the City of Oak Ridge Wastewater Treatment Facility could be the most significant point source, other nonpoint sources, including runoff from agricultural and commercial development, may also be important.

2. BIOLOGICAL MONITORING TASKS

Because of the complex nature of the effluent discharged to EFPC and the temporal and spatial variability in the composition of the effluent (i.e., temporal variability related to various pollution abatement measures that will be implemented over the next several years and spatial variability due to additional pollutant inputs downstream of NHP), a multi-tiered, integrated approach to biological monitoring has been developed as the basis of the EFPC BMAP. The Program consists of four tasks: toxicity monitoring (Sect. 2.1), bioaccumulation studies (Sect. 2.2), biological indicator studies (Sect. 2.3), and instream monitoring (Sect. 2.4). These tasks combine well-established monitoring methods with more innovative state-of-the-art techniques to establish regulatory compliance.

2.1 TOXICITY MONITORING (TASK 1)

2.1.1 Introduction

Chemical analysis has traditionally been used to characterize ambient water quality in streams receiving effluents. However, toxicity testing is a useful supplement or even a preferred alternative to chemical analysis in many situations because (1) it is rapid and economical; (2) many chemical constituents of complex effluents are difficult to analyze; (3) some toxic constituents may be unsuspected and therefore not measured; (4) toxicological interactions among effluent constituents and between those constituents and other compounds in the receiving water are unpredictable; and (5) toxicity testing gives a direct indication of potential hazards to aquatic life.

Application of toxicity testing to effluent monitoring and water quality management is still a new approach (EPA 1985). Because methods for implementing the EPA policy on biomonitoring have not been finalized, this task attempts to develop a toxicity testing program that could be adapted by others in the future.

The purpose of this task is to test the water and sediments from EFPC, NHP, selected point sources, and reference streams for toxic effects on aquatic organisms and ecosystem properties. Conditions at the beginning of the program will be documented, and changes in toxicity resulting from implementation of the Water Pollution Control Program (Sect. 1.1) will be determined. Additional information on the toxicity of specific Oak Ridge Y-12 Plant effluent streams, obtained under toxicity evaluation programs associated with Toxicity Control and Monitoring Plans specified elsewhere in the NPDES permit, will be integrated with BMAP toxicity results in order to develop a more complete picture of conditions in EFPC.

2.1.2 Study Design

The toxicity monitoring task consists of seven subtasks (Table 4). The NPDES monitoring point at the outfall of NHP will be the major sampling location for evaluating the toxicity of discharges from the Oak Ridge Y-12 Plant and for documenting changes in effluent toxicity over time. Four subtasks (1a-1d) will measure the toxicity of water leaving NHP. Three subtasks (1e-1g) will measure the toxicity of water and sediments at sites on EFPC above and below NHP and on selected reference streams (probably tributaries to EFPC).

Subtasks 1a, 1b, 1d, and 1e involve two newly-developed "mini-chronic" toxicity tests, the 7-day fathead minnow (*Pimephales promelas)* larval growth test (Norberg and Mount 1985) and the *Ceriodaphnia* life-cycle test (Mount and Norberg 1984). Subtask 1c involves acute (48-h) tests with the same species. Recommended practices for effluent toxicity testing (Peltier and Weber 1985) will be followed where applicable.

Each test with NHP effluent or ambient EFPC water will include a full dilution series unless no toxicity is found in full-strength samples. The lowest-observed-effect concentration (LOEC), no-observed-effect concentration (NOEC), median lethal concentration (LC50), and median effective concentration (EC50) will be calculated for each test in which a dilution series is used. These calculations will

Table 4. List of subtasks related to toxicity monitoring

Subtask No.	Title
1a	Effluent Toxicity Tests
1b	Toxicity Persistence Tests
1c	Effluent Variability Study
1d	Point-Source Toxicity Evaluation
1e	Ambient Toxicity Tests
1f	Sediment Toxicity Tests
1g	Periphyton Community Effects Studies

be based on data for survival, growth, and (for *Ceriodaphnia*) reproduction in test samples, control water, and/or samples from reference streams.

Because there is no upstream receiving water at the Oak Ridge Y-12 Plant for use as dilution water or control water in the toxicity tests, dechlorinated tap water will be used for testing fathead minnows and hard reconstituted water will be used for *Ceriodaphnia*. Natural variability in the reference streams precludes their use for dilutions or controls when results must be compared over time, as is the case for most of this task.

If most test organisms are found to survive routinely in full-strength NHP effluent or EFPC water for the duration of each mini-chronic or acute test, only undiluted samples will be tested. Data will then consist of values for mortality, growth, and (for *Ceriodaphnia*) reproduction in undiluted samples, in control water (see below), and in water from reference streams.

Sodium pentachlorophenate (PCP) will be used as a reference toxicant (Peltier and Weber 1985). The responses of fathead minnow larvae and *Ceriodaphnia* neonates to PCP will be evaluated monthly using 24-hr acute tests (cf. Horning and Weber 1985) to determine the sensitivity of the test animals reared in the Oak Ridge National Laboratory (ORNL) Environmental Sciences Division's toxicology laboratory. Results of biomonitoring tests will be considered invalid and the tests will be repeated if (1) the reference toxicant's LC50 (concentration needed to kill 50% of the test population) falls outside of the acceptable range (i.e., ± 2 SD of the running mean), and (2) minnow growth or *Ceriodaphnia* fecundity is systematically and unusually low in all of the ambient water samples tested within a particular test period.

2.1.3 Description of Subtasks

Subtask 1a: Effluent Toxicity Tests

The objective of this subtask is to develop a long-term record of EFPC toxicity at the outfall of NHP. Samples will be tested monthly with both fathead minnows and *Ceriodaphnia*. Twenty-four-hour composite

samples will be taken daily for replacement of water during the test. If the first two tests indicate an LOEC greater than 50%, subsequent tests will include only full-strength effluent.

Subtask 1b: Toxicity Persistence Tests

Tests will be performed twice a year to measure the persistence of toxicity in samples from the NHP outfall. Twenty-four-hour composite samples will be taken daily. Two tests will be set up, one with fresh samples (as in Subtask 1a), and the other with samples that have been aerated at room temperature for 96 h. Either fathead minnows or *Ceriodaphnia* (whichever is found to be more sensitive to unaged water) will be used for these tests.

Subtask 1c: Temporal Variability Study

To determine the short-term temporal variability of NHP toxicity, a series of acute (48-h) toxicity tests with either *Ceriodaphnia* or fathead minnows will be conducted on grab samples taken periodically from the outfall of NHP. Continuous data on conductivity, pH, or other easily measured water characteristics will be examined before the study begins to determine an appropriate sampling interval for assessing effluent variability. Median lethal concentrations (LC50) will be estimated.

Subtask 1d: Point-Source Toxicity Evaluation

As information is developed on the magnitude and potential sources of toxicity in EFPC from the Oak Ridge Y-12 Plant and elsewhere (e.g., through testing related to the Toxicity Control and Monitoring Plans; see Sect. 2.1.1), acute or mini-chronic tests will be conducted as needed on specific point-source inputs above or below NHP. This testing (with either fathead minnows or *Ceriodaphnia*) will supplement data from other sources (e.g., Toxicity Control and Monitoring Programs) and enable diagnosis of toxic conditions within EFPC.

Subtask 1e: Ambient Toxicity Tests

This subtask will measure ambient toxicity at various points along EFPC and in reference streams. Water will be tested four times per year, using mini-chronic tests with both fathead minnows and *Ceriodaphnia*. Daily grab samples will be taken; if possible, sampling times will be consistent with the measured flow in the stream, so that the same parcel of water will be sampled at different points as it moves down the channel. Sampling sites will be selected after an initial survey; they will probably include sites at the NHP outfall, near the DOE Oak Ridge Reservation boundary, below the downtown area of Oak Ridge, above and below the City of Oak Ridge Wastewater Treatment Facility, at the USGS gaging station, at the mouth of EFPC, and at the mouth of Poplar Creek.

Subtask 1f: Sediment Toxicity Tests

Sediment samples from sites within EFPC and reference streams (probably the same sites as in Subtask 1e) will be tested by exposing first-instar midge larvae (*Chironomus tentans*) in flow-through systems containing sediment and dechlorinated tap water. Survival, growth, and behavioral effects (e.g., burrowing and avoidance) will be monitored.

Subtask 1g: Periphyton Community Effects Studies

Wooden troughs will be placed at several sites along EFPC and a fraction of the stream flow will be diverted through them continuously. Periphyton (the community of organisms colonizing the surfaces of the troughs) will be monitored; community biomass, productivity, and species composition will be determined.

2.1.4 Sampling

Water will be collected from sites on EFPC and elsewhere and transported to the Aquatic Ecology Laboratory at ORNL for bioassays. Refrigerated 24-h composite samples will be taken for Subtasks 1a, 1b, and 1d, and grab samples will be used for Subtasks 1c and 1e. Sediments

will be sampled by using cores or dredges as appropriate for each site (Subtask 1f). Several sediment samples will be taken at each site and combined for testing.

2.1.5 Facilities

Water Supplies

Dechlorinated process (tap) water is available for culturing bioassay organisms and as dilution water for tests with fathead minnows and *Chironomus*. Results of a recent chemical analysis of this water are presented in Table 5. This water source has been used since 1980 in the Aquatic Ecology Laboratory and has been found acceptable for growth and reproduction of *Daphnia magna*, fathead minnows, and *Chironomus tentans*. *Ceriodaphnia* will be reared and tested in aged hard reconstituted water prepared from deionized distilled water (Peltier and Weber 1985).

Temperature Controls

Environmental chambers will be used for rearing and testing daphnids and for tests with fathead minnows. Water baths will be used to control the temperature of fish stock cultures.

Instrumentation

Equipment available for this project includes recording thermometers, pH meter, dissolved oxygen meter, conductivity meter, DOC analyzer, compound and dissecting microscopes, and balances.

Computers/Statistics

IBM 3033 and DEC-10 mainframes will be used to analyze and archive data. An IBM personal computer will be used for data entry and report preparation. Programming and statistical assistance are available.

2.2 BIOACCUMULATION STUDIES (TASK 2)

2.2.1 Introduction

East Fork Poplar Creek water and sediments downstream from the Oak Ridge Y-12 Plant contain metals, organic chemicals, and radionuclides

Table 5. Analysis of dechlorinated process water[a]

Parameter	Concentration (mg/L)
Calcium	33.8
Magnesium	10.0
Potassium	1.6
Sulfate	25.5
Total organic carbon	1.8
Phosphorus	0.2
Cadmium	<0.05[b]
Chromium	0.5[b]
Mercury	<0.005[b]
Lead	<0.2[b]
Zinc	70.0[b]
Total residual chlorine	<1.0
Alkalinity	103.0
Hardness	126.0
Free CO_2	2.8
pH	7.5[c]
Conductivity	230[d]

[a]Sampled on April 4, 1985; analyzed by ORNL Analytical Chemistry Division.
[b]μg/L.
[c]pH units.
[d]μsecond/cm.

discharged over many years of operation. Fish from EFPC contained elevated levels of mercury (Van Winkle et al. 1984) and polychlorinated biphenyls or PCBs (TVA 1985), and may also contain elevated levels of other contaminants. In the next several years, advanced wastewater treatment measures will be implemented at the Oak Ridge Y-12 Plant. If such measures are effective, plant discharges will be less significant as a source of contamination to stream biota, and levels of contamination in fish should decline.

