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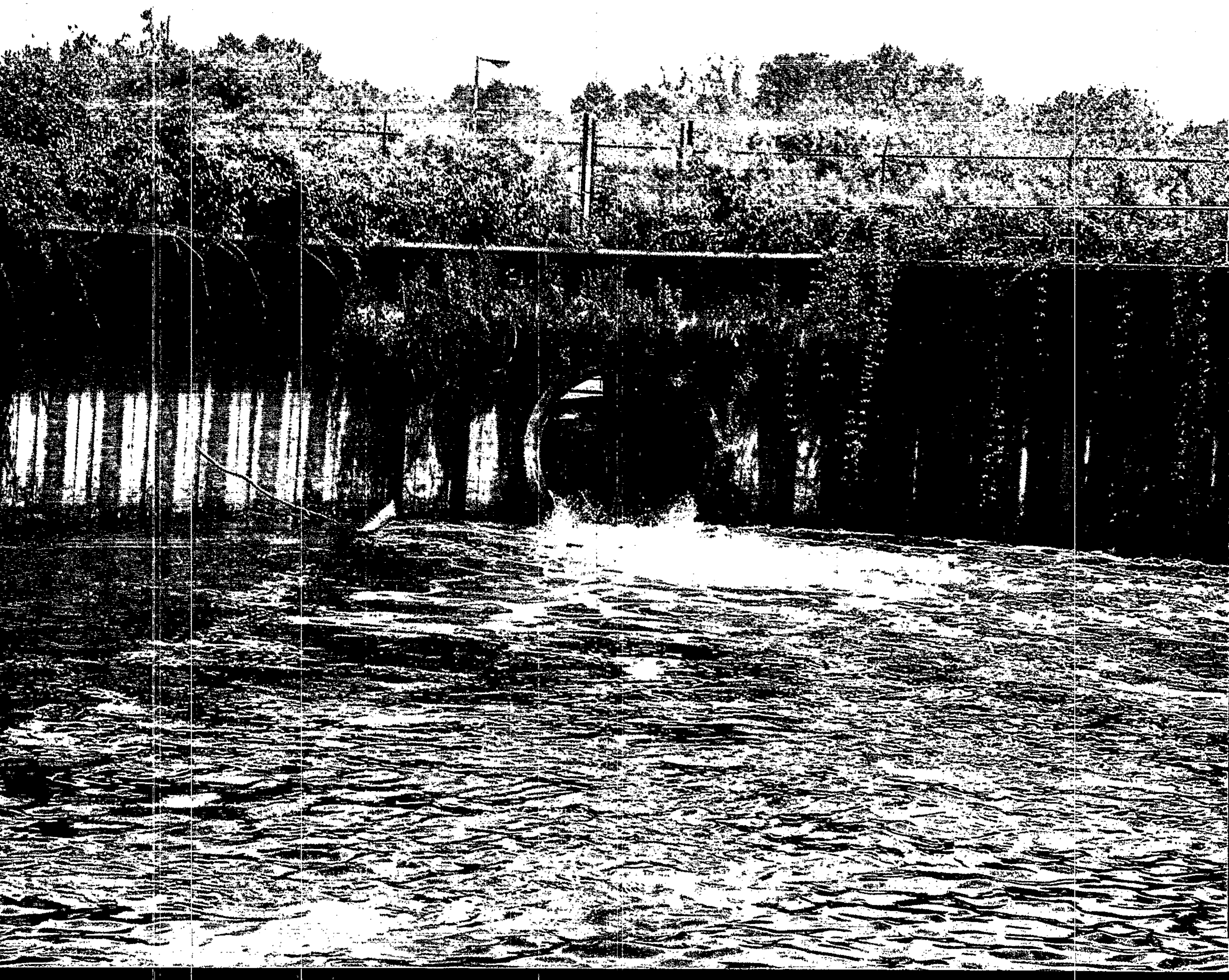
Biomonitoring to Achieve Control of Toxic Effluents



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Biomonitoring to Achieve Control of Toxic Effluents

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Foreword

When the Cuyahoga River caught fire at Cleveland, Ohio on June 22, 1969 the river immediately became a symbol of the degraded state of the nation's surface waters. Since then, significant efforts have been made to improve the river's water quality, and the Cuyahoga is a much cleaner river than it was 18 years ago.

Downstream from Akron, Ohio, the Cuyahoga flows through rural surroundings and the Cuyahoga Valley National Recreation Area before reaching Cleveland, where it discharges into Lake Erie. It is a shallow, swift-flowing river that has the potential for being an excellent warm water habitat, which is the river's designated use. The physical habitat necessary for supporting a healthy fishery is present and the oxygen levels in the free-flowing portions of the river are consistently high. In 1984, however, the few fish that were found in a 16-km stretch of the river downstream of Akron were juveniles of pollution-tolerant species (white suckers and creek chubs), most of which had lesions, eroded fins, or skeletal deformities. Few of the fish were expected to live to adulthood.

The Akron Publicly Owned Treatment Works (Akron POTW) discharges its effluent at the point in the river where a severely depleted fish population was first observed. The Akron POTW is a secondary treatment facility for 2.2 to 4.4 m^3/s (50 to 100 mgd) of industrial and domestic waste. At certain times during the year, the wastewater comprises up to 60 percent of the total flow of the river.

This report provides a case study of how water quality-based toxicity control procedures can be combined with chemical analyses and biological stream surveys to achieve more effective water pollution control. It describes how regulatory agencies used laboratory toxicity testing and biological stream surveys to confirm that the Akron POTW causes the Cuyahoga's water quality problem downstream from Akron, and that effluent toxicity, rather than conventional or nonconventional pollutants, causes the effects observed. It describes the toxicity testing and chemical analysis procedures that researchers used to search for toxicants responsible for the effluent toxicity. Finally, the report presents sample permit limit derivation for the Akron POTW. This derivation is presented for educational purposes only and does not represent an official EPA regulatory action. Derivation calculations are followed sequentially through to recommendations which outline the toxicity tests that would be most valuable to monitor the toxicity of the Akron POTW's effluent.

Contents

1. Introduction

This case study describes the water quality-based toxicity control procedures that researchers used to address a serious water quality problem on the Cuyahoga River in northeastern Ohio. The objective was to provide National Pollutant Discharge Elimination System (NPDES) program managers, NPDES permit writers, and water quality specialists with an example of how toxicity testing can be used to address pre-identified toxic water quality problems.

1.1 Regulatory Background

During the 1960s and 1970s the major focus of pollution control was conventional pollutants — oxygen-demanding materials, heat, and suspended solids — which were causing severe degradation of rivers, lakes, and streams. Industries installed technologies to control the discharge of these types of pollutants and billions of dollars were invested in publicly owned treatment works (POTWs). Progress was so dramatic that by the 1980s, point source contributions to conventional pollution problems were considered largely under control. The focus of point source abatement programs then shifted to the control of toxic chemicals.

In regulating toxic discharges, water pollution control agencies have usually used a "chemical-specific" approach. Typically, a regulatory authority would require a large industrial discharger to monitor and submit data on the concentrations at which the priority pollutants' list toxicants occurred in its wastewater discharge. The concentrations reported were often based on one effluent sample per week or month. To keep laboratory testing costs low, regulators would allow multipollutant scans with detection levels for individual compounds of about 50 to 100 parts per billion — levels higher than the toxic effect concentrations of some priority pollutants.

For many dischargers, regulatory authorities set concentration limits on compounds based on state water quality standards. Many such standards limit only the concentration of 10 or fewer metals and other inorganics, and five or fewer organic pollutants. This occurs when states have adopted numerical criteria based on EPA's Red Book values from 1976 (1), although narrative water quality standards are now available.

Nationwide, certain categories of industrial dischargers are required to meet minimum treatment levels (e.g., best available technolgy [BAT], best possible technology [BPT]) for a list of conventional and priority pollutants that have been found in the effluent discharged by most facilities in that category. These treatment standards do not necessarily relate to acceptable concentrations to prevent toxicity to aquatic life. Only POTWs that receive wastes from many industrial users, or users in certain industrial categories, must analyze their effluent for toxicants. Using this chemical-specific methodology, regulators have reduced the discharge of the priority list toxicants and of other toxicants with similar physical and chemical properties by millions of pounds annually. For many dischargers this level of control has reduced effluent toxicity to acceptable levels. Limited toxicity testing by the U.S. Environmental Protection Agency (EPA), some states, and the regulated community, however, has shown that controlling conventional pollutants and the 126 priority pollutants does not reduce or eliminate the toxic effects of all discharges. In particular, large, multiprocess industrial facilities and POTWs that treat industrial plant wastewaters may have effluents laden with nonpriority list toxicants. These toxicants may significantly contribute to the degradation of some surface waters. In less complex situations a discharger's effluent may contain a few nonpriority list toxicants in concentrations that adversely affect the instream biota and exceed water quality standards.

A chemical-by-chemical approach to controlling nonpriority toxic pollutants can be inadequate for several reasons. Many of these compounds cannot be detected by gas chromatography/mass spectroscopy (GC/MS) or other widely available detection methods. Of the subset of toxicants that can be detected by GC/MS analysis, complex wastewaters often contain many more than can be identified using the most comprehensive mass spectroscopy libraries. For many of the compounds that can be detected and identified, little or no definitive aquatic toxicology data are available. These difficulties point up the need for a more direct and cost-effective method for toxicity assessment and control.

1.2 The Cuyahoga River Study Area

The Cuyahoga River's water quality problems downstream from the Akron POTW represent a typical case of the need for advanced water quality-based pollution controls for certain dischargers on certain receiving waters. The Akron POTW is a well-operated municipal wastewater treatment facility that achieves significant reductions in conventional and toxic pollutant levels through sophisticated treatment processes (see Sections 2.5 and 3.1.1). Yet the condition of the Cuyahoga River downstream of the Akron POTW as a fishery is poor and its recreational and aesthetic value is lowered.

At the time of this study, several factors indicated that the Akron POTW was causing the water quality problem, and that toxic rather than conventional or nonconventional pollutants were producing the adverse effects on water quality. These factors were:

- Industrial wastes comprised about 14 percent of the influent to the Akron POTW. These wastes may have contained many toxic pollutants which exhibited inhibitory effects to the biota at very low concentrations.
- GC/MS scans of the effluent showed several organic pollutants in the effluent. The pollutants causing the toxicity may not even have been seen in these scans because their low concentrations were undetectable or they could not be extracted for analysis.
- The Akron POTW effluent constituted up to 60 percent of the river's flow at certain times of the year.
- The few fish found in the river exhibited characteristics such as lesions and other deformities associated with exposure to toxicants.

The Akron POTW was selected for the case study as a result of cooperative efforts between various Federal and state agencies. The Ohio EPA, U.S. EPA Office of Research and Development (ORD), Cincinnati, and U.S. EPA Region V identified the toxicity problem as described in Chapter 2. A reconnaissance trip was conducted by U.S. EPA Environmental Research Laboratory (ERL), Duluth, and others to confirm the site selection and to refine the study plan for an on-site test program. The results of this field reconnaissance trip are presented in Chapter 3. The evolution of the study plan (Chapter 4) is presented in view of the site selection data, reconnaissance data, and site description. Chapter 5 contains the results of the on-site testing of effluents and ambient river water. The toxicity source investigation for identifying toxic components and to suggest appropriate treatment technologies is presented and used to make sample NPDES permit recommendations for the Akron POTW (Chapter 7). Technical methods are not included in this document but are referenced as appropriate.

2. Site Selection Process

2.1 Identification of the Cuyahoga River as Having a Water Quality Problem

Ohio environmental regulatory agencies (e.g., Department of Health and Ohio EPA) and other cooperating groups have recognized problems and have monitored conditions in the Cuyahoga River at intervals over the last 40 years. During that time, the discharge of municipal and industrial wastes has been substantially reduced and there have been documented improvements in the chemical water quality of the river (2,3,4,5,6,7). In 1960, dissolved oxygen (DO) concentrations measured in the Cuyahoga downstream from Akron showed that a 27-km segment of the river between Akron and the Canal Diversion Dam (near river kilometer [RK] 32) was anoxic (see Glossary) (4). Since that time, the DO profile has steadily improved and 1984 to 1985 results show that DO is no longer a limiting factor to the biota (8, 9). Likewise, the instream average ammonia concentrations downstream from the Akron POTW have declined from >5 mg/L in 1969 to <1 mg/L in recent years (8,9).

The segment of the Cuyahoga River downstream from the City of Akron was termed grossly polluted in the 1960s (4). Benthic macroinvertebrate sampling was conducted in 1973, 1974, and 1980 by researchers at the University of Akron, with results indicating that the macroinvertebrate communities in the river between Akron and Independence, Ohio were predominantly pollution-tolerant organisms, primarily pulmonate snails *(Physidae)*, oligochaetes, and midges *(Chironomidae)* (see Figure 2-1) (10, 11). Conditions were blamed primarily on pollutants originating in the Akron area of the river.

Historical data on the fish communities in the Cuyahoga River downstream from Akron are rather sparse. Fish were not observed or collected at any of 12 sampling sites between the Akron POTW (RK 62) and Independence, Ohio (RK 23), in the summer of 1967 survey (4). Intensive sampling in the early 1970s between the Akron POTW and Peninsula, Ohio (RK47) yielded no fish, while downstream from the dam in Peninsula, a fish community composed only of 19 species representing three families (*Cyprinidae, Catastonidae, Centrarchidae*) was found (12, 13).

U.S. EPA Region V data analyses from 1981 indicated a water quality problem in the Cuyahoga due to the Akron POTW. Concentrations of phthalate esters, phenols, and ammonia in ambient water samples were greater than state standards at the three ambient stations nearest to, and downstream of, the Akron POTW (the Ohio phenol standard is set to prevent taste and odor problems, not toxicity). A number of actions were initiated to bring the Akron POTW into compliance with its NPDES permit limits. These included improvements in sludge handling, primary settling, aeration, final settling, and phosphorus removal. As a result, the Akron POTW made a number of structural and process changes to improve the efficiency of operation and treatment.

In 1984 Ohio EPA conducted a biological impact assessment on the main stem of the Cuyahoga. The results of this study indicated a 16-km reach where fish collections (species and individuals) were much reduced. This reach traversed a rural area, including a portion of a national park and recreation area. The Akron POTW is located at the upstream end of this 16-km reach.

2.2 1984 and 1985 Fish Survey Data

Water quality data collected in 1984 confirmed that the chemical/physical condition of the Cuyahoga River downstream from Akron had greatly improved (2). Along with improved DO and lower ammonia concentrations, the data also revealed that levels of other conventional pollutants (e.g., biochemical oxygen demand [BOD], suspended solids [SS]) and chlorine and heavy metals met or nearly met (less than 10 percent frequency of exceedance) Ohio EPA water quality standards for warm water aquatic life habitat.

Physical habitat conditions were excellent and judged to be fully capable of supporting a warm water fish community (2). The biological data, however, revealed that the river was nearly devoid of fish for 27 km downstream of the Akron POTW. The fish survey was conducted along a 104-km segment of the river in 1984 and along a 72-km segment in 1985. Two ecological indices were used to characterize the overall health of the fish community and the river system in 1984. The profile of the fish community using the Index of Well-Being illustrated a rapid decline downstream from the Akron POTW (Figure 2-2) (14). The loss of nearly all fish just downstream of the Akron POTW and the failure of the fish community to recover to upstream levels indicated a persistent toxic influence. A similar pattern and severity of impact was also apparent using the Invertebrate Community Index (ICI) (See Section 2.3) (13). Some component of the Akron POTW discharge was limiting the fish fauna, although the conditions measured in 1985 were slightly improved (Figure 2-2). The predominant species were juvenile white suckers and creek chubs (13). Observations of fin erosion, lesions, and external deformities on the fish collected in 1985 added more evidence of a serious environmental stress in the Cuyahoga River downstream from Akron (9).

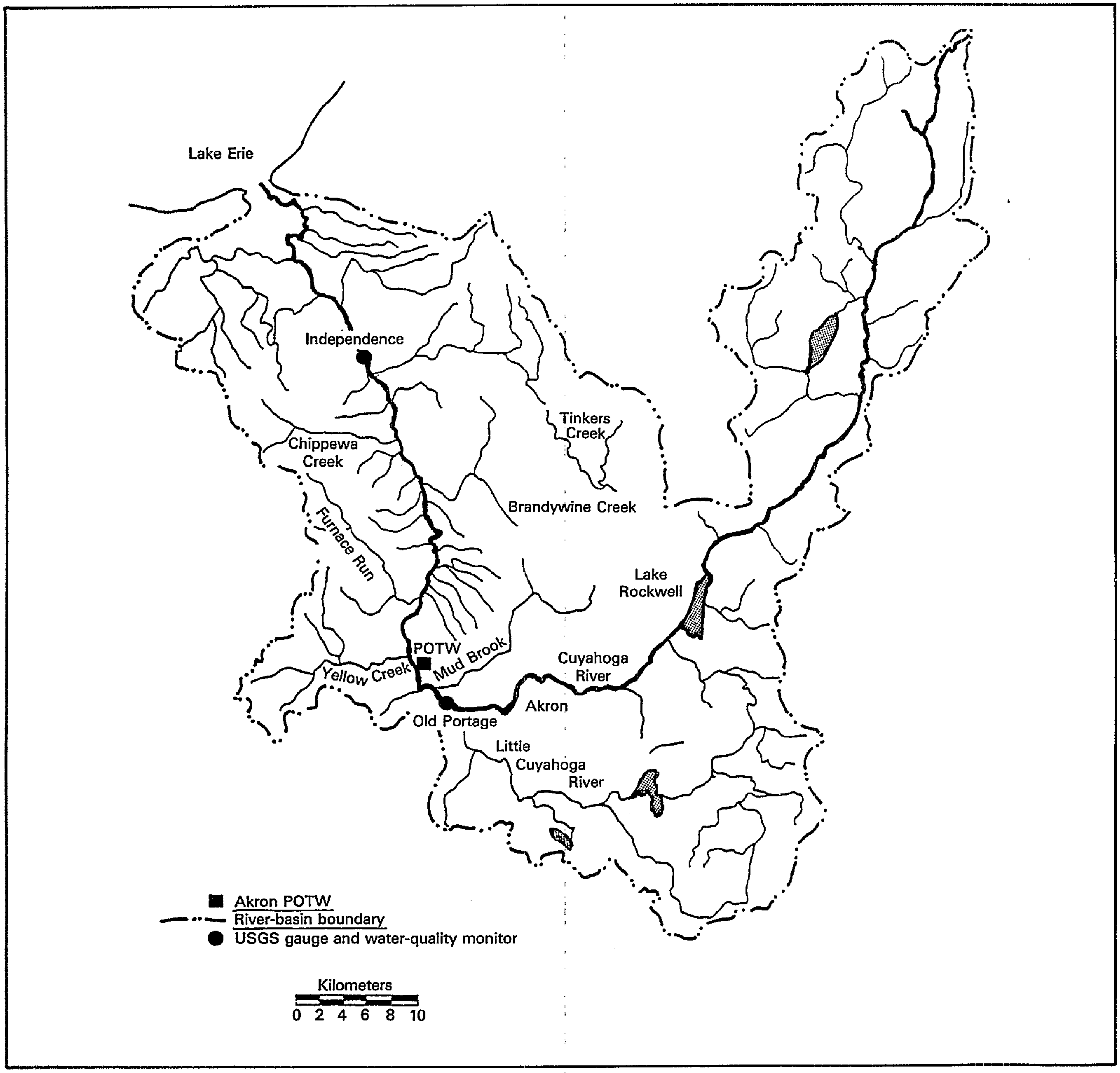


Figure 2-1. The Cuyahoga River Basin (19)