The anticipated decline in residue levels in fish could be complicated by factors acting to alter the availability of contaminants in the water column and sediments to uptake by aquatic biota (bioavailability). Thus, it is possible that contaminants currently discharged from the Oak Ridge Y-12 Plant may be present in a form relatively unavailable to aquatic biota (e.g., sorbed to suspended solids). While reducing suspended solids, waste treatment could possibly reduce aqueous concentrations of contaminants even more, resulting in an increase in the bioavailability of some contaminants. If this were the case, residue levels in fish might decline less than anticipated.

The sediments of EFPC contain elevated levels of contaminants. These can act as a source of continuing contamination of the biota through the mobilization of contaminants back into the water via diffusion and resuspension, or by accumulating in sediment-dwelling organisms which are then eaten by organisms higher up the aquatic food chain. The degree to which sediments act as a continuing source of contaminants will be affected by both the chemistry of EFPC and the species composition and function of its biological community. Since both stream chemistry and species composition are likely to change as a result of remedial actions at the Oak Ridge Y-12 Plant, the dynamics of sediment-to-biota transfer of contaminants are also likely to change. Thus, there is a need to (1) monitor the bioaccumulation of contaminants in EFPC biota throughout and following the period in which advanced waste treatment measures alter water quality and (2) understand the dynamics of contaminant accumulation in EFPC biota, with particular emphasis on bioavailability and the role of sediments as a continuing

source of contaminants. Such an understanding, coupled with the bioaccumulation monitoring, should provide a capability for predicting the environmental consequences of various remedial actions.

2.2.1.1 Effects of Physical/Chemical Factors on Uptake and Bioaccumulation

Uptake and bioaccumulation depend on physical and chemical as well as biological factors. Partitioning of contaminants between dissolved and solid phases within an aquatic environment can affect accumulation because contaminants associated with different physical phases (e.g., dissolved in water or bound to particles) exhibit different rates of uptake by aquatic organisms (McCarthy 1983, Landrum and Scavia 1983, Muir et al. 1983). In *Daphnia magna*, for example, polycyclic aromatic hydrocarbons (PAHs) dissolved in the water are taken up 10 times faster than are PAHs bound to suspended particles (McCarthy 1983).

The interactions between dissolved and particle-bound contaminants are well recognized; however, there is another, less obvious sorbent that can affect the environmental fate of some organic contaminants. This additional sorbent is dissolved organic matter (DOM), such as naturally occurring organic substances or components of complex mixtures of contaminants such as oily sludges (Boehm and Quinn 1973, Carter and Suffet 1982, Landrum et al. 1984, Southworth and Keller 1984, McCarthy and Jimenez 1985a). DOM competes with particles for binding of PAHs and can reduce the expected accumulation of contaminants in the sediment and stabilize the compounds in the water column (J. F. McCarthy and M. C. Black, Environmental Sciences Division, Oak Ridge National Laboratory, personal communication; Southworth and Keller 1984). The presence of DOM can also result in a large decrease in bioaccumulation because organic contaminants bound to DOM are essentially unavailable for uptake by either fish or aquatic invertebrates (McCarthy 1983, Leversee et al. 1983, McCarthy and Jimenez 1985b, McCarthy et al. 1985). The presence of DOM may act, therefore, to decrease the biological risk of those contaminants having the greatest potential for transfer to man via aquatic food chains.

Consideration of three physical phases of contaminants (i.e., dissolved, bound to particles, and bound to DOM or other dissolved sorbents) will be particularly important in understanding both the accumulation and remobilization of pollutants from the sediment. Because the organic contaminants identified in EFPC (PAHs and PCBs) have a high affinity for DOM and suspended particulates, sorptive interactions will be of particular importance in understanding contaminant behavior and bioaccumulation in EFPC.

2.2.1.2 Effects of Biotic Factors on Bioavailability

Biological factors also influence bioavailability. The potential for accumulation of contaminants by an organism is related to the lipid (fat) content of the organism and the hydrophobicity of the compound (meaning literally "water hating"; compounds that are hydrophobic exhibit a much higher affinity for nonpolar solvents such as lipids or octanol, than for polar solvents such as water)(Neely et al. 1974, Roberts et al. 1977, Veith et al. 1979, Kenaga and Goring 1980). Thus, seasonal or stress-related changes in lipid reserves (Adams and McLean 1985, Adams et al. 1985a) are likely to influence the body burden (Thomann and Connolly 1984). Physiological factors also control bioaccumulation. Changes in respiratory demand due to pollution-induced stress or to normal physiological and seasonal factors such as temperature, digestive activity, reproduction, food-seeking activity, and growth may influence the rate at which contaminants are accumulated (McKim and Goeden 1982, McKim and Heath 1983, Thomann 1981, Niimi and Oliver 1983).

The body burden will also be controlled by the rates at which contaminants are eliminated or detoxified by the organism. These rates will depend on the chemical structure of the pollutant, temperature, feeding rates, fish lipid content, and induction of the detoxification enzymes (Roberts et al. 1977, Bruggeman et al. 1981, Fagerstrom et al. 1974). Because of the complexity of the interactions between environmental and physiological factors, changes in effluent discharges at the Oak Ridge Y-12 Plant will not necessarily be reflected in commensurate changes in pollutant body burdens in EFPC organisms. For

example, if pollutant discharges were decreased in late spring, body burdens might still be higher in summer than before the remedial action if increased food availability enhanced lipid reserves and high temperatures enhanced contaminant uptake through increased ventilation rates. The impact of remedial actions on bioaccumulation must be interpreted, therefore, in the context of a suite of physicochemical, physiological and ecological interactions. For this reason, it is essential to develop an understanding of how these factors will influence contaminant bioaccumulation in EFPC in order to provide informed guidance on the impact of various proposed or instituted remedial actions at the Oak Ridge Y-12 Plant.

2.2.1.3 Effects of Complex Mixtures of Contaminants

The preceding discussion of bioavailability is based almost exclusively on studies with individual contaminants; very little is known about the influence of complex mixtures of pollutants on the sorptive or biological behavior of individual components of the mixture. In EFPC, a wide suite of pollutants has been demonstrated (Hoffman et al. 1984). While sorptive interactions of organic contaminants with particles or with DOM may not be affected by the presence of complex mixtures, such mixtures will almost certainly influence the biological uptake of individual components, due either to direct effects on membrane transport or to secondary effects due to alteration of physiological functions by other components of the mixture. Any physiological response to pollution stress, either in terms of a specific response or a general stress syndrome, will be likely to alter the bioaccumulation of contaminants in EFPC.

2.2.2 Objectives

The following are objectives of the EFPC bioaccumulation studies.

1. To determine what materials present in EFPC water accumulate to unacceptable levels in stream biota and to determine the effects of remedial actions on accumulation of contaminants from this source.

2. To determine the importance of contaminated sediments as a source of continuing contamination of EFPC biota and to determine the effects of remedial actions on the accumulation of materials from sediments.
3. To develop an understanding of the critical factors that control the transport, fate, and bioavailability of contaminants in EFPC.
4. To combine data from laboratory and field studies with computer models already developed at the Environmental Sciences Division to develop a capability to predict the effect of remedial actions on the accumulation of contaminants in biota of EFPC.

To accomplish these objectives, the bioaccumulation component (Task 2) of the EFPC BMAP will consist of three major subtasks:

2a. Identify materials that accumulate in biota of EFPC and document changes in the accumulation of contaminants in biota following implementation of advanced waste treatment measures at the Oak Ridge Y-12 Plant

2b. Quantify dynamics of bioaccumulation of contaminants in EFPC biota, with particular emphasis on the sources and bioavailability of contaminants.

2c. Develop a predictive capability for estimating the integrated body burden of EFPC biota based on laboratory physiological and uptake experiments and field-collected data on animal feeding types, rates of ingestion, growth rates and population structure, and seasonal factors such as temperature and lipid level in fish.

2.2.3 Description of Subtasks

2.2.3.1 Accumulation of Contaminants by EFPC biota (Subtask 2a)

Since virtually all chemical substances found in aquatic biota are at concentrations in excess of those found in the ambient environment, bioconcentration factors (BCF, the ratio of the concentration of a substance in biota to its concentration in water) are of limited value in defining accumulation for a monitoring study. The criteria to be

are comparison with controls from uncontaminated waters, comparison with contaminant levels measured in previous years, and comparison of the estimated daily intake of contaminants by people ingesting fish from EFPC with the estimated allowable daily intakes. The latter screening procedure has been used in a previous study of sediment contamination in EFPC (Hoffman et al. 1984). A similar analysis is currently underway, using fish contaminant levels measured by TVA in 1984 (B.G. Blaylock, Environmental Sciences Division, Oak Ridge National Laboratory, personal communication). Results of this study will be used to help select compounds for intensive monitoring. Where Federal or State guidelines or limits exist for levels of specific contaminants [e.g., Food and Drug Administration (FDA) action limits on mercury and PCBs], these will also be used for comparison against observed concentrations in EFPC biota.

The fish species most likely to be the major elements of a sport fishery in EFPC are bluegill (*Lepomis macrochirus*), redbreast sunfish (*Lepomis auritus*), and possibly carp (*Cyprinus carpio*). These species are found throughout the length of EFPC, and are readily taken by anglers or bowfishermen. Advantages of including bluegill in the study are its relatively short life span, which yields a more consistent exposure history within the group of fish sampled, its adaptability to the laboratory environment, and the presence of a recent historical record of contamination of this fish in EFPC, based on studies from 1982 and 1984 (Van Winkle et al. 1984, TVA 1985). The redbreast sunfish occupies a similar ecological niche to bluegill and is more uniformly distributed in EFPC. It would probably be more important in any sport fishery that develops in the stream. It is also more likely to remain abundant if NHP is bypassed in the future, since the pond possibly serves as a critical nursery area that supports the abundant bluegill population in EFPC immediately downstream.

Carp are much less abundant in EFPC than either sunfish species. However, most individuals within the population appear to be large. While mercury and other metals do not appear to occur at higher levels in carp than in other species in EFPC (TVA 1985), PCBs measured in carp in the same study exceeded the FDA action limit and were more than a factor of ten above levels measured in redbreast sunfish and bluegill

(TVA 1985). This may reflect higher historical levels of PCB contamination, longer exposure due to the possible older age of these fish, high lipid content of carp, or migration of fish from other areas.