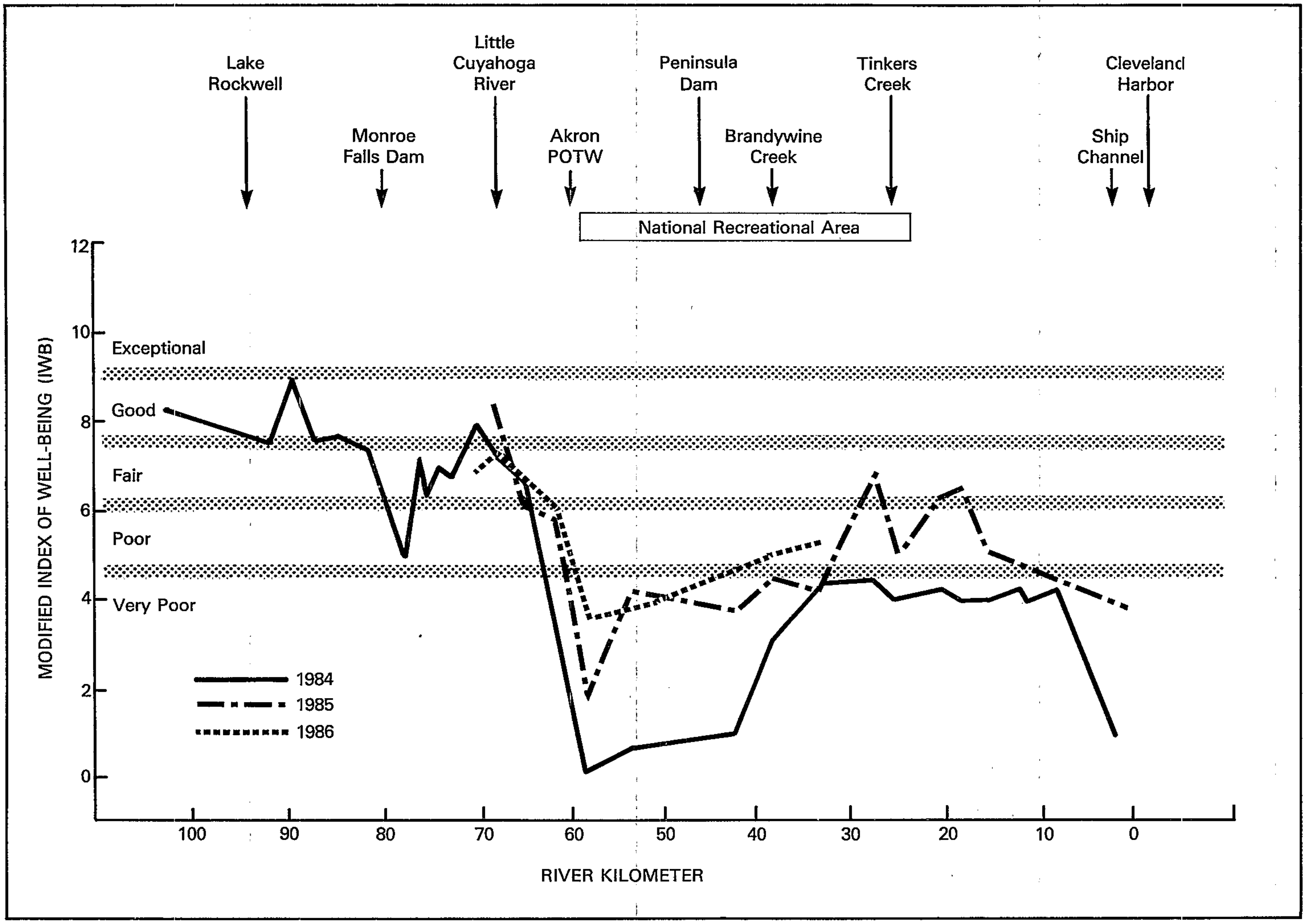


Figure 2-2. Profile of the Cuyahoga River Fish Community Using a Modified Index of Well-Being (17)

2.3 1984 Benthic Invertebrate Survey Data

Invertebrate samples were collected in the middle and lower reaches of the river by Ohio EPA in 1984 (8). Eleven stations were located at positions upstream of the Little Cuyahoga River, upstream from the Akron POTW, and at numerous locations downstream from the Akron POTW for approximately 48 km. Longitudinal profiles of taxa richness, Shannon-Wiener diversity index values, and taxonomic composition are shown in Figures 2-3 and 2-4.

In addition, Ohio EPA developed the Invertebrate Community Index (ICI) to measure aquatic invertebrate community health. This index is composed of eight measures of taxa and density, and results in ICI values are designed to reflect stream water quality. Invertebrate data from 1984 yielded ICI values that reflected good water quality upstream of the Akron POTW at the confluence with the Little Cuyahoga River. Reduced ICI values reflected poor to fair water quality downstream of that confluence and for 44 km downstream of the Akron POTW (Figure 2-5). This decline was first observed at a station between the confluence and the Akron POTW, indicating that another discharger may have influenced the water quality, although toxicity data do not show any effect upstream of the Akron POTW (see Sections 2.5 and 3.2). An intermediate area near the confluence

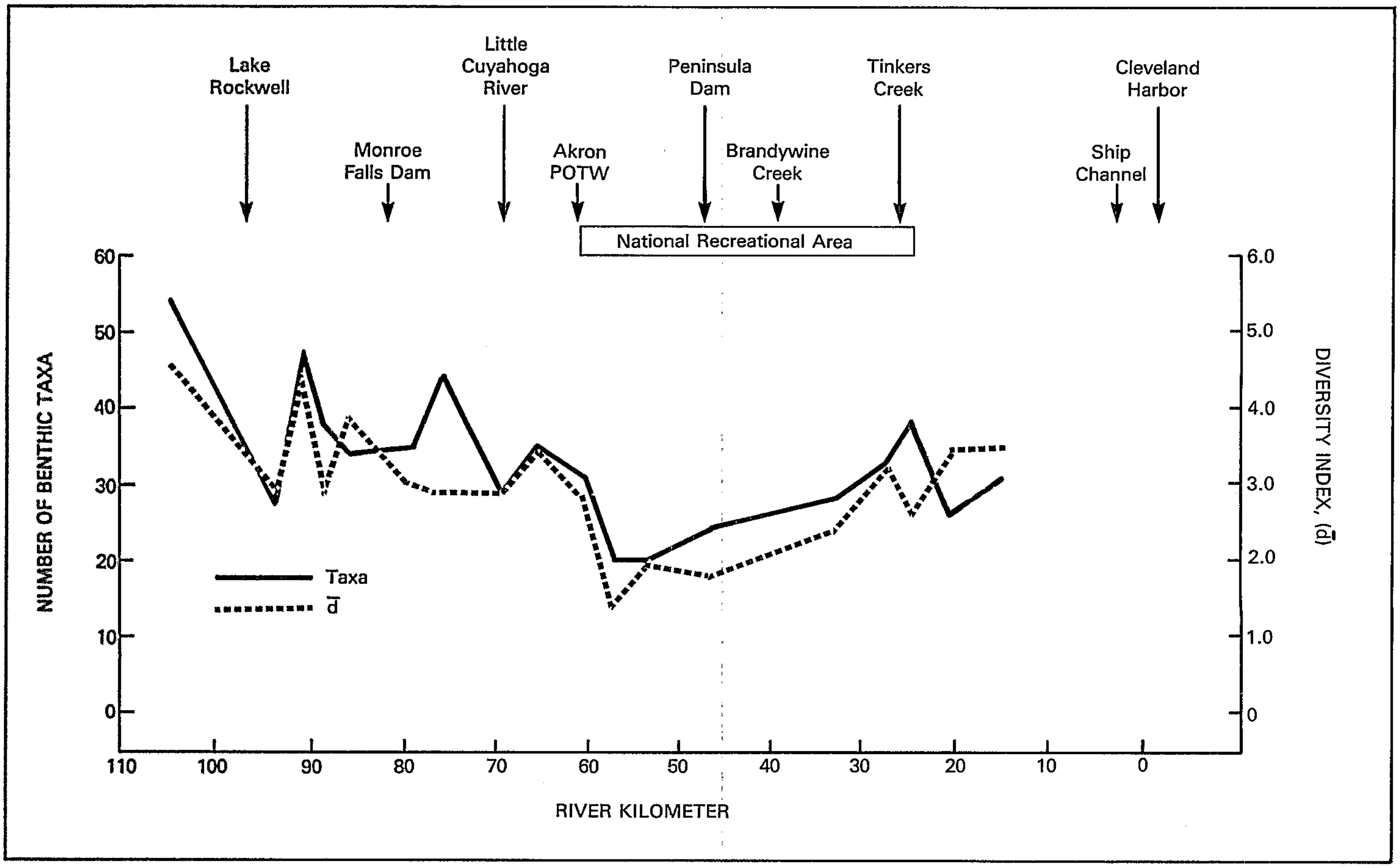


Figure 2-3. Profile of the Number of Benthic Taxa and Benthic Diversity on the Cuyahoga River

with Tinkers Creek had better ICI values, indicating some recovery, but that dissipated within 8 km.

A good invertebrate community predominated by pollution-intolerant and pollution-intermediate mayflies, caddisflies, and midges was collected at the station upstream from the Little Cuyahoga River. Substantial compositional changes in the community (from insect to noninsect dominated) initially occurred downstream from the Little Cuyahoga followed by further changes downsteam from the Akron POTW. Less dramatic declines also occurred in taxa richness, density, and diversity over this area of the Cuyahoga River. Predominant organisms at nearly all the sites downstream of the Akron POTW were the pulmonate snail genus *Physella,* the mayfly species *Bactis intercalaris,* and the midges *Cricotopus bicinctus, Polypedilum (Polypedilum) fallax* group, and *Conchapelopia.* This association of invertebrates has not been typically encountered in other Ohio rivers with heavy organic loadings from POTWs. In addition, the typical community response to organic contamination (i.e., degradation followed by various stages of downstream recovery) did not occur downstream of th Akron POTW. Relatively little community change was detected for nearly 32 km downstream of the Akron POTW.

2.4 Evaluation of Stream Surveys

Recent sampling has indicated much improved water quality conditions in the Cuyahoga River. Yet fish surveys have rarely caught fish downstream of the Akron POTW (Section 2.2), and benthic invertebrate surveys of this same area have indicated a community change (Section 2.3). Treatment improvements had succeeded in reducing levels of conventional pollutants (e.g., SS)