Based on the above information, the monitoring program will initially focus on bluegill (*Lepomis machrochirus*), redbreast sunfish (*Lepomis auritus*), and carp (*Cyprinus carpio*). If the initial study indicates that bluegill and redbreast have similar levels and patterns of contaminant accumulation, either one species will be dropped or both will be sampled as a single population.

Because contaminated sediments may be an important part of several pathways of accumulation, benthic biota should be included in the monitoring program. Crayfish (*Cambarus* spp.) appear to be the only benthic organism that may enter the human food chain, and its use as human food is uncommon in this region. Levels of metals in crayfish from EFPC were similar to levels found in fish (TVA 1985). Bivalves do not appear to be common, if present, in EFPC. However, such organisms have several properties useful in a bioaccumulation study. They are relatively poor at metabolizing many organic contaminants that are rapidly metabolized in fish and thus accumulate substances such as PAHs. Because they live in the sediments, they also can be an indicator of the bioavailability of sediment-bound contaminants.

Contaminant levels in benthic organisms that are major elements of the aquatic food chain are needed for the modeling part of Task 2 (Sect. 2.2.3.3). The appropriate organisms to sample for this purpose will be determined from stomach analyses of fish collected in Task 4 (Sect. 2.4.3). In the absence of a compelling human health problem from ingestion of crayfish, the organisms from those analyses will be used to represent the benthic fauna. Bivalves (probably *Corbicula*) will be introduced in enclosures to monitor the bioavailability of PAHs and other sediment-bound contaminants.

Biota will be collected from EFPC between the outfall of NHP and a site approximately 800 m downstream. This section of stream receives little input of additional water and wastes and thus most unambiguously indicates the impact of discharges from the Oak Ridge Y-12 Plant.

Reference (control) samples will be taken from Brushy Fork and/or Beaver Creek, that drain predominantly agricultural watersheds containing some residential development. While not pristine sites, they are typical of many unpolluted waters near metropolitan areas of East Tennessee which are used by sport fisherman. Other reference streams will be sampled if necessary. Additional controls will be run by using animals maintained in the laboratory. These will have no prior exposure to pollutants and will represent minimum expected levels of contaminants.

Analytical Program

(1) Organics. Based on analyses of levels of contamination found in sediments and biota of EFPC (Hoffman et al. 1984, TVA 1985) and on the physical-chemical properties of the contaminants included in these analyses (Callahan et al. 1979), a relatively small number of organic compounds are likely to be found in biota at detectable levels. Three groups of hydrophobic compounds (those whose properties would result in bioconcentration) were found in detectable levels in sediments: PAHs, PCBs, and phthalate esters. Only PCBs were found to contaminate fish in EFPC (TVA 1985). Results of these earlier studies provide a good basis for screening out all organics except PCBs as needing extensive monitoring. Bivalve studies of PAH bioavailability will aid in understanding the biological transport of this group of compounds.

Since the chemistry of effluent streams entering EFPC above NHP will change as new waste treatment systems come on-line, a periodic broad spectrum analysis of organic contamination of fish will be conducted. Analyses will be conducted by gas chromatography/mass spectrometry, with focus on those compounds that have the potential to bioaccumulate. Such studies will be conducted approximately 6 months after actions are taken that may result in major changes in effluent chemistry, or every 2 years in the absence of such changes.

(2) Metals. Only mercury occurred at elevated levels (relative to controls) in EFPC biota in 1984 (TVA 1985). A risk analysis based on those data is currently underway at ORNL and may indicate several other

metals to consider, such as thallium and beryllium. If so indicated, additional metals will be included in the post remedial action monitoring program described below.

Changes in effluent chemistry could affect the concentrations and bioavailability of metals in EFPC. Therefore, a broad suite of metals will be measured in EFPC biota 6 months after actions are taken that may result in major changes in effluent chemistry, or every 2 years in the absence of such changes.

(3) Radionuclides. The maximum concentrations of radionuclides measured in EFPC fish by TVA in 1984 were all below reporting levels suggested by the Nuclear Regulatory Commission for radioactivity in environmental samples taken in the vicinity of nuclear power plants (TVA 1985). Levels in EFPC fish were generally somewhat higher than those observed in fish from control sites. Radionuclides occurring at elevated levels in EFPC fish were cesium-137, cesium-134, and technetium-99. Only cesium-137 was within a factor of 10 of the maximum permissible concentration in fish calculated by Hoffman et al. (1984). This radionuclide along with mercury and PCBs will be monitored twice annually. A broad spectrum of radionuclides will be monitored when other broad spectrum chemical analyses are conducted.

Documentation of Changes in the Accumulation of Contaminants in EFPC Biota Following Implementation of Remedial Actions

When advanced waste treatment measures are implemented at the Oak Ridge Y-12 Plant, levels of contamination in EFPC water will be altered, which may result in a decrease in contaminant levels in biota. The rate at which contaminant levels in biota decrease will be determined by the biological turnover time of the contaminant, the rate of replacement of older organisms with younger ones, and the role of sediments as a source of continuing contamination. Long-term monitoring of the biota may be necessary to observe a return to near background levels of contamination in biota, especially if residual contamination in sediments is an important source.

(1) Sampling Native Biota. Bluegill, redbreast sunfish, and carp will be sampled twice yearly from sites in EFPC between NHP and the mouth of the creek. The organisms will be analyzed for those materials identified in the previous surveys. Initially, mercury and PCBs will be monitored, and other substances will be included if warranted. This monitoring will continue until all remedial actions are complete and levels of contamination in biota have stabilized.

(2) Introduced Organisms. A possible deficiency in using resident organisms to document the effects of remedial actions on the accumulation of contaminants by biota is the residual burden of long-lived contaminants in native fish. Thus, even if exposure drops dramatically, contaminant levels in individual fish may not drop immediately. In order to more rapidly and conclusively ascertain the effect of new treatment facilities, it may be necessary to introduce marked fish into EFPC to monitor the accumulation of low-turnover contaminants. An ORNL study is currently underway in EFPC using introduced organisms (bluegill x green sunfish hybrids; both species occur naturally in EFPC) (J. W. Elwood, Environmental Sciences Division, personal communication). If results of that study demonstrate that the use of introduced organisms provides information unavailable from monitoring resident biota, such methods will be included in the monitoring program.

2.2.3.2 Critical Factors in Transport, Fate, and Bioavailability (Subtask 2b)

The second subtask will investigate the sources of contaminants that accumulate in biota of EFPC with the goal of developing an understanding of the critical factors that control the transport, fate, and bioavailability of contaminants in EFPC. The sediment of EFPC contains elevated levels of many contaminants (Hoffman et al. 1984). Elevated contaminant levels in biota may result from the transfer of contaminants from the sediment to the biota as well as from newly discharged material present in the water. In order to assess the present role of the Oak Ridge Y-12 Plant discharges as a source of

contamination to biota, the relative importance of the sediment as a source of pollution must be known. The experimental work will focus on organic contaminants, since a great deal is already known about the environmental behavior of metals and radionuclides. This subtask will be composed of three parts:

(1) examination of the factors controlling the abiotic transport and fate of contaminants, including the binding to and remobilization from the sediment;

(2) examination of the abiotic and biotic factors that influence the bioavailability of contaminants; and

(3) measurement of contaminant uptake from EFPC water and sediment into biota to validate and refine parameters derived from the experimental work in Parts 1 and 2 above.

Evaluation of Factors Controlling Contaminant Transport and Fate (Subtask 2b, Part 1)

The sediments of EFPC are now contaminated with a variety of pollutants (Hoffman et al. 1984). Even if remedial actions at the Oak Ridge Y-12 Plant reduce or eliminate further inputs to the system, it is unclear whether contaminants will remain associated with the sediment and act as a continuing source of pollution for biota in EFPC. Conversely, if the contaminants are remobilized from the sediment, this source of persistent contamination may be ameliorated by the remedial actions. To better understand the processes involved in binding of materials to and remobilizing from the EFPC sediment, two hypotheses will be tested: (1) partitioning equilibrium can be predicted from the hydrophobicity of the contaminant and the affinity of contaminants for binding to sediment and DOM and (2) the presence of complex mixtures will not affect the partitioning of individual components between the different physical phases.

The affinity of contaminants for binding to sediment or to DOM from EFPC will be measured by filtration or centrifugation for sediment and by equilibrium dialysis (Carter and Suffet 1982, McCarthy and Jimenez 1985a) or vapor-phase analysis for DOM. The effect of DOM on the

binding to particles from EFPC will also be determined for a variety of contaminants identified in EFPC. Three-phase binding studies (water, sediment, and DOM) will be conducted using a modified closed-loop vapor stripping technique to minimize potential artifacts arising from physical separation of the phases prior to analysis. Such studies will test the validity of the first hypothesis and develop predictive relationships to estimate the partitioning of these contaminants under observed or anticipated conditions in EFPC. Of particular interest is the role of DOM on binding and dissociation in the bedded sediment, which is a major source for continued release of sequestered pollutants. If the contaminants released into the water column are actually bound to DOM migrating out of the interstitial water, the impact of this remobilization on bioaccumulation may be far less than might be anticipated since bound pollutants are less available for uptake (McCarthy 1983, Leversee et al. 1983, McCarthy and Jimenez 1985b, McCarthy et al. 1985).

Three-phase binding studies will be continued using effluents from the Oak Ridge Y-12 Plant and water from EFPC. If the second hypothesis is true, binding affinities of individual compounds will be unchanged by the presence of the mixture.

Evaluation of Factors Influencing Bioavailability of Contaminants (Subtask 2b, Part 2)

The results of the studies described above (Subtask 2b, Part 1) will provide the capability to predict the relative concentrations of contaminants in the different physical phases. In Part 2 of this subtask, the bioavailability of pollutants associated with each of these phases will be examined. The role of biological and physiological parameters on the uptake and bioavailability of contaminants will also be investigated. Two hypotheses will be tested.

(1) The bioavailability of any organic contaminant to aquatic organisms will vary, depending on the physical phase with which it is associated; total uptake can be predicted from the concentration of contaminant from each source compartment and the rate coefficient describing the uptake from each compartment.

(2) the presence of complex mixtures will alter the uptake of individual components from water or by ingestion in a manner that can be predicted from physiological and bioenergetic principles.

This part of Subtask 2b will involve experimental work to examine processes of biological uptake to develop a capacity to predict the consequences of future remedial actions at the Oak Ridge Y-12 Plant. The information on the rates of uptake and the biotic and abiotic interactions that control bioaccumulation developed in this subtask will be tested and validated by comparison with the data to be collected in Part 3 of Subtask 2b. The conceptual framework for development of the predictive capability is explained in Subtask 2c. Predictions of contaminant body burden based on results of the experimental work will be compared to those observed in the validation studies to test the accuracy and completeness of the parameters describing biological uptake from the different physical phases. By iteration of these comparisons, the parameters used to predict bioaccumulation will be refined and improved so that they can be used with confidence to forecast the probable impacts of present and future remedial actions at the Oak Ridge Y-12 Plant.

The rates of uptake of contaminants associated with each physical phase will be determined either by pharmacokinetic measurements of the uptake and elimination of radiolabeled contaminants (Southworth et al. 1979, McCarthy 1983, McCarthy and Jimenez 1985b) or by direct measurement of the mass transfer of contaminants using a fish metabolic chamber (McKim and Goeden 1982; see Sect. 2.3.2.3). Rate coefficients describing the uptake of contaminants in the water, sediment, and DOM can be estimated from these data using an iterative least-squares fitting procedure based on equations derived from multicompartment models. These data will be supplemented with more specific experiments to clarify the importance of environmental and seasonal variables on the rate of uptake. Experiments in which contaminated food or sediment is fed to fish will measure the efficiency with which contaminants are assimilated by the digestive tract. The effect of variables such as the chemical structure of the contaminant, the type of digestive system (which varies with fish species), fish lipid levels, and temperature

will be examined. Physiological factors which control the efficiency with which contaminants are transferred into the gills will be examined using a fish metabolic chamber (McKim and Goeden 1982).

Since complex mixtures are likely to alter biological uptake of contaminants present in complex mixtures, gavage and metabolic chamber studies will be repeated with mixtures of contaminants and with the Oak Ridge Y-12 Plant effluent and EFPC water to determine the effect of mixtures on the efficiency with which compounds are transferred across the gills or assimilated by the gut. If mixtures do alter uptake, the underlying mechanisms will be examined so that a rational basis can be developed for predicting changes in bioaccumulation.

Measurement of Contaminant Uptake from EFPC Water and Sediments (Subtask 2b, Part 3)

These studies will measure the uptake of existing contaminants in the water and sediments of EFPC and will be used to confirm and validate the accuracy of the preceding estimates of the parameters describing bioaccumulation of contaminants derived in Parts 1 and 2 of Subtask 2b. To measure the accumulation from water, fish and mussels will be maintained in tanks receiving a constant flow of EFPC water as its only water source. Organisms will be sampled periodically over a 90-day period and analyzed for contaminants identified in Subtask 2a. Control organisms will be maintained under similar conditions in tanks receiving well water as a water source. Uptake rates and bioconcentration factors will be calculated, and the equilibrium levels of contamination in the experimental organisms will be compared with levels in resident organisms in EFPC.

To measure the contribution of the sediment to bioaccumulation, depositional sediments from EFPC will be placed in tanks identical to those used in the aqueous uptake experiment. Fish and mussels will be maintained in the tanks and analyzed for contaminants at the same intervals as in the aqueous uptake experiment. A constant flow of water will be maintained through the tanks, providing a water renewal per unit mass of sediment that is substantially less than typical of EFPC. Control studies will use uncontaminated sediments from a reference site.

Uptake rates will be determined and compared with uptake directly from the water to ascertain the relative importance of the two pathways.

The measured rates of uptake from the EFPC water and sediments provide only a snapshot of the rates of bioaccumulation under a limited set of environmental and physiological conditions. By themselves, these data cannot give an accurate representation of the bioaccumulation potential over an annual cycle, nor can they be relied on to forecast the impact of remedial actions. They will be used primarily to test and validate the more dynamic and flexible predictions derived from the experimental studies in Parts 1 and 2 of Subtask 2b. Given the environmental and physiological conditions that existed during the validation uptake studies, the experimentally determined parameters will yield estimates for the bioaccumulation of EFPC contaminants by biota. If these estimates agree with those measured in the validation studies, confidence in the predictive capabilities will be increased. Failure to achieve accurate predictions will force refinement of the experimental parameters or reevaluation of the completeness of the experimental description until accurate predictions are achieved. By this iterative process among elements of this task, the relevance of the experimental predictions will be validated for conditions in EFPC. With this validation, predictions of the long-term effects of remedial actions under dynamic natural conditions can be accepted with a much higher degree of confidence.

2.2.3.3 Bioaccumulation Models for Estimating Response to Remedial Actions (Subtask 2c)

The third subtask will apply previously developed bioaccumulation models to the field and laboratory data collected in Tasks 3 and 4 of the BMAP in order to predict the response of the biota to various remedial actions at the Oak Ridge Y-12 Plant. A dynamic model for bioaccumulation is required because most problem chemicals have slow elimination rates and, consequently, only slowly approach new steady states.

Bioenergetics-based models of fish growth have been adapted to predict contaminant fluxes through fish populations. These detailed

models account for the influence of fish size, water temperature, and food quantity and quality on the major physiological processes influencing growth (consumption, respiration, egestion, excretion, and reproduction). Such models are useful for estimating metabolic rate and the rate of consumption of several dietary items, and thence, the contaminant uptake from water and from different food organisms.

This subtask will be composed of two parts: (1) application and calibration of the models to the field and laboratory data collected in Tasks 3 and 4 and (2) estimation of the time course of contaminant concentrations in EFPC biota in response to various remedial action scenarios.

Model Calibration (Subtask 2c, Part 1)

The previously developed bioaccumulation models will be applied to the site-specific field conditions in EFPC and to the laboratory experiments to attempt to predict the contaminant concentrations in biota from concentrations in water and sediment. Use of a bioenergetics-based model will allow certain differences among sampled individuals to be accounted for, such as species, age, typical diet, lipid level, and temperature history. These data will be available from Tasks 3 and 4 (Sects. 2.3 and 2.4, respectively). Such accounting for sample differences should increase the usefulness of the field data from Subtask 2a and permit increased precision in estimates of parameters from laboratory experiments. As additional information becomes available from Subtask 2b, model parameters and processes will be refined, and the model will be calibrated to the field data from EFPC.

Model Simulations (Subtask 2c, Part 2)

Once a calibrated model is available for contaminant levels in EFPC biota, simulations will be done for various remedial-action scenarios. Estimates will be made for each scenario of the time course of the contaminant levels in different organisms, including the time required to reach a given fraction (e.g., 90%) of a new steady state. This

calibrated model can be used to guide continuing remedial actions and provide a basis for cost-benefit analyses and decisions on any future action.

2.3 BIOLOGICAL INDICATORS OF CONTAMINANT-RELATED STRESS (TASK 3)

2.3.1 Introduction

Biological indicators can be defined as selected components or variables of organisms, populations, or communities that respond in a variety of biologically meaningful ways to changes in the environment (Tables 6 and 7). Certain indicators can reflect the response of major ecosystem components to stress without requiring detailed, costly, time-consuming, and long-term measurements. For example, the liver condition of fish is easy to measure and may reflect, in many situations, the health of a fish, thus allowing inferences to be made about the overall condition of not only fish populations but also their associated biotic communities.

In Task 3, biological indicators will be used to evaluate the responses of fish populations and communities in EFPC to the Oak Ridge Y-12 Plant effluents and the changes in effluent chemistry over time. Selection and application of these indicators will focus on four major questions: (1) What will be the effects of water quality changes on the biological communities of EFPC? (2) As various wastewater treatment facilities come on-line, what will be the additional or cumulative effects, if any, of these facilities on the biotic integrity of EFPC? (3) What is the relative contribution of other potential pollutant sources (e.g., City of Oak Ridge Wastewater Treatment Facility) to the ecological status of EFPC? (4) What are the effects of periodic short-term discharges of high levels of contaminants to EFPC? Application of selected biological indicators along with instream monitoring, toxicity testing, and bioaccumulation studies can be used to address these questions and to demonstrate that the effluent limitations established for the Oak Ridge Y-12 Plant protect the use of EFPC for growth and propagation of fish and aquatic life.

Table 6. List of representative biological indicators that have been used to investigate the biochemical, physiological, and tissue-level response of organisms to various pollutants

Indicator	Pollutant or stress	Nature of response	Reference
Biochemical level			
Serum glucose	Kraft mill effluent	Elevated concentrations	McLeay 1977
	Reduced pH-acid rain	Elevated concentrations	Adams et al. 1985b
	Dieldrin	Elevated concentrations	Silbergeld 1974
	DDT, endrin, HCB, atrazine	Elevated concentrations	Gulth and Hanke 1984
Serum cholesterol	Aldrin, DDT, HCB, toluene	Decreased concentrations	Gulth and Hanke 1984
	Reduced pH-acid rain	Elevated concentrations	Adams et al. 1985b
Serum cortisol	Cu	Elevated concentrations	Schreck and Lorz 1978
	DDT, atrazine, HCB, toluene	Elevated concentrations	Gluth and Hanke 1984
Serum electrolytes	PCBs, DDT, low PH, Cu	Electrolyte homeostasis	Kinter et al. 1972, Caldwell 1974
(Na, K, Mg)	Cd	Inhibits ionic regulation	Rao et al. 1979, Thurberg et al. 1973
Serum protein	Oil, Cd	Impaired protein metabolism	Schafer 1961, Syed et al. 1979
	Aldrin, DDT, endrin, HCB	Impaired protein metabolism	Gluth and Hanke 1984
Steroid hormones	PCBs	Impaired reproduction	Freeman et al. 1981
Reproductive hormones	Cd	Impaired reproduction	Sangalang and Freeman 1974
Lysosomal enzymes	Heavy metals, organics	Cellular damage	Allison 1979, Moore and Stebbina 1976
RNA/DNA ratio	Cd, Zn	Impaired growth	Kearns and Atchison 1970
Liver enzymes	PCBs	Induction of specific enzymes	Addison 1981
(Mixed function	PAHs	Induction of specific enzymes	Paine and Fancey 1981
oxidases)	Heavy metals	Complexation with metallothionein	Harrison and Lam 1985
	Crude oils	Induction of specific enzymes	Chambers 1979
Physiological level			
Ventilation rate	Acetone, Cd, Pb, Cu, Zn	Impaired respiration	Majewski et al. 1978
Coughing rate	Phenol, NH_3, cyanide, DDT	Impaired respiration	Morgan 1977
Respiration rate	Heavy metals	Impaired respiration	Hughes 1981
Tissue and organ level			
Liver condition	Polluted river water	Hyperplasia-enlargement	Poels et al. 1980
	Crude oil	Decrease in size	Chambers 1979
Liver glycogen	Kraft mill effluent	Decreased glycogen	Oikari and Nakari 1982
	Aldrin, DDT, endrin, HCB, toluene	Decreased glycogen	Gluth and Hanke 1984
Body lipid	Strip mining	Decreased body fat	Stair et al. 1984
	Reduced pH	Decreased body fat	Steigen and Raddum 1981
	Increased temperature	Decreased body fat	Adams et al. 1985a
Histology	Cd,Pb crude oil	Impaired gonadal development	Yevich 1981
	Reduced pH	Lesions in skin and kidney	EPA 1971, EPA 1979
		Adrenal gland dysfunction	Mudge et al. 1977

Table 7. Environmental Protection Agency documents related to the use of various biological indicators in toxicant effects research