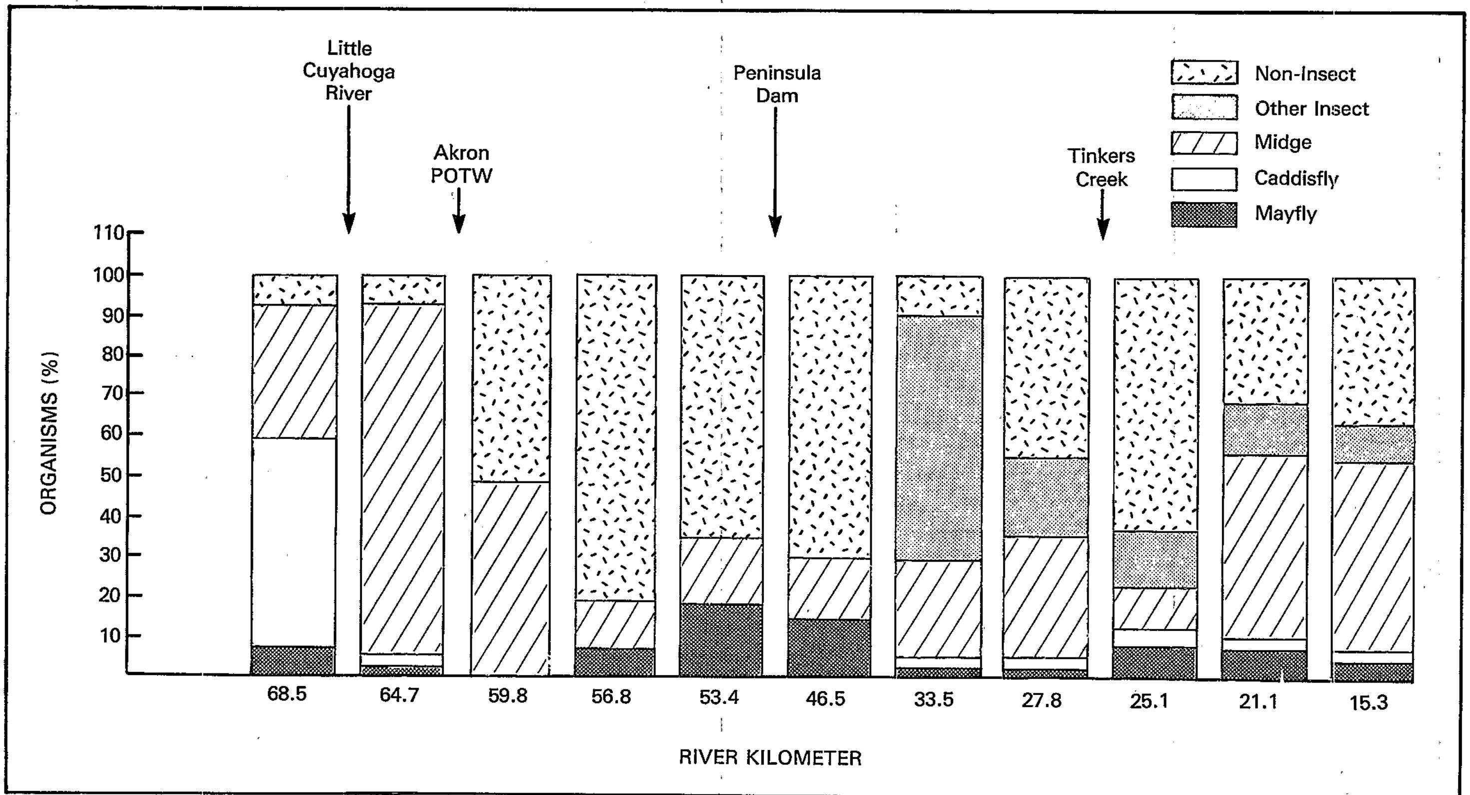


Figure 2-4. Benthic Invertebrate Community Composition From Selected Stations on the Cuyahoga River (7)

discharged to the river; however, a corresponding gradual improvement in aquatic organism populations or community composition compared to upstream conditions was not observed. In 1985 Ohio EPA began to examine the possibility of other types of pollutants such as toxicants.

2.5 Previous Effluent Toxicity Surveys

A separate survey of six Ohio POTWs was conducted by U.S. EPA ORD Cincinnati in 1984 and 1985. The results of this survey indicated that of the six POTWs, the Akron plant was receiving the most toxic raw wastewater for treatment (15). Results of toxicity tests on fathead minnows (*Pimephales promelas*) and a cladoceran (*Ceriodaphnia dubia*) also showed reduced toxicity with treatment, but no observed effect concentration (NOEC) values were still 10 to 100 percent. These data indicated that the treated effluent discharged by the Akron POTW could still be toxic. The data did show variable toxicity, but since the effluent often constituted a large portion of the Cuyahoga River flow, the effluent could have been a cause of the observed stream effects. The State of Ohio water quality standards limit acute toxicity within the mixing zone to less than rapidly lethal conditions, regardless of the quantity of water available for dilution.

The ORD-Cincinnati chemical analyses indicated that trace metal concentrations were reduced in the effluent and that conventional pollutant removal requirements were met (16). This indicated that the Akron POTW was treating its influent and achieving large reductions in concentrations of metals and extractable organics, and that plant performance was not an obvious cause of effluent toxicity or adverse biological effects. Investigative efforts were then concentrated on determining the variability of the effluent toxicity, the magnitude of the toxicity, ambient toxicity, and eventually the identification of toxicants in the effluent.

Since the effluent discharge composed a large portion of the Cuyahoga River flow (from 20 to 60 percent), there was probably insufficient initial dilution to remove toxicity. In addition, examination of plant operating records showed that when influent volumes were too great, the plant would bypass raw or partially treated

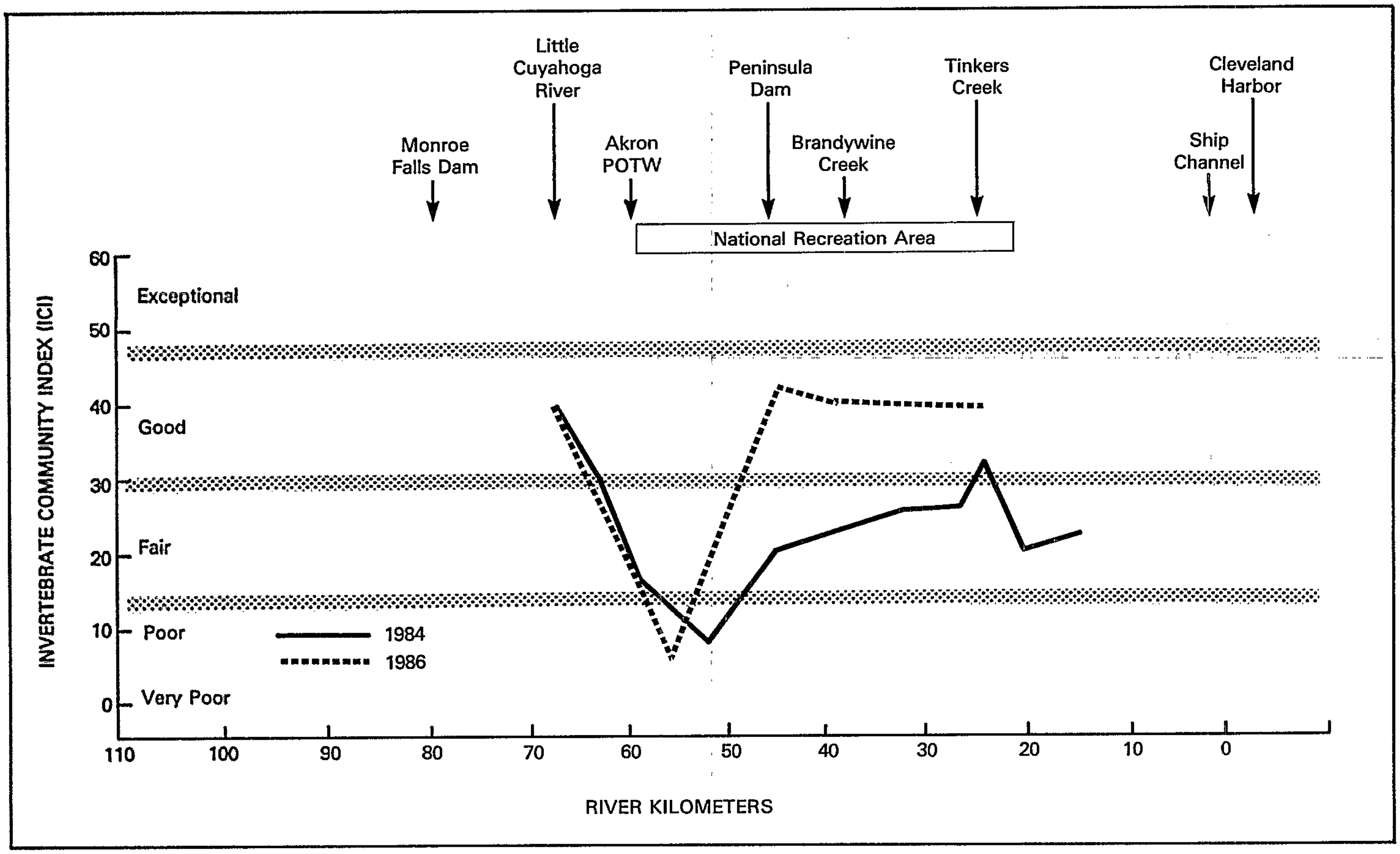


Figure 2-5. Profile of the Cuyahoga Macroinvertebrate Community Using an Invertebrate Community Index (17)

wastewater into the Cuyahoga River. Bypassing after a rain may have produced a diluted but possibly toxic discharge. As a result of the observed instream effects on fish and benthic organisms as well as effluent toxicity, the Akron POTW was selected for a detailed toxicological evaluation.

2.6 Post-Study, 1986 Stream Survey Data

Ohio EPA repeated their previous sampling for invertebrate and fish data during the summer of 1986. ICI values demonstrated a similar pattern to that found in 1984, showing lower water quality downstream of the Akron POTW. The decline in ICI values downstream of the Akron POTW, however, occurred over a much shorter distance, and the maximum values up- and downstream of the Akron POTW, were greater in 1986 than in 1984 (Figure 2-5).

Fish sampling showed increased numbers and increased diversity in the Cuyahoga in 1986. In addition, Ohio EPA noted better use of available habitat by the fish (14). In contrast to 1985 data, adult white suckers were found in 1986 and in general, fish abundance increased as distance downstream from the Akron POTW increased (14). Fish community recovery can also be observed over time and with distance from Akron POTW using the Index of Well-Being (Figure 2-2).

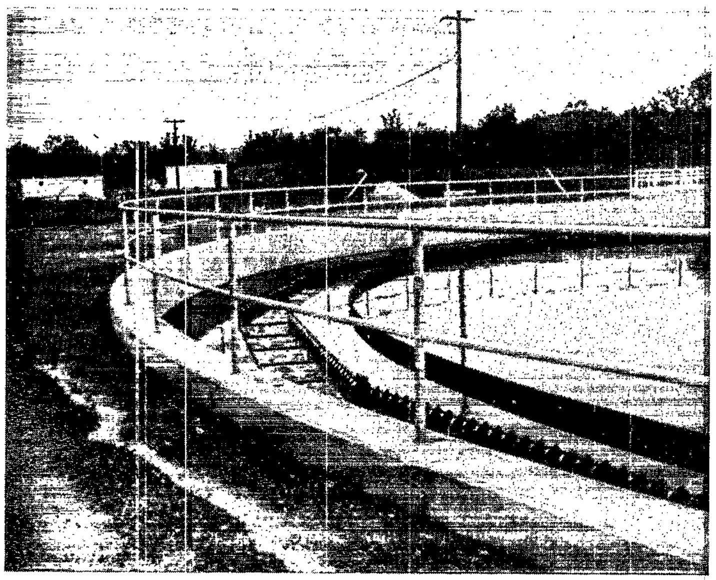
The Ohio EPA noted that these improvements were incremental and the Cuyahoga River biological communities were poor and still reflected toxic effects (14). Effluent toxicity tests during the summer of 1986 resulted in LC50 values >100 percent effluent for *Ceriodaphnia* (Table 6-1). This absence of effluent toxicity should aid in the continuation of improvements to the Cuyahoga River. The Ohio EPA will sample again during the summer of 1987 for water quality, invertebrate community health, and fish community health to document any further improvements to the Cuyahoga River and to verify the absence of acute toxicity noted in 1986.