Document title	Principal indicators	Pollutant or stress	Reference
Procedures for measuring cough (gill purge) rates of fish	Gill purge rates	Heavy metals, pesticides, mixed effluents	Drummond and Carlson 1977
Assessment of clinical procedures to evaluate liver intoxification in fish	Liver enzymes, histology, plasma protein	Carbon tetrachlorine, monochlorobenezene	EPA 1979
Toxic effects of water soluble pollutants on freshwater fish	Oxygen consumption, plasma electrolytes, hematocrit	Inorganic and organic mercury	EPA 1977
Assays of toxic pollutants by fish blood	Morphology and composition of blood cell population	Cu, Cd	EPA 1976
Toxaphene effects on reproduction, growth, and mortality of brook trout	Bone collagen, reproductive success	Toxaphene	EPA 1975
Evaluation of an index of biological integrity: Temporal variability and regional application in the Midwest	Trophic composition, species richness, fish condition	Water quality and habitat degradation	Karr 1981, Karr et al. 1984

Water quality criteria are commonly used not only to determine compliance by water-user facilities but also to assess potential environmental effects. Many of these compliance criteria are based on short-term toxicity tests which determine the time required (usually hours) to elicit various levels of mortality in standard test organisms. This approach may be generally acceptable for screening toxicant effects on a short-term basis (i.e., hours or days), but in most cases these criteria may be relatively ineffective in predicting or evaluating the effects of chronic levels of pollutants on long-term responses of biological populations and communities (i.e., growth, reproductive potential, trophic level status, abundance, and diversity)(NRC 1981). Capabilities are needed, therefore, to identify and quantify responses of aquatic organisms and communities to sublethal chronic exposures before adverse, irreversible, or obvious undesirable effects on populations are realized (Sastry and Miller 1981, Adams and McLean 1985). In this regard, biological indicators can serve as early warning signals of impending ecosystem change, allowing time for remedial action to be initiated.

2.3.1.1 Advantages of Biological Indicators

The principal advantages of biological indicators are that they (1) can provide early warning signals of potential ecological effects because of their sensitivity to environmental change; (2) can be used to identify mechanisms underlying observed effects at higher levels of organization, including changes in population growth, reproductive success, and age structure; and (3) are easy to measure and cost effective for long-term monitoring. Each of these is discussed in detail below.

1. Early Warning Signals of Potential Environmental Stress. Because many of the more important components of aquatic ecosystems may respond slowly to environmental change (i.e., reproductive potential and shifts in species composition and abundance) (Fig. 2), by the time these long-term responses are observed in an ecosystem, undesirable effects may have occurred. These longer-term responses may take place over

periods of years or several biological generations (Fig. 2), and within these time periods, a variety of environmental factors, both natural and man-induced, can influence the integrity of biological communities. Therefore, because many environmental factors can contribute to and influence an ecological response, the major causes of any observed long-term ecological change may not be readily identified and quantified by measuring only long-term response variables such as fish species abundance and diversity. Some of the biological indicators to be used in this study respond within the same time frame as the environmental changes (primarily water quality) of concern, and because they are immediate reflections of that change, serve as early warning signals of potential environmental effects. With bioindicators, changes observed (beneficial or adverse) in the biotic integrity of EFPC may be directly related to specific changes in environmental quality. For example, some of the indicators can be used to evaluate the effects of (1) progressive on-line additions of various wastewater treatment facilities at the Oak Ridge Y-12 Plant and (2) short-term inadvertent discharges of contaminants. Because many of the biological parameters (such as abundance and diversity of organisms) normally measured in compliance monitoring programs , change relatively slowly over periods of years or generations, their usefulness for serving as an early warning system of potential environmental change is limited.

2. Identification of Mechanisms Underlying Long-Term Effects. As emphasized above, some biological indicators respond within the same time frame as the environmental change or stress that is being investigated, while other responses occur more slowly over periods of biological generations or years (Fig. 3). The bioindicators selected for this study can simultaneously measure biological changes over a gradient of response times from relatively short term to long term (Fig. 3). Measurements of responses over this gradient permit long-term changes in ecological systems to be correlated with the principal factors responsible for those long-term changes. For example, in the initial phases of Subtask 3a, we plan to measure a suite of selected short-term indicators, such as rapid-response biochemical variables of

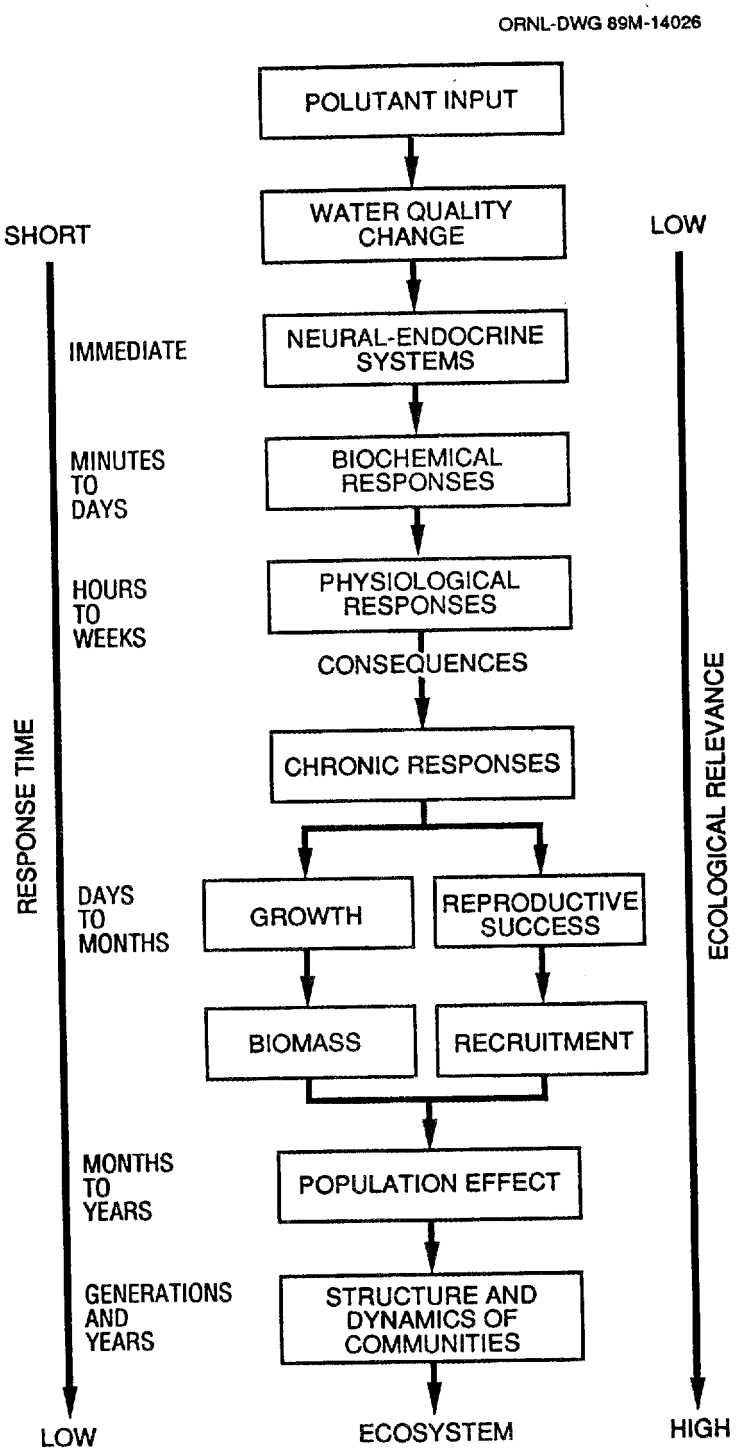

Fig. 2. Biological responses to elevated contaminant levels in surface waters.

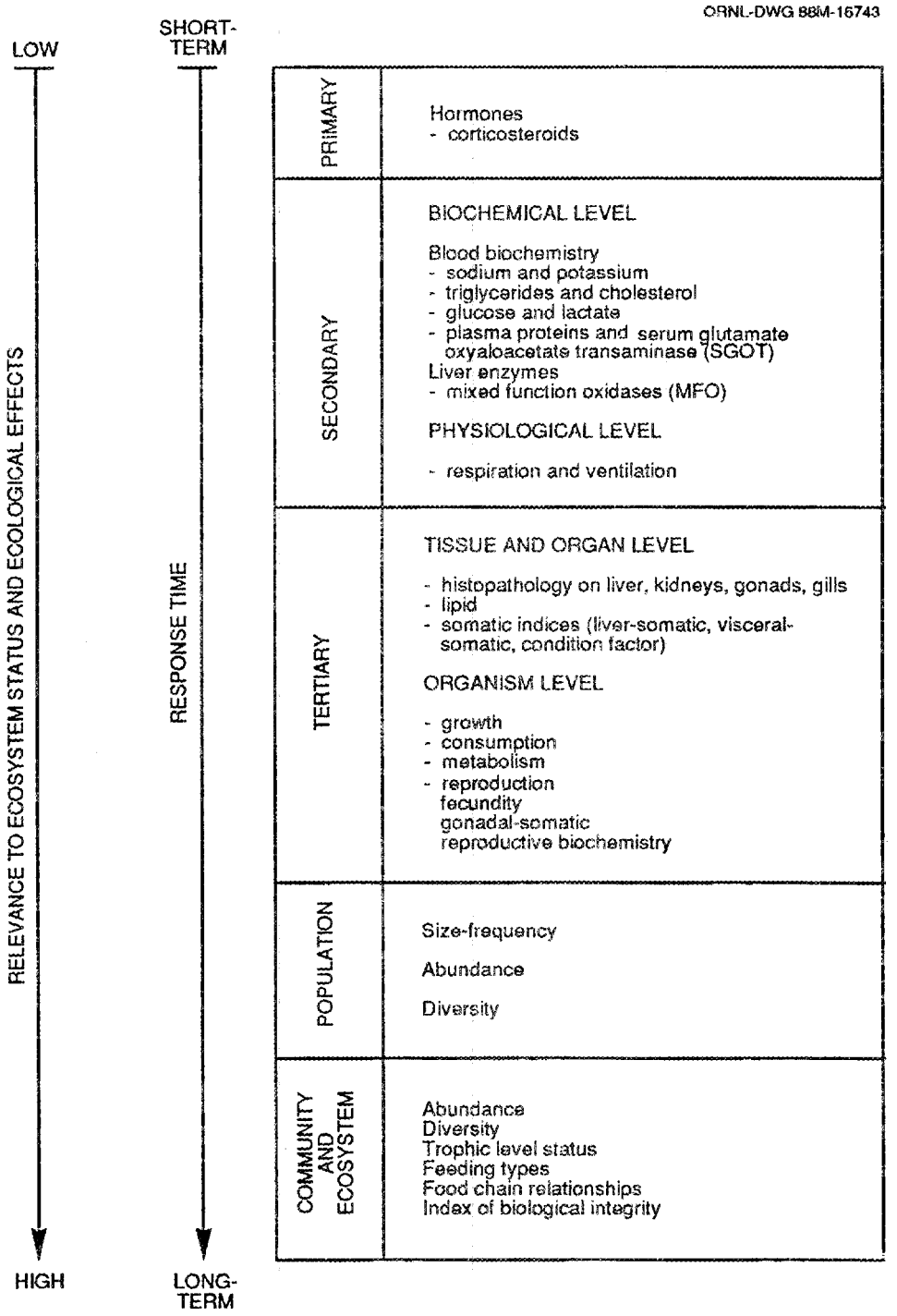

Fig. 3. Biological indicators to be measured in Task 3, illustrating the gradients in response time and relevance to ecosystem status and effects.

fish, along with more intermediate-term responses, such as fish health (e.g., liver condition, histology of selected organs, quantity and quality of lipid storage). With this approach, biological indicators can be correlated with more ecologically relevant long-term responses such as fish growth, reproduction, and changes in fish community abundance and diversity (Figs. 2 and 3). Mechanisms primarily responsible for the long-term population and community level changes can also be identified and quantified.

3. Cost Effectiveness and Ease of Use. Most of the biological indicators developed in Subtask 3a and applied in Subtasks 3b and 3c will be relatively easy to measure and should be cost-effective for application and use in long-term biological monitoring. Once the relationship and biological significance between the short-term indicators (e.g., liver condition) and the more long-term responses (e.g., growth, species abundance, etc.) are developed, these short-term and easily measured indices should provide reliable documentation of the ecological status of EFPC during the entire period of the NPDES monitoring program.

2.3.2 Description of Subtasks

The bioindicator studies consist of three major subtasks: (3a) selection of biological indicators for use in Subtasks 3b and 3c, (3b) application of selected indicators in the field for long-term instream monitoring and for manipulative field experiments, and (3c) application of selected indicators in the laboratory for testing effects of various effluent concentrations on the response of target fish species.

2.3.2.1 Selection of Biological Indicators (Subtask 3a)

Numerous biological indicators have been used to evaluate biological responses to a variety of environmental pollutants (Tables 6 and 7). The primary objective of this subtask will be to select a subset of these indicators that can be used to identify and quantify the effects of the Oak Ridge Y-12 Plant effluents on the integrity of biotic

communities in EFPC. A list of the indicators that will be measured initially is given in Table 8. They represent a comprehensive series of biological responses along a gradient of relative short-term to long-term responses (Figs. 2 and 3). For example, in the early stages of this subtask, fish will be sampled at four to six stations from the NHP outfall downstream to below the City of Oak Ridge Wastewater Treatment Facility (Fig. 1 and Table 3). For fish sampled from all areas, measurements will be taken of (1) biochemical parameters, such as biochemistry of the blood and metabolic variables; (2) tissue and organ-level indicators, such as energy storage, histopathology, and various fish condition indices; and (3) organism-level response parameters, such as growth, reproductive capacity, and food consumption (Fig. 2). In conjunction with these measurements, information on fish abundance and diversity collected at the same monitoring sites as part of Task 4 (Sect. 2.4) will be used as indicators of population and community-level responses (Fig. 2). The indicators listed in Fig. 3 fall along a response gradient from relatively rapid responses at the primary and secondary levels of organization to relatively long-term responses at the population and community levels. These indicators also fall along a gradient of increasing ecological relevance for evaluating effects. For example, blood biochemical variables have the advantage of being relatively rapid indicators of environmental change, but their potential ecological relevance for evaluating changes in fish abundance and diversity is relatively low. By measuring a continuum of response-time indicators, we can establish the ecological relevance of short-term effects by correlating and relating them to the long-term chronic population and community-level effects (Fig. 2).

Following initial studies, a subset of the most informative and biologically relevant indicators will be selected for use in subsequent long-term biological monitoring of EFPC and for the experimental field and laboratory studies (Subtasks 3b and 3c). The most useful indicators for investigating community-level responses to environmental change will be determined by applying appropriate statistical tests such as correlation and discriminant analysis.

Table 8. Biological indicators to be measured in Tasks 3a-3c, the biological responses or conditions reflected by by each indicator, and key references that describe the use and application of each indicator

Response level	Indicators to be measured	Responses or conditions reflected by indicators	Key references
Organism			
Primary	Hormones		
	-Corticosteroids	Immediate environmental change or stress initiates other biochemical responses	Hill and Fromm 1968 Mazeaud et al. 1977
Secondary	Biochemical level		
	-Blood biochemistry		
	-Sodium and potassium	Electrolyte homostatis	Eddy 1981
	-Triglycerides and cholesterol	Lipid metabolism	Adams et al. 1985a
	-Glucose and lactate	Carbohydrate metabolism	Silbergeld 1974
	-Plasma proteins and serum glutamate oxyaloacetate transaminase	Protein and amino acid metabolism	Freeman and Idler 1973
	-Liver enzymes		
	-Mixed function oxidases	Induction of enzymes and metabolism of toxic compounds	Lech et al. 1982
	Physiological level		
	-Respiration and ventilation	Total metabolic response	Hughes 1981, Drummond and Carlson 1977
	-Multivariate biochemical evaluation	Total metabolic response	Adams et al. 1985b
Tertiary	Tissue and organ level		
	-Liver glycogen	Energy reserves; energy utilization	Hinton et al. 1982
	-Liver and body lipid	Energy reserves; energy mobilization	Shulman 1974
	-Somatic indices (liver-somatic, visceral-somatic, condition factor	General organism health; energy availability	LeCren 1951, Anderson and Gutreuter 1983, Heidinger and Crawford 1977, Adams and McLean 1985, Wedge and Anderson 1978
	Histology		
	-Liver	Liver dysfunction	EPA 1971, EPA 1979
	-Gills	Respiratory dysfunction	Plonka and Neff, 1969
	-Kidney	Excretory and endocrine dysfunction	Mudge et al. 1977, Yevich 1981
	-Pathological assessments	Effects of aquatic pollutants	Hinton et al. 1977, Hinton and Couch 1985

Table 8. (continued)

Response level	Indicators to be measured	Responses or conditions reflected by indicators	Key references
Tertiary (cont.)			
	Organism level		
	-Growth	General overall condition; integrator of all effects acting on an organism	Waters 1977; Larkin 1978
	-Consumption	Food availability; effect of organism on food resources	Kitchell et al. 1977
	-Metabolism	Total metabolic response to environment	Hughes 1981
	-Reproduction		
	-Fecundity	General reproductive success	Zalewski 1979; DeVlaming et al. 1982
	-Gonadal-somatic index		Billard et al. 1981
	-Reproductive biochemistry		
Population	Size/age distribution	Reproductive and recruitment success	Anderson and Gutreuter 1983; Kuman and Adams 1977
	Abundance	Intraspecific competition	Sale 1979
Community and Ecosystem	Abundance	Intraspecific and interaspecific competition; reproductive and recruitment success; food and habitat availability	Sale 1979
	Diversity		Fausch et al. 1984
	Trophic level status		Karr 1981
	Feeding types		
	Food chain relationships		Schlosser 1982

2.3.2.2 Application of Biological Indicators in the Field (Subtask 3b)

The selected set of biological indicators developed in Subtask 3a will be used as a basis for (1) monitoring and evaluating the long-term effects of the Oak Ridge Y-12 Plant effluent on the biotic integrity of EFPC and (2) conducting manipulative field experiments with target fish species.

Long-Term Monitoring

In addition to the instream monitoring component (Task 4) of the BMAP for the Oak Ridge Y-12 Plant (Sect. 2.4), the biological indicators developed in Subtask 3a will be used to evaluate both the short- and long-term responses of the EFPC biological communities to discharges from the Oak Ridge Y-12 Plant over the entire 5-year monitoring period. During this period, each indicator will continually be reevaluated as to the quality and quantity of information it provides relative to the primary objectives of the BMAP. Some indicators initially identified as important may be deleted, while others may be implemented and incorporated into the long-term monitoring program.

Manipulative Field Experiments

Manipulative field experiments will be employed for two purposes: (1) to test for potential effects of the Oak Ridge Y-12 Plant effluents on selected fish species and (2) to evaluate the relative effects of other pollutant sources on the biotic integrity of EFPC. Biological indicators will be used to test for potential effects on the response of selected fish species by exposing fish from nonaffected (control) areas to potentially affected stressed areas in EFPC and by determining if fish from potentially stressed areas in EFPC recover when placed in unaffected, control areas. Designation of control and "stressed" areas of EFPC will be based on data from our initial monitoring studies in Subtask 3a, on information from the instream monitoring program (Sect. 2.4), and on existing water quality data. Control areas may include nonaffected streams within the EFPC watershed, such as Mill Branch, Gum Hollow Branch, and Pinhook Branch (Fig. 1).

Live fish captured in the stressed areas and transported to control areas and fish captured in control areas and transported to stressed areas will be confined to small sections of EFPC by semipermanent enclosures. To account for the effects of handling stress on transported fish, identical capture and handling procedures will be performed on separate groups of fish confined in enclosures in the same section of stream where they were originally captured. After accounting for handling stress, responses observed in fish transferred from control areas to experimental areas should indicate the level and degree of organism-level effects incurred due to discharges from the Oak Ridge Y-12 Plant. Conversely, by examining recovery responses in fish transferred to control areas from stressed areas, the degree of stress or change originally experienced by an organism due to effluent discharges from the Oak Ridge Y-12 Plant can be evaluated. No significant responses in fish transferred to either area would suggest that plant discharges were having no effect on the biotic integrity of EFPC, at least at the individual organism level.

For evaluating the relative contribution of other potential pollutant sources to the ecological health of EFPC, manipulative caging experiments will be conducted by placing control fish in tributary streams suspected of being polluted (non-Y-12 Plant sources) and monitoring their response using selected bioindicators.

2.3.2.3 Application of Biological Indicators in the Laboratory (Subtask 3c)

This subtask will utilize those biological indicators selected from Subtask 3a and refined in Subtask 3b to investigate the responses of fish exposed to varying concentrations of the Oak Ridge Y-12 Plant effluents under controlled laboratory conditions. This subtask has three components: (1) integration with various aspects of Tasks 1 and 2, (2) effects testing, and (3) challenge or performance tests.

Integration with Toxicity Tests and Bioaccumulation Studies

As an integrated component of the laboratory and field toxicity tests and bioaccumulation studies proposed in other sections of the

BMAP, those indicators selected in Subtask 3a will be measured along with the standard variables measured in the toxicity tests and the bioavailability studies (Sects. 2.1 and 2.2, respectively). With this integrated approach, our indicators can be correlated with and related to fish survival, growth, and reproduction. Physiological and organism-level responses will be related to changes in bioaccumulation through bioenergetics models as described in Sect. 2.2. The relationships among the biological indicators, body burden of contaminants, and toxic response will also be examined.