3. Site Description and Reconnaissance Data

3.1 Site Description

3.1.1 The Akron POTW

The Akron POTW is on the eastern bank of the Cuyahoga River located northwest of Akron, Ohio at approximately RK 62. The service area is approximately 194 km^2 and contains an estimated population of 382,000. Wastewater flow to the Akron POTW is 35 percent residential, 14 percent industrial, and 51 percent infiltration plus stormwater and miscellaneous flows (18). The area is served by a 1,100 km separate and combined stormwater and sewage collection system.



Wastewater settling basin (chlorine contact chamber and EPA mobile laboratory in background).

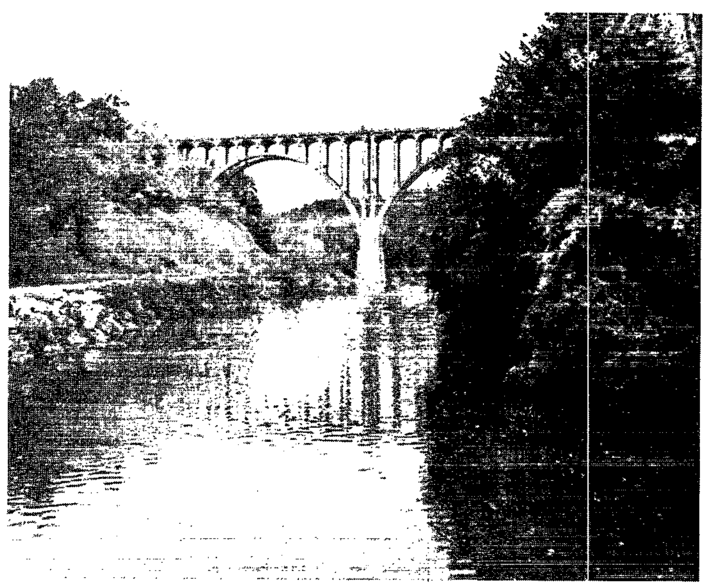
The original plant was constructed in 1928. Since then, major revisions have been made so that the treatment processes now consist of grit removal, screening, preaeration, chemical treatment for phosphorus removal, primary settling, aeration (secondary treatment), final settling, and chlorination. The plant is capable of bypassing influent (raw wastewater) prior to entering the plant. In addition, flows in excess of 6.6 m^3/s (150 mgd) may be shunted after grit removal to the equalization basin and then to the chlorine contact basin before discharge. The process schematic diagram illustrates the waste treatment and indicates the locations of bypassing (Figure 3-1). The partially treated wastewater is chlorinated with the final effluent only during the seasonal chlorination period of May 1 to October 31. The effluent has its greatest effect from June to October when it often constitutes more than 20 percent of the river flow.

Influent (raw) and partially treated wastewater bypasses occur irregularly. The Akron POTW Monthly Operating Reports indicate that 1980 to 1985 total raw bypass volumes varied from 1,134,000 m^3/yr to 11,340,000 m^3/yr (300 to 3,000 mil gal annually) (19). Annual volumes of partially treated wastewater bypasses are probably similar, but totals are not available since the volumes are not reported during the seasonal chlorination period of May 1 to October 31. The bypass volumes alone exceed 10 percent of the Cuyahoga River flows (Chapter 5).

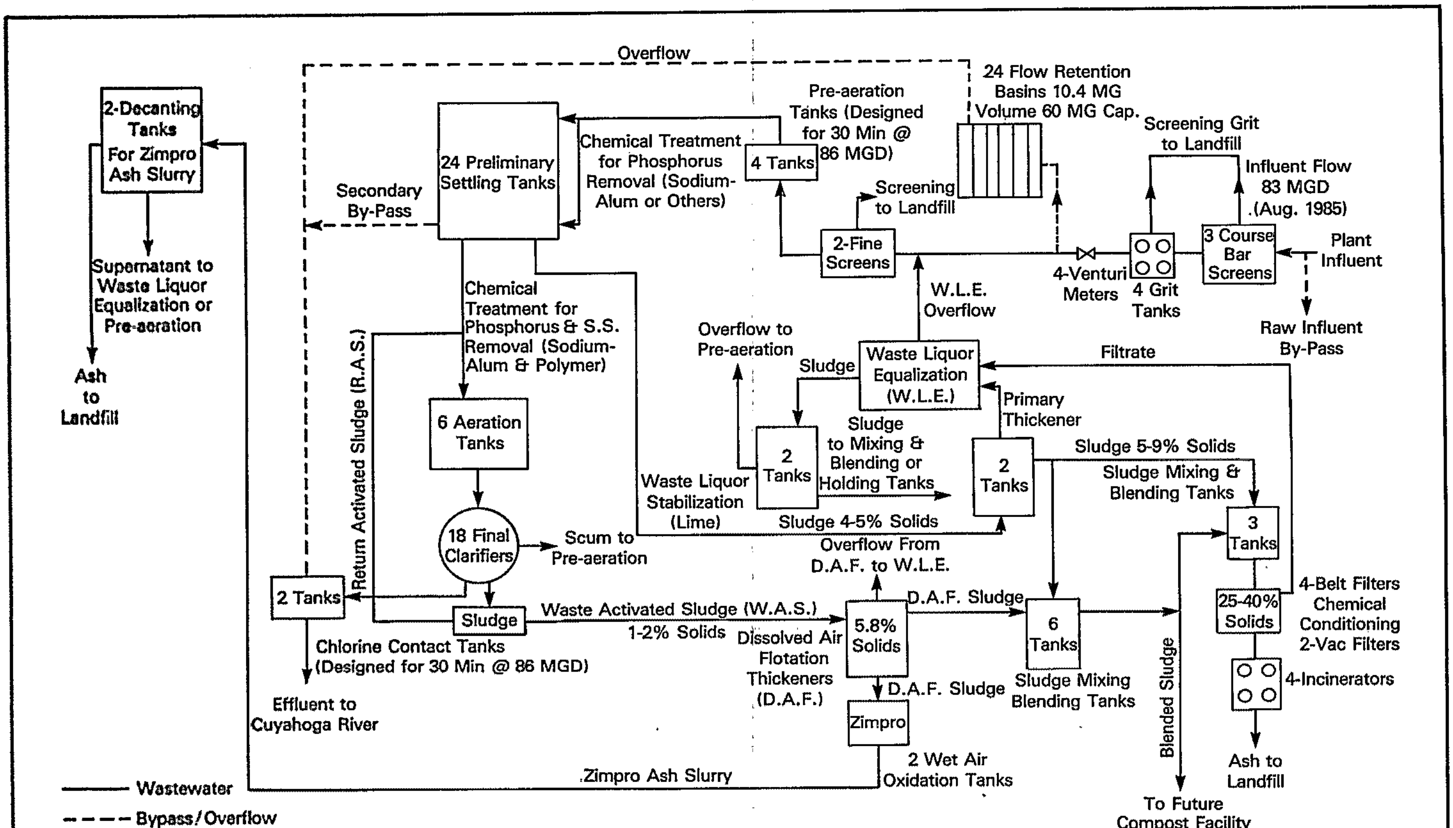
3.1.2 The Cuyahoga River

The Cuyahoga River has a designated use as a warm water habitat, capable of supporting warm water species. The river flows north into Lake Erie from headwaters in Geauga County to the Akron area and through rural Ohio to Cleveland (Figure 2-1). The river runs through the Cuyahoga Valley National Recreation Area for much of the length of the study area. The river is shallow and the water is sufficiently clear to reveal that silty sediments have coated the river bottom in some areas. Such sediments may remove some toxicants from the water column, placing them in greater proximity to benthic organisms and perhaps at higher concentrations than in the water column.

The study area encompassed portions of the middle and lower reaches of the Cuyahoga. The middle reach extends from Lake Rockwell to the Akron POTW, and two of the major tributaries are in the study area (Little Cuya-



Cuyahoga River downstream of Station 10.



SOURCE: City of Akron Water Pollution Control Division.

Figure 3-1. Schematic Diagram of the Akron POTW Processes

hoga River and Mud Brook). Water quality is considered to be degraded in the middle reach (20). The lower reach extends from the Akron POTW to Lake Erie and includes the Cuyahoga Valley National Recreation Area. The portion of the study area in the lower reach has five tributaries: Yellow Creek, Furnace Run, Brandywine Creek, Chippewa Creek, and Tinkers Creek (Figures 2-1 and 3-2). A 1980 Ohio EPA report considered the waters from the Akron POTW to Furnace Run to be grossly polluted (21).

Near the Akron POTW the river is approximately 7 m wide and has had a mean flow at Old Portage of 12.1 m^3/s over the 59-year period 1925 to 1984 (7). During the low flow periods in the summer, the Akron POTW discharge returns much of the water removed from the Lake Rockwell water supply reservoir for use in the Akron area and this contributes as much as 60 percent of the river flow (19). Minimum flows of 0.7 m^3/s have been recorded twice at Old Portage in the last 59 years (7), where the 7Q10 value for September to November is 1.27 m^3/s based on 39 years of data (22). Downstream at the Independence, Ohio gauging station the mean flow has been 23.3 m^3/s, nearly twice that measured at Old Portage. The 7Q10 value for the period of September to November is 1.92 m^3/s, based on 44 years of data at the Independence gauge (22).

The Cuyahoga River and its tributaries receive point source discharges from various industrial and commercial operations. On the Cuyahoga River proper, Ohio EPA has issued NPDES permits to 32 dischargers (one of which is the Akron POTW). There are point source discharges to Mud Brook, Yellow Creek, Furnace Run, Brandywine Creek, and Tinkers Creek, as well as to other tributaries. Field stations were located by each of these four tributaries during the full-scale evaluation. In addition, a landfill adjacent to the Akron POTW has a small intermittent discharge to the Cuyahoga River.