Effects Testing

Sublethal responses of target fish species to varying concentrations of the Oak Ridge Y-12 Plant effluent will be examined under controlled laboratory conditions. As measures of fish response, the same suite of biological indicators selected in Subtask 3a and refined for laboratory use in Subtask 3c will be utilized. Water will be collected from EFPC at various locations below NHP to represent various dilution levels. Responses of fish in the laboratory to these various effluent dilutions will be compared to responses of fish collected in the field from areas with similar effluent concentrations.

Responses of fish to the Oak Ridge Y-12 Plant effluent will be measured in specially designed plexiglass metabolic chambers (Fig. 4). These chambers have been used to measure sublethal responses of fish to chronic exposure to a variety of toxicants. The chamber illustrated in Fig. 4 may have to be modified somewhat to accommodate smaller fish such as bluegill. With this chamber, a suite of biochemical and physiological indicators can be measured continuously without sacrificing the fish. Principal indicators that can be easily measured include blood biochemistry, respiration and ventilation rate, and metabolic detoxification processes in the liver and gills. For each effects test representing exposure to a specific dilution level, similar biological measurements will be taken from fish in control chambers.

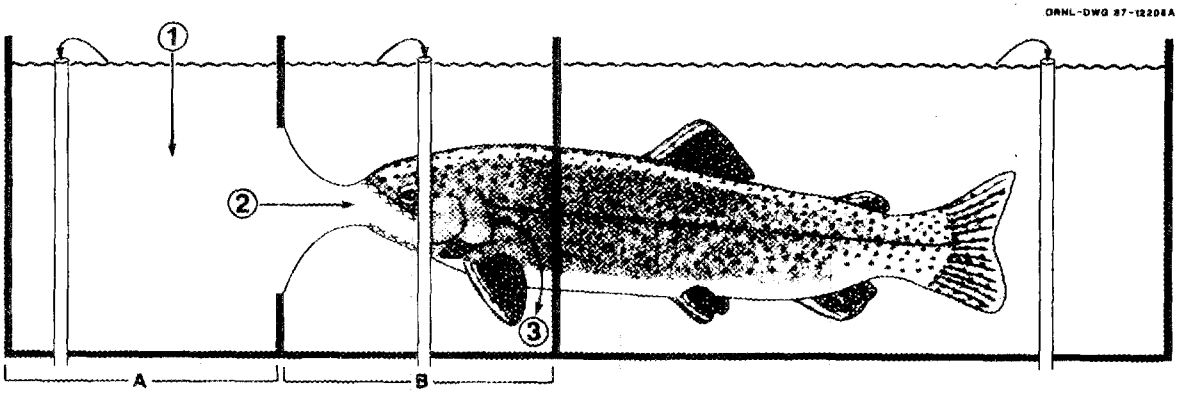

Fig. 4. Diagram of metabolic chamber that will be utilized in Subtasks 2b and 3c. Toxicant enters Chamber A at (1) and water (including toxicant and O_2) is pumped over the gills (2) and expelled into Chamber B (3) via ventilatory action of the fish.

The metabolic chambers will also be used to evaluate the effects of periodic short-term discharges of contaminants on selected organisms and bioindicators. Response tests will be performed on fish exposed to different effluent concentrations for varying periods of time. The exposures will simulate possible short-term effluent releases with higher-than-normal contaminant levels.

Challenge or Performance Tests

As an integral part of the effects testing described above, laboratory challenge tests will be performed to measure the tolerance of fish to stress. According to Wedemeyer and McLeay (1981), an assessment of fish health should not be restricted only to an examination of internal changes within an animal (such as biochemical and physiological changes), since these responses may not in themselves provide sufficient information with which to appraise the overall condition of an organism. A combination of appropriate blood and other tissue analysis (such as histopathology) with standardized stressor challenge tests is needed to provide the most meaningful assessment of fish health.

Fish that display a negative response to the effects testing will be subjected to challenge tests to determine if their ability to tolerate stress has been reduced. Decreased tolerance may indicate, for example, that the probability of survival has been reduced (Wedemeyer et al. 1976). Some of the challenge or performance tests that could be conducted include (1) tolerance to hypoxia (Carter 1962), (2) scope for activity (Sprague 1971), (3) disease challenge tests (Wedemeyer and McLeay 1981), and (4) leukocrit stress test (McLeay and Gordon 1977).

2.3.3 Experimental and Sampling Design

2.3.3.1 Field Studies

Selection of biological indicators, as discussed in Subtask 3a, along with long-term monitoring and manipulative experiments outlined in Subtask 3b will constitute the field work required in Task 3 of the BMAP. For these field studies, sampling and experimental (manipulative) stations in EFPC will be located at various distances downstream of the NHP outfall in representative pool and riffle habitats. Because the

four major tasks that comprise the BMAP complement each other to various degrees, location of these stations will be coordinated with the instream monitoring, toxicity monitoring, and the bioaccumulation studies.

Potential sampling sites are shown in Fig. 1 and described in Table 3. Field sampling will be conducted approximately five to six times per year. The temporal sampling sequence will probably be: (1) one early and one late spring sample because environmental and biological conditions change more rapidly in the spring than any other time of year, (2) mid-summer, (3) mid-fall, and (4) mid-winter. As with selection and placement of sampling stations, sampling frequency and time will be closely coordinated with those of the other tasks.

The number of samples collected at each station for each date will be based on the information derived from initial studies. Determination of optimum sample size for each sampling period and each of the indicators listed in Fig. 3 will be based on appropriate statistical procedures applied to the data from Subtask 3a and also on information from other studies (Table 8). For each sampling period and station, those indicators listed in Fig. 3 will be measured along with the population- and community-level parameters included as part of the instream monitoring task (Sect. 2.4).

2.3.3.2 Laboratory Studies

Water for the laboratory bioassays, bioavailability, and effects testing studies will be collected at the same field sampling sites identified above (Fig. 1). Effects testing will be conducted on approximately the same temporal schedule as the field monitoring to determine the contribution of seasonal changes such as temperature to fish response to the Oak Ridge Y-12 Plant effluents. As emphasized in Subtask 3c, biological indicators to be measured will be those that reflect short-term responses to various levels of effluent dilutions, such as blood biochemical variables, liver enzymes, and metabolic parameters.

2.4 INSTREAM MONITORING (TASK 4)

2.4.1 Introduction

The instream monitoring task will involve field sampling of the benthic invertebrate and fish populations in EFPC. The objectives of these studies are (1) to characterize spatial and temporal patterns in the distribution and abundance of these populations downstream of NHP and (2) to document the effects of new wastewater treatment facilities on community structure and function. Intensive sampling to characterize the benthic invertebrate and fish communities will be conducted during the first year. Based on the results of these and other studies (see Sects. 2.1 and 2.3), sampling frequencies and locations may be modified, as appropriate, to assess the effects of various waste treatment facilities on the biotic communities of EFPC (Objective 2) during the next 4 years.

Analysis of the structure and function of benthic invertebrate communities provides a useful tool for evaluating the ecological effects of effluent discharges to streams (Sasson 1981). Because of their low motility and relatively long life cycles (several months to more than a year), benthic invertebrates integrate changes in the physicochemical environment of streams. Many benthic species spend a considerable portion of their life cycle on or in stream sediments, resulting in long-term exposure to contaminants that accumulate in the sediments. Consequently, biological monitoring that includes studies of benthic organisms can be considerably more informative than methods that rely solely on water quality analyses but ignore the potential synergistic effects often associated with complex effluents.

The majority of the studies included in the BMAP focus on fish and include all levels of organization from individual tissues to communities (Fig. 2). Fish communities are comprised of species that represent several trophic levels, and species that comprise the potential sport fishery in EFPC (e.g., bluegill and redbreast sunfish) are at or near the end of food chains. Consequently, they integrate the effects of water quality and habitat degradation on lower trophic levels (e.g., benthic invertebrates) which are utilized for food. Because of

these trophic interrelationships, the well-being of fish populations is often used as an index to water quality (e.g., Weber 1973, Greenson et al. 1977, Karr 1981). Moreover, statements about the condition of the fish community are better understood by the general public (Karr 1981), an especially important consideration in EFPC where fishing is presently prohibited due primarily to elevated levels of mercury in several species (Van Winkle et al. 1984, TVA 1985).

Instream sampling of the periphyton communities, as outlined in preliminary discussions with staff of the EPA and the TDHE, is not included as a component of the initial instream characterization studies. Instead, periphyton studies will be conducted with wooden troughs and diverting a small portion of the flow in EFPC through them (Sect. 2.1.3). Should the results obtained from these microcosm studies (to be conducted during the second year of the BMAP) indicate a need for additional instream sampling, such a program would be initiated at that time.

2.4.2 Benthic Invertebrates

Benthic invertebrate populations will be sampled monthly from designated riffle areas at six locations on EFPC (Fig. 1 and Table 3). The results obtained during the first year will be used to evaluate the possibility of reducing the sampling frequency in subsequent years. To accurately estimate species richness, qualitative samples will be collected in April from a variety of microhabitats (e.g., leaf packs, undercut banks, pools, and riffle areas outside the study riffles) in EFPC and several reference (control) streams. Quantitative sampling at the latter sites, which are located both within and outside of the DOE Oak Ridge Reservation, will be conducted quarterly. Using multiple control sites with less frequent sampling provides a better basis for comparison with impacted sites than intensive sampling of a single control stream. Because species composition of the benthic invertebrate communities may vary widely between watersheds, the use of multiple reference streams provides an estimate of this variability in community structure between streams.

Benthic invertebrates will be collected from riffle areas at each site with a 0.1 m^2 Hess bottom sampler fitted with a 363-m mesh collection net. Five randomly selected samples will be taken at each site and preserved in 10% formalin. In the laboratory, benthic organisms will be separated from debris in white enamel pans and identified to the lowest practicable taxonomic level (i.e., species wherever possible); oligochaetes will be identified to family. A blotted wet weight of each taxon will be obtained to the nearest 0.1 mg.

Additionally, one to five species will be selected as target species for estimating secondary production via direct methods. For these target species, head widths will be measured to the nearest 0.1 mm and sorted into size classes, and a blotted wet weight will be taken to the nearest 0.01 mg for each size class.

Nonparametric statistics will be used to compare species richness, abundance (total number of individuals per 0.1 m^2), and diversity between the EFPC sites and the control sites. Estimates of secondary production for the target species will be obtained by standard methods (i.e., removal summation, instantaneous growth, or size frequency) (Waters 1977, Krueger and Waters 1983). Secondary production of the remaining species will be estimated indirectly from published data on production/biomass ratios (Waters 1977, Krueger and Waters 1983).