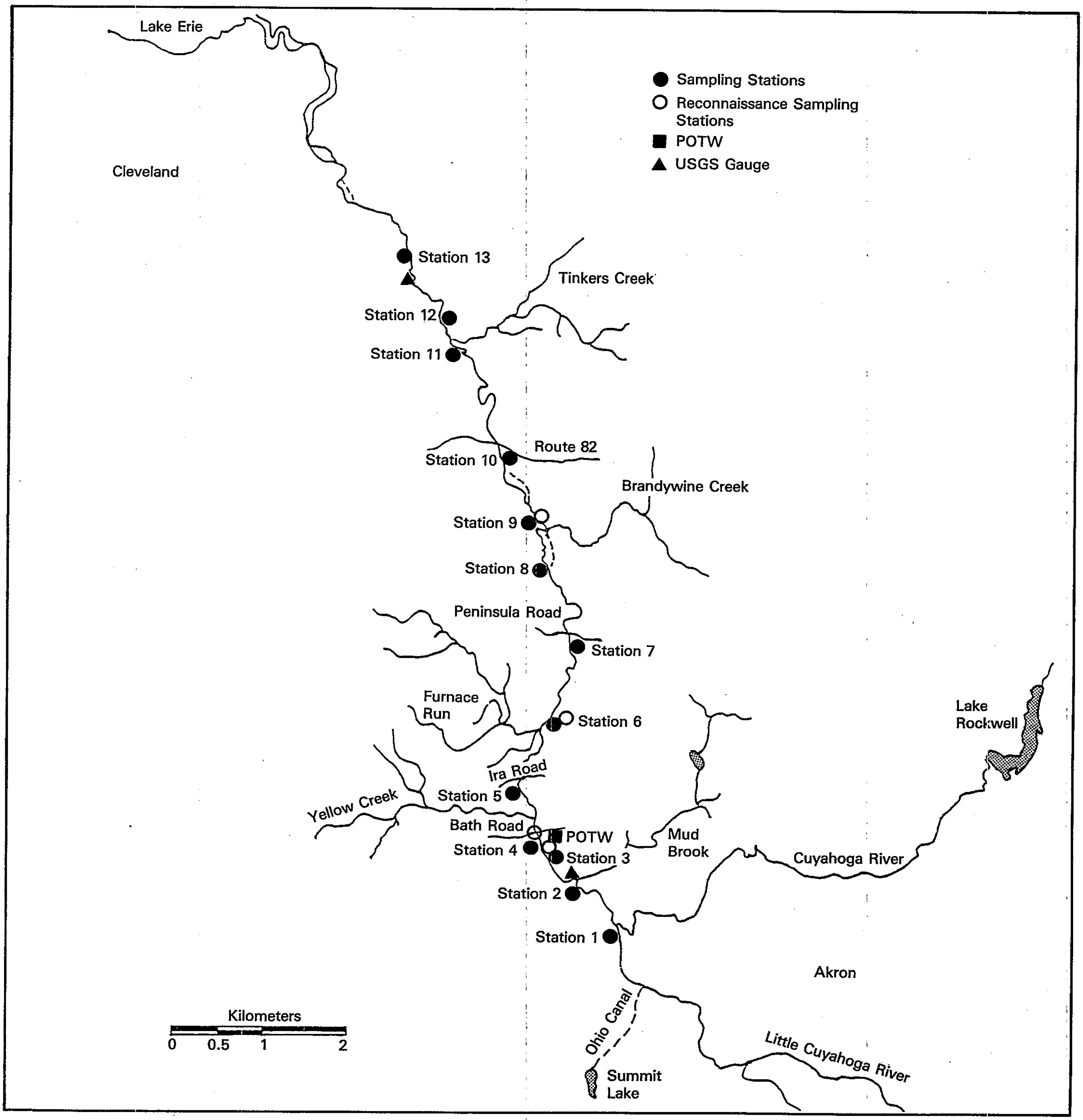


Figure 3-2. Location of Sampling Stations and Tributaries in Study Area on the Cuyahoga River, Ohio

3.2 Initial Sample Collection During Site Reconnaissance

After the Akron POTW site was identified as having a water quality problem probably due to toxics, an on-site visit was planned for ERL-Duluth to conduct initial toxicity tests. By the time the reconnaissance visit was arranged, the following was known:

- The Akron POTW effluent had been shown to be toxic to fathead minnows and *Ceriodaphnia* (U.S. EPA Cincinnati data).
- Ambient water collected just upstream of the Akron POTW had been shown to have no toxic effects on fathead minnows (15) and concentrations of metals up- and downstream of the outfall were similar (ORD-Cincinnati data).
- The effluent contains measurable levels of extractable organics and metals (ORD-Cincinnati data).
- The effluent had been cited as causing a water quality problem due to ammonia and phenol levels which exceeded Ohio state standards (23). However, recent water quality data on ammonia and DO concentrations had shown improved conditions, but had not indicated any relationship to the observed biological conditions downstream of the Akron POTW since these had not improved commensurately (Ohio EPA data).
- The discharge volume of the Akron POTW is large compared to the Cuyahoga River flow. At typical flows of 2.9 m^3/s (67 mgd), the Akron POTW discharge contributes more than 30 percent of the mean river discharge. During low river flows the effluent may comprise up to 60 percent of the flow. This typical flow level also represents over twice the flow of the Cuyahoga River under 7Q10 conditions at Old Portage gauge.
- The Little Cuyahoga River joins the Cuyahoga River several kilometers upstream of the Akron POTW and may supply toxic materials.

A reconnaissance trip was conducted from July 15 to 17, 1985, to familiarize the ERL-Duluth staff with the site and to collect effluent and ambient water samples. Samples of river water were collected from four areas near the eventual location of Stations 3, 4, 6, and 9 during the reconnaissance site visit (Figure 3-2). These particular stations were selected to obtain samples from just upstream and downstream of the outfall (Stations 3 and 4), several kilometers downstream at the confluence with Furnace Run (Station 6), and farther downstream at the confluence with Brandywine Creek (Station 9). These ambient stations were selected to include the 16-km area that Ohio EPA reported as having a markedly reduced fish community. Grab samples were collected on July 16, 1985 and shipped to ERL-Duluth to determine toxicity to *Ceriodaphnia* (Table 3-1). A 24-hour composite Akron POTW effluent sample was collected on July 17 and shipped to ERL-Duluth to determine toxicity to fathead minnow larvae and *Ceriodaphnia* (Tables 3-2 and 3-3). The ambient and effluent toxicity tests were conducted in accordance with accepted chronic toxicity testing protocols. This general testing information is available from U.S. EPA (24). Specific test methods for *Ceriodaphnia* are described in Mount and Norberg (25) and for fathead minnows in Norberg and Mount (26). Statistical analyses were conducted (27) with a Dunnett's two-tailed t-test for the effluent data and Tukey's Honestly Significant Difference Test (28) for the ambient data. The fathead minnow data were statistically analyzed using MINITAB © (Pennsylvania State University, 1982) to determine a t-statistic for comparison with Dunnett's two-tailed t-test.

Table 3-1. Reconnaissance Sample Analysis for Ambient Toxicity Measured by *Ceriodaphnia* Exposed to Cuyahoga River Water, July 16, 1985

Station	Mean Young/Female	95% Confidence Interval	7-Day Survival (%)
3	15.4	13.9-16.9	100
4	16.6	15.5-17.6	90
6	14.4[a]	12.8-16.0	20[a]
9	7.5[a]	4.6-10.5	70
Control[b]	17.3	15.9-18.7	100

[a]Value is significantly lower than the control at $P < 0.05$.
[b]Dilution water from Lester River, Duluth, MN.

3.3 Site Reconnaissance Toxicity Test Data

Tests using *Ceriodaphnia* and fathead minnows were conducted off-site (at ERL-Duluth) using the static renewal method on the samples shipped from the reconnaissance trip. The composite samples were less than 24 hours old including travel time, when testing was begun. Sampling locations are described in Section 3.2 and in Figure 3-2. Routine water chemistry data were collected during the effluent toxicity tests. Dissolved oxygen ranged from 4.3 to 8.9 mg/L. The range of pH values was 6.9 to 8.4.

3.3.1 Data Analysis and Interpretation

A seven-day *Ceriodaphnia* reproductive potential test was conducted on the four Cuyahoga River samples (Table 3-1). Highest young production occurred in the con-

Table 3-2. Effluent Variability Test Results for *Ceriodaphnia* Exposed to Akron POTW Effluent, July-August 1985

Exposure Concentration	Mean Young/Female	95% Confidence Interval	7-Day Survival (%)
		July 17	
Control[a]	17.3	15.9-18.7	100
1	16.7	15.3-18.1	100
3	15.5	14.0-17.0	100
10	16.4	15.6-17.3	90
30	14.9	13.7-16.1	80
100	16.5	15.4-17.6	100
	NOEL > 100% effluent		
		July 26	
Control[a]	20.2	18.6-21.8	100
1	18.8	17.1-20.5	100
3	19.1	17.4-20.7	90
10	19.1	18.1-20.2	100
30	15.5[b]	13.8-17.2	100
100	0[b]	—	0[b]
	NOEL = 10% effluent		
		August 16	
Control[a]	16.5	14.3-18.7	100
1	15.9	12.1-19.5	90
3	17.7	14.8-20.6	100
10	18.3	15.9-20.7	100
30	14.7	11.2-18.2	100
100	0[b]	—	0[b]
	NOEL = 30% effluent		

[a]Dilution water from Lester River, Duluth, MN.
[b]Value is significantly lower than the control at $P \leq 0.05$.

Table 3-3. Effluent Variability Test Results for Fathead Minnows Exposed to Akron POTW Effluent, July-August 1985

Exposure Concentration	Survival (%)	Mean Survival (%)	Dry Weight (mg)	Mean Dry Weight[a] (mg)
		July 17		
Control[b]	100	100	0.537	0.451
	100		0.364	
1	100	95	0.527	0.444
	90		0.360	
3	70	75	0.531	0.514
	80		0.496	
10	80	90	0.364	0.468
	100		0.572	
30	90	90	0.448	0.486
	90		0.523	
100	100	100	0.409	0.448
	100		0.487	
	NOEL > 100% effluent			
		August 15		
Control[b]	90	80	0.437	0.518
	70		0.599	
1	90	90	0.539	0.440
	90		0.340	
3	100	95	0.319	0.328
	90		0.337	
10	90	85	0.348	0.409
	80		0.470	
30	78[c]	89	0.302	0.379
	100		0.455	
100	100	90	0.365	0.360
	80		0.354	
	NOEL > 100% effluent			

[a]Initial fry weight: 0.078 mg, July 18, 1985; 0.086 mg, August 15, 1985.
[b]Dilution water from Lester River, Duluth, MN.
[c]n = 9

trol and at Station 4, which was the closest downstream station to the Akron POTW outfall. Significantly lower ($P < 0.05$) young production was observed in the two farther downstream stations. Survival followed the same pattern, with lowest values at Station 6 (significantly lower than the control at $P < 0.05$) and at Station 9. These stations are located 7.8 km and 22 km downstream of the Akron discharge. In contrast, survival near the Akron POTW outfall at Station 4 was 90 percent. In addition, the following tests were conducted on the composite Akron effluent samples (Tables 3-2 and 3-3):

- Seven-day *Ceriodaphnia* reproductive potential test (The NOEL was >100 percent effluent).
- Seven-day fathead minnow larval growth test (The NOEL was >100 percent effluent).