2.4.3 Fishes

The fish communities at six sites on EFPC (Fig. 1 and Table 3) will be sampled three times (May, September and March) during the first year to estimate population size (densities in numbers and biomass per unit area) and production, by species. Sampling will be conducted over designated reaches approximately 100 m in length. In addition, qualitative sampling above and/or below each study reach will be conducted in the spring and fall.

All sampling will be conducted by using two Smith-Root Model 15A backpack electrofishers. These units utilize self-contained, gasoline-powered generators capable of delivering up to 1200 volts of pulsed direct current. Population estimates will be based on the

removal method (Carle and Strub 1978) with a minimum of three consecutive passes at each site. Fish will be anesthetized with MS-222 (tricane methanesulfonate), identified, measured to the nearest 0.1 cm (total length), weighed to the nearest 0.1 g (for fish less than 100 g) or gram (for individuals more than 100 g), and returned to the stream. For target species (bluegill, redbreast sunfish, and carp), scales will be taken for age determination. The instantaneous growth rate method (Ricker 1946) will be used to calculate annual production, by year class, of the target species (either bluegill or redbreast sunfish, depending upon their distribution and abundance as determined by the initial population sampling). For all other species, production will be estimated by the method of Garman and Waters (1983).

Using the approach and procedures described above, data will be available to estimate parameters at the community level (diversity, production, and abundance), the population level (length-weight relationship, production, and abundance), and for individual age classes of the target species population (instantaneous growth rates and mortality rates, mean condition factor, production, and abundance). However, information on only two parameters (age and condition) is available at the level of individuals within the population. To correct this deficiency and strengthen the correlation between the instream monitoring task with its focus on communities and populations and the bioindicator studies (Task 3) with their focus on the individual, several additional parameters of the target species (either bluegill or redbreast sunfish) will be measured in the field during the second year of the BMAP.

Growth rates of individuals in the population will be estimated by periodic sampling of tagged fish. A sample of fish from each of several age classes will be collected by electrofishing and tagged; length and weight will be recorded and scales will be taken for age determination prior to release. Sampling will be conducted at approximately 3-month intervals during the spring and summer to minimize the effects of electroshocking on growth (Gatz et al. 1986). Continuous strip-chart recordings of water temperature will be obtained with a Peabody Ryan Model J90 thermograph at each site. Because this procedure for

estimating growth rates requires (1) marking a large number of individuals initially and (2) intensive sampling thereafter to obtain a sufficient number of fish representing all age classes, the study will be conducted at a subset of the six sampling sites shown in Fig. 1 and at a control site.

Another parameter of individuals in a population is fecundity, or the number of ripening eggs in the female just prior to spawning (Bagenal 1978), which provides a measure of potential reproductive success. Fecundity of either bluegill or redbreast sunfish will be estimated by gravimetric subsampling (Bagenal and Braum 1978) at the same sites selected for the individual growth rate studies. The number of eggs per female will be expressed as a function of fish length and age at each site.

Finally, an evaluation of feeding preferences will be conducted on the same species used in the growth rate and fecundity studies. To obtain data on diet composition, including variability in composition as a function of sampling location and season, stomachs will be flushed by inserting a small plastic tube (0.5-cm O.D.) into the gullet and repeatedly injecting water into the stomach using a rubber bulb with one-way valves. This nondestructive sampling technique is the same as that used by Loar (1985) and is similar to the procedure described by Seaburg (1956). Taxonomic identification of stomach contents will be made to the lowest practical taxon (genus, if possible). Additional, but more qualitative sampling of other species may also be required to establish trophic relationships that are poorly documented in the literature. Together, the studies on food habits of fishes in EFPC will provide general information on trophic interrelationships that can be used to construct community food webs or to evaluate biotic integrity (e.g., Karr et al. 1984).

In summary, information obtained from the instream monitoring studies will be used to determine whether the effluent limits specified in the NPDES permit for the Oak Ridge Y-12 Plant protect the classified uses of EFPC, specifically the use of the creek for "growth and propagation of fish and other aquatic life" (TDPH 1978). The data will also be used in support of the studies outlined in Sects. 2.2 and 2.3.

For example, correlations between results of the bioindicators studies and field measurements of growth rates or fecundity can be examined to determine the feasibility of using bioindicators as a cost-effective approach to long-term monitoring of fish populations. In addition, studies of age structure and population densities will be used together with results of the contaminant bioaccumulation studies to assess present and future impacts of the Oak Ridge Y-12 Plant discharges on the potential EFPC sport fishery. Finally, information generated by all four tasks can ultimately be used to evaluate the effects of new wastewater treatment facilities on the ecological health of EFPC (Sect. 2.5). Identification of possible causal factors responsible for any adverse effects that are observed can be performed using both chemical analyses and diagnostic laboratory bioassays on influent streams upstream of the treatment plant. In this way, corrective measures can be designed and implemented over much shorter time periods than would be possible if only instream monitoring of the biotic communities was used to determine effects.

2.5 SUMMARY AND SYNTHESIS

The proposed EFPC BMAP consists of four major tasks that reflect different but complementary approaches to evaluating the effects of the Oak Ridge Y-12 effluents on the biotic integrity of stream communities. The evaluation of effects is focused on both regulatory compliance, as required by Part III(c), Special Condition No. 7, of the NPDES permit for the Oak Ridge Y-12 Plant, and documentation of the ecological changes associated with several new wastewater treatment facilities that will come on-line over the next 4 years. The four tasks include (1) toxicity monitoring, (2) bioaccumulation studies, (3) biological indicator studies, and (4) instream monitoring of benthic invertebrate and fish communities.

Integration of the four tasks as a function of the flow of information between them is shown in Fig. 5. For example, information obtained on the benthic invertebrate communities in EFPC (Task 4) can be compared with the results of the sediment bioassays (Task 1). Data on

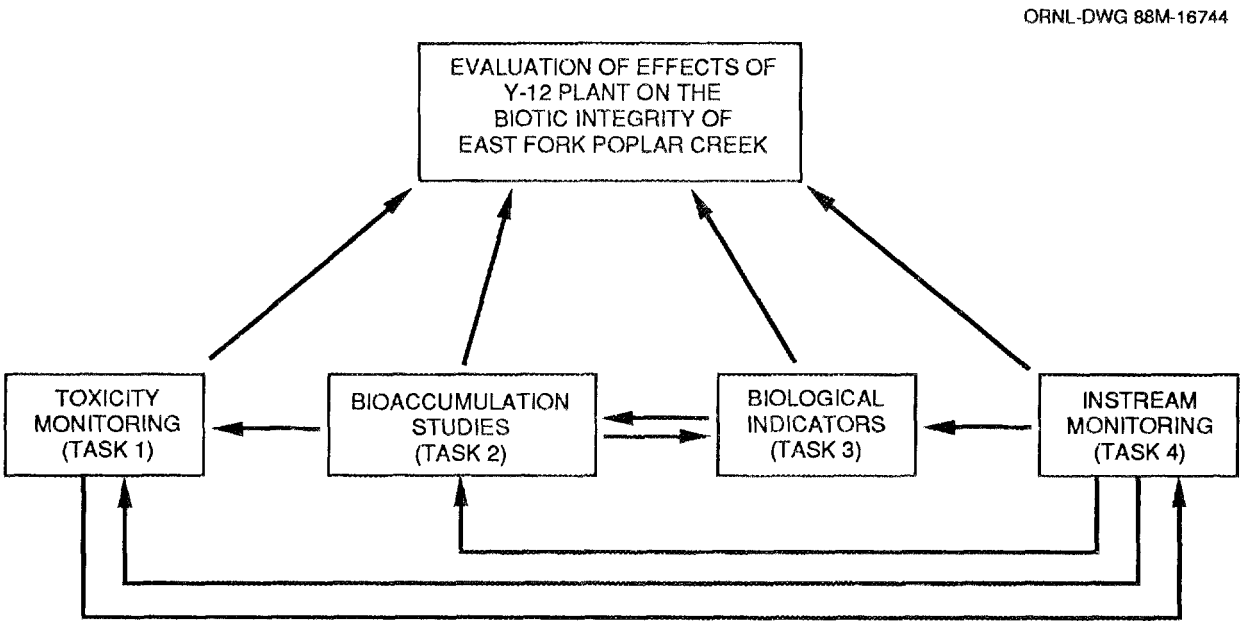

Fig. 5. Integration of the four major tasks comprising the East Fork Poplar Creek Biological Monitoring and Abatement Program. Arrows represent pathways of information flow.

fish population abundance, growth, and reproduction (Task 4) can be correlated with both the results of the fish toxicity tests and the results from the biological indicator studies (Task 3); short-term responses at the tissue or organ level can be correlated with long-term responses at higher levels of organization (e.g., populations), as measured by instream monitoring. Also, physiological measurements (e.g., metabolism) obtained in Task 3 will be used in the bioenergetics modeling component of Task 2 to predict contaminant bioaccumulation. Likewise, toxicant body burdens measured in Task 2 will assist in the interpretation of the results of toxicity tests and in determination that exposure periods are appropriate (Task 1).

Components of the BMAP will also be integrated with other environmental monitoring programs at the Oak Ridge Y-12 Plant. Water quality data that are collected to meet the requirements of the NPDES permit will be used to interpret the ecological information obtained from the BMAP. As stated in Sect. 2.1.2, the NPDES monitoring station at the outfall of NHP will be the major sampling location for evaluating the toxicity of discharges from the Oak Ridge Y-12 Plant and for documenting changes in effluent toxicity over time. Consequently, the water quality data collected at this station can be compared with the results of the toxicity studies (Subtasks 1a-1e, Sect. 2.1.3) to identify those variables that could be responsible for observed mortality or reductions in growth of the test species. Water quality data from this and other effluent monitoring stations above NHP, as well as results of the Toxicity Control and Monitoring Program, will be used in the point-source toxicity evaluation described in Subtask 1d.

We will also use the routine NPDES monitoring data from the outfall of NHP to (1) characterize the water quality of upper EFPC below NHP; (2) identify contaminants that may be associated with various biological indicators in fish collected from this site (see Task 3, Table 6); and (3) identify potential sources of impact on species composition of the benthic invertebrate and fish communities in upper EFPC. A long-term NPDES water quality data base consisting of weekly measurements at the outfall of NHP can also be used to document changes in ambient conditions that may be correlated with temporal changes in community

composition and in contaminant levels (e.g., mercury) of EFPC biota. Data collected during the PCB Monitoring Program will be used both to evaluate PCB levels in the environment for comparison with PCB levels in fish and to identify potential sources of PCB contamination.

In view of the location of the plant at the headwaters of EFPC, the temporal and spatial complexity of the effluent discharged from the Oak Ridge Y-12 Plant, and the absence of background ecological studies, any biological monitoring that is conducted for the purposes of ensuring compliance with applicable regulations and of evaluating the effects of remedial actions must utilize several approaches. The four tasks outlined in Sect. 2.0 represent an integrated approach to monitoring that combines well-established monitoring methods with more innovative techniques to establish regulatory compliance and ensure environmental restoration and protection.

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