These three sets of results did not, by themselves, show that the Akron POTW effluent was toxic.

The second effluent toxicity test using *Ceriodaphnia* did show toxicity at 30 and 100 percent concentrations (Table 3-2). The NOEL was 10 percent. A third *Ceriodaphnia* test resulted in young production of 14.7 to 18.3 per female and high survival until the 100 percent exposure concentration, where there was no young production or survival (Table 3-2). The NOEL was 30 percent.

The second effluent toxicity test using fathead minnow larvae showed lowest mean survival in the control (Table 3-3). Consequently, the NOEL was >100 percent.

Results from this second series of preliminary tests differed from the first test series results. *Ceriodaphnia* young production and survival were significantly lowered at the 30 and 100 percent effluent concentrations. In contrast, fathead minnow larvae exhibited little or no change in survival or weight with increasing exposure concentrations.

The combined results of all the preliminary effluent tests indicated that: 1) there did appear to be differences in the toxicity of the effluent with time; 2) the effluent was sometimes toxic to *Ceriodaphnia* at concentrations of 30 percent and greater; and 3) *Ceriodaphnia* were equally or slightly more sensitive to the toxic agents in the effluent than fathead minnow larvae. Evidence suggested the need for a more complete toxicity assessment. Chapters 4 and 5 illustrate the development, execution, and results of that assessment.

4. Development Of The Study Plan For Toxicity Assessment

4.1 Introduction

Having identified the Akron POTW as a probable source of water quality impact to the Cuyahoga River, the next task was to quantify the toxicity. This section discusses the development of the procedures used to identify and quantify the source of toxicity.

As discussed in Chapters 2 and 3, it appeared likely that toxicants were damaging the fish and invertebrate populations in the river below Akron. The available field data and toxicity screening data suggested that the Akron POTW was a source of this toxicity despite good treatment of the influent to reduce concentrations of metals and extractable organics (Section 2.5).

A regulatory authority can use two approaches to assessing and controlling effluent toxicity. The traditional approach is to identify the pollutants being discharged, compare their concentrations instream to existing state and Federal water quality standards, and set limits for each of the pollutants exceeding the standard. The other approach is to analyze the toxicity of the whole effluent and set limits either on the parameter toxicity (e.g., the effluent shall not exhibit a NOEL of less than 25 percent) or require the identification of the causative agents of the measured toxicity and limit these toxicants individually. This approach is accomplished by using toxicity testing to find the chemicals causing the effect. As it was possible that other sources such as leaching landfills, combined sewer overflows, bypasses of the Akron POTW, or other tributaries were causing or contributing to the problem, it was necessary to analyze the toxicity of the Akron POTW effluent and analyze the pattern of ambient toxicity in the receiving water. It was recognized that direct analysis of other potential toxicant sources might also be appropriate.

The whole effluent toxicity testing approach was the best assessment technique for analyzing the Akron POTW's effluent because:

1. Available data showed that the Akron effluent is a highly complex mixture of compounds that varies over time. Identifying each of the compounds that could cause toxicity would be time-consuming and costly.
2. Even if all the specific toxicants could be identified, there was likely to be little information on the toxicity of most of them. Few state or Federal standards exist for many pollutants.
3. The impact of effluent toxicity depends on many factors, particularly the interaction in the water column of the toxicants and such other water quality parameters as suspended solids, pH, alkalinity, and hardness. Most individual pollutants limits do not take into account such interactions (except ammonia, which considers pH, and some metals). Limiting toxicity accounts for these water quality parameters and permit limits are set to protect aquatic life.

4.2 Effluent Sampling And Toxicity Analysis Plan

The effluent toxicity testing procedure, was designed considering that:

1. Effluent toxicity data were needed for comparison with instream effluent concentrations (IWC) to determine if the effluent caused instream toxicity. The toxic effect from continuous discharge of an effluent must be determined through toxicity testing.
2. Because the Akron POTW effluent dominates the Cuyahoga's flow, aquatic organisms are exposed to relatively high concentrations of effluent over long periods (during low flow, high exposure may last for days). Chronic toxicity tests, which measure toxic effects over a species' lifespan, had to be performed. These tests simulate the exposure to which the organisms are subjected in the stream.
3. Testing one organism, which may not be sensitive to the toxicants in the effluent, could lead to an erroneous conclusion that the effluent is not toxic. Therefore, to avoid a false negative conclusion two to three aquatic organisms representing fish, invertebrates, and algae should be tested (24). The range of sensitivity of test species is thus measured.

   Finding a sensitive test species is important in the permitting process (24). Once identified, the regulatory authority can use the most sensitive of the test species in a Toxicity Source Investigation (TSI) and for permit compliance monitoring.
4. To determine the effects on aquatic biota, the toxicity tests should simulate effluent/receiving water interaction at the point of discharge. Therefore, dilution water for the tests should be taken directly upstream of the discharge point. To measure the inherent toxicity of an effluent to avoid interactions with natural waters or upstream sources of contaminants, a standard laboratory dilution water should be used. This is especially important in situations with multiple discharges.
5. All potential sources of toxicity associated with the Akron POTW had to be analyzed. In this case, pre- and postchlorination, bypass/overflow, and landfill leachate wastewaters.

6. Wastewaters should be sampled to simulate exposure. For the continuously discharged effluent, composite sampling simulates the chronic exposure conditions experienced by the resident biota. For bypass situations, the acute toxicity associated with intermittent flows of short duration are best simulated using multiple grab samples collected over the duration. The effect of the landfill leachate was also assessed using grab samples. Grab samples for pre- and postchlorination are sufficient because chlorine is reactive in water and should exhibit toxicity only for a short (acute) period of time.
7. Knowledge of the variability of the effluent's toxicity is needed to assess toxic impact. Since the Akron POTW effluent was known to be variable, toxicity testing was to be repeated as often as practicable from samples collected over a period of time, to observe potential changes in effluent composition and thus in effluent toxicity.

4.3 Ambient Sampling And Toxicity Analysis Plan

It is often helpful for a regulatory authority to perform ambient toxicity tests to develop a toxicity profile of the stream segment under study. Ambient toxicity tests:

- Will demonstrate whether instream toxicity occurs
- May locate the source(s) of toxicity
- May determine the fate/persistence of toxicity.

For regulatory purposes, a sampling station should be placed at the perimeter or downstream edge of the mixing zone to determine chronic effects. Since this case study was conducted for research purposes, such a station was not precisely located, but was placed beyond the mixing zone edge at the nearest access point. A dye dilution study should be conducted to properly locate stations with respect to the effluent plume and to measure the concentration of effluent instream. An anlysis of this type was not within the scope of work for this project.

In ambient toxicity testing the river does the mixing for the dilutions. The regulatory authority only needs to know the concentration of the effluent instream and locations of other sources of dilution or potential toxicity. Sampling should be conducted when possible during critical low flow periods so that dilution levels will match those specified in the water quality and toxicity standards. Otherwise, toxicity test results will reflect conditions of higher effluent dilution and ambient samples may be so dilute as to have no toxic effects.

Ambient sampling stations should be established at the following locations:

1. A control station upstream of the point of discharge and, if possible, any other potential sources of toxicity.
2. A station just upstream of the discharger and downstream of other potential sources of toxicity to determine upstream toxicity levels.
3. A station as close as possible to the point of complete mixing where the concentration of effluent is greatest after mixing, but before the effluent toxicity may begin to decay.
4. Several stations at points of increasing distance downstream of the point of discharge (in this case the Akron POTW) to measure the decay in toxicity of the sampled effluent, the effect of other sources of toxicity, and the dilution or additional pollution that occurs as tributaries flow into the stream under study. These stations are generally placed immediately above points where other sources of water enter the river to identify and separate any tributary influences on water quality. Where tributaries are known to receive discharges of toxicants should be stations located up- and downstream of their confluences.

The same biological species used in the effluent toxicity tests should be used in the ambient toxicity tests. Sampling should be conducted to best simulate the exposure received by the resident biota. As discussed, composite samples provide the best duplication of chronic exposure.

4.4 Ambient River Station Descriptions

The Cuyahoga River banks are generally steeply sloped and wooded. The ambient stations were placed either along these wooded banks or on bridge foundations, with one exception (Table 4-1). At Station 6, because of the difficult access to the bridge foundation, surface samples were collected below the bridge using a polyethylene bucket hung from a rope.

Station locations were selected based on the principles described in Section 4.2, with some modifications because of nearby discharges, topography, and accessibility. Three stations were located upstream of the Akron POTW: one immediately upstream of the Akron POTW, and two others each upstream of a tributary confluence with the Cuyahoga. The furthest upstream location is the control and reflects all water quality influences upstream of the study area. Samples from the next two

Table 4-1. Station Locations and Descriptions

Station Number	River Kilometer	Description
1	68.1	Upstream of Little Cuyahoga River confluence with Cuyahoga River on the Cuyahoga River control station
2	64.6	Upstream of confluence of Mud Run with Cuyahoga River, 50 m upstream of USGS gauging station at Old Portage
3	61.1	At POTW, 100 m upstream of outfall
4	60.3	Upstream of Bath Road bridge and landfill drainage confluence with Cuyahoga River, 100 m downstream of POTW outfall
5	56.8	Upstream of Ira Road bridge
6	53.2	Furnace Run confluence with Cuyahoga River
7	46.8	Upstream of Peninsula Road bridge
8	42.6	Downstream of Boston Road bridge
9	38.8	Upstream of Brandywine Creek confluence with Cuyahoga River at Vaughn Road bridge
10	33.5	Upstream of Route 82 bridge at Station Road
11	27.8	Upstream of Tinkers Creek confluence with the Cuyahoga River at Fitzwater Road
12	25.1	Downstream of Tinkers Creek confluence with Cuyahoga River at Hillside Road
13	21.2	Downstream of Old Rockside Road

stations downstream reflect all water quality influences, including tributaries downstream of the control, yet still upstream of the Akron POTW (see Figure 3-2.)

Ten stations were located downstream of the Akron POTW, one just downstream of the outfall and the other nine covering the 16-km area identified by Ohio EPA. These nine stations were approximately equidistant and located to identify any downstream tributary effects. Sampling Stations 11 and 12 were set just upstream and downstream of the Tinkers Creek confluence with the Cuyahoga River, respectively. This was done because Tinkers Creek was known to receive discharges from four small POTWs.

There were some complications with the placement of the composite water samplers. At Station 2, an intermittent discharger was discovered at the desired station location so the sampler was moved about 50 m upstream to the opposite river bank. At Station 5, because there was an old culvert on the river bank, the composite sampler was repositioned upstream (~145 m) as a precautionary measure. At Station 7, the composite sample was moved about 12 m upstream from a rusted drainpipe with a very low volume outflow which smelled of sewage.

In general, ambient samples were collected as 24-hour composites to further examine any day-to-day variability in toxicity. However, due to access difficulties and lack of equipment, grab samples were collected at Stations 6 and 13.

Drainage pipe at Station 7. Water sampler was located upstream.

4.5 Toxicity Testing Procedures

The mobile toxicity testing laboratory and staff members from ERL-Duluth began effluent and ambient toxicity testing on September 26 and ended on October 3, 1985.[a]

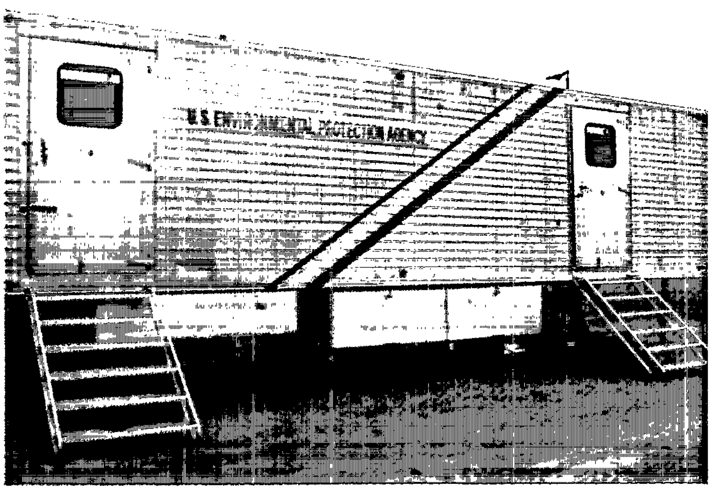
The mobile laboratory was parked at the Akron POTW so that there was sufficient electricity and water, security, 24-hour access, and proximity to POTW operation and management personnel. This location provided access to plant operations and allowed bypass sample testing and observation of effluent variations.

Three test organisms were selected for the toxicity testing procedures—the fathead minnow, *Ceriodaphnia,* and a nonoperculate snail, *Aplexia* sp. The test methodology for *Aplexia* is still under development. The methods of Mount and Norberg (25) and Norberg and Mount (26) were used for the other test species. Each of these tests is considered a short-term chronic toxicity test.

Inside EPA's mobile laboratory. Ceriodaphnia *and fathead minnow tests are being set up.*

4.5.1 Effluent Sample Tests

Toxicity data collected previously by ORD-Cincinnati had indicated that the chronic toxicity effect concentration (NOEL) of the effluent was similar to its projected instream waste concentration at low flow periods (Section 2.3). Therefore, concentrations of 50 and 75 percent were added to the usual dilution series (100, 30, 10, 3, and 1%) to bracket the expected toxicity concentration of the wastewater. For the effluent tests, dilution water was collected upstream of the point of discharge at Station 3. Since ambient testing was also being conducted, any toxicity contribution from upstream could be accounted for in the analysis of the toxicity results. Further, based on the State of Ohio's fish survey, the fish population upstream of the Akron POTW was in very good condition. Upstream toxicity, therefore, was expected to be negligible.

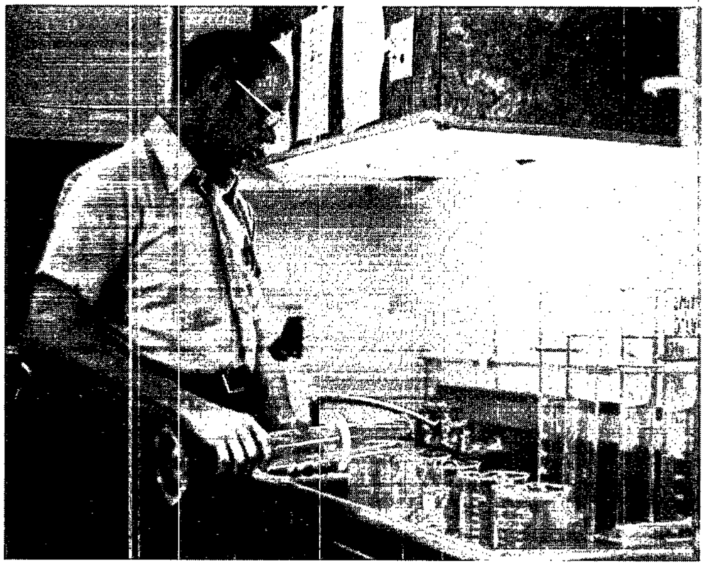


The EPA mobile laboratory for ambient and effluent toxicity testing.

Both effluent and ambient samples were collected as 24-hour composite samples using commercial, battery-powered samplers (except at Stations 6 and 13). Sampling was conducted during low flow, but not 7Q10 conditions (Section 3.1.2 and Table 5-4). Each day the organisms were transferred into a new sample so that over the period of seven days they were exposed to seven different samples.

Several different types of bypasses occur at the Akron POTW. One is a straight bypass of raw sewage to the river when rainfall precludes treatment of the entire volume of wastewater entering the Akron POTW. One bypass event of this type occurred during the course of this study. The overflow basin was allowed to discharge for several hours before a grab sample was taken so that a

[a]Toxicity testing was performed on-site, although all data could have been generated off-site had samples been provided. This would have significantly lowered costs.



Making effluent dilutions for use in toxicity tests.

sample representative of bypassed wastewater after the first flush would be obtained. This sample was tested for acute toxicity since it was from a short-term event (approximately four hours) resulting in short-term exposure. No further bypassing samples were tested for toxicity. Another type of bypass occurs when influent from the primary settling basins is diverted around the secondary treatment system and into the chlorine contact basin. This partially treated wastewater is mixed with the final effluent, so effluent toxicity testing during this time was considered to account for this type of bypass.

Variability of the effluent's toxicity was assessed in two ways. First, the tests were conducted over a seven day period to measure variation in toxicity. Second, effluent toxicity tests were repeated six times over six months, beginning with a sample collected in July 1985 and ending with a sample collected in December 1985. These samples were 24-hour composite samples collected onsite and shipped to the ERL-Duluth. The toxicity test conducted on the bypass of raw sewage also assessed effluent variability since this potential source of increased toxics contribution of the Akron POTW was measured.

4.5.2 Ambient Toxicity Tests

The ambient toxicity tests were conducted using seven daily samples of 100 percent of the sample (i.e., tests were renewed daily with new sample water). Test organisms were exposed to whatever concentration of the effluent occurred at each sampling station, plus other ambient conditions that existed in the river.

Sampling locations are described in Table 4-1. The area of interest along the Cuyahoga River was originally defined from Ohio EPA's data which indicated a 16-km stretch of river from the Akron POTW downstream where fish and benthic populations were greatly reduced. An upstream area was used as a control and an area farther downstream was also included. Ambient toxicity analysis was terminated at Station 13, below which several large-volume tributaries and effluents entered the river.

Due to the high volume flow of the effluent into the Cuyahoga, instantaneous, complete mixing was assumed to occur at the outfall. This assumption was confirmed when a green dye spill from an indirect industrial discharger was observed in the plant effluent. Acting as an unintentional dye dilution study, this green effluent mixed at the point of discharge and colored the entire river near the Akron POTW for approximately 24 hours. The green effluent was observed to cross the Cuyahoga almost perpendicular to the direction of flow and reflect back toward midstream where an eddy formed. Downstream of this eddy, the green color was greatly diminished.

5. Toxicity Testing Results and Data Interpretation

5.1 Introduction

Cuyahoga River and Akron POTW effluent samples were collected, following the study plan described in Chapter 4. Fathead minnows, *Ceriodaphnia*, and *Aplexia* were the three organisms used as toxicity detectors in the effluent and ambient toxicity tests. Statistical analyses were conducted according to procedures described in Section 3.2 and were typically used in the Complex Effluent Toxicity Testing Program report series, unless specified otherwise.

Adding effluent dilutions to fathead minnow test chambers for sample renewal.

5.2 Akron POTW Effluent Toxicity Results

Fathead minnows were not as sensitive as *Ceriodaphnia* to the Akron POTW effluent. Exposure to 100 percent effluent did not affect fathead minnow survival, although mean weight was significantly lower ($P < 0.05$) than the control. The NOEL is 75 percent effluent for the fathead minnow (Table 5-1) and the Acceptable Effluent Concentration (AEC) is 86.6 percent effluent.

Effects on survival and mean young production were observed for *Ceriodaphnia* (Table 5-2). The NOEL is 10 percent effluent and the AEC is 17.3 percent.

A trial test using *Aplexia* indicated no effluent effects on survival, although mean larval snail weight decreased with increasing exposure concentration (Table 5-3).

The Akron POTW effluent (in all concentrations) produced extreme convulsive behavior in *Ceriodaphnia* for the first three to four days of exposure before mortality began. The *Ceriodaphnia* were so convulsive that they were difficult to capture when being transferred during the sample renewal process. In almost every case, adult mortality occurred when a brood of young was produced (day 4 of the test). As adults molt at the time of brood release and are known to be more sensitive at that time, this behavior is

Table 5-1. Effluent Toxicity Test Results for Akron POTW Using Fathead Minnows, September 26–October 3, 1985

Exposure Concentration (% Effluent)	Mean Weight (mg)	Standard Error ±	Mean Survival (%)
Control[a]	0.397	0.021	90
1	0.303	0.022	85
3	0.395	0.021	100
10	0.365	0.022	85
30	0.303	0.021	95
50[c]	0.337	0.020	95
75[c]	0.314	0.021	90
100	0.291[b]	0.020	100
	NOEL = 75% effluent		
	AEC = 86% effluent		

[a]Station 3 water.
[b]Significantly lower ($P \leq 0.05$) than the control.
[c]Additional dilutions to typical series to ensure better resolution of toxic level.

Adding dilutions to test cups for effluent toxicity test using Ceriodaphnia.

not unusual. The young appeared normal and were alive when the daily observation was made, but they were not kept to see how long they would have survived.

It is important to record how and when test organisms are affected in the tests. Symptoms enable biologists to better interpret toxic impact. For example, knowing that *Ceriodaphnia* adults died in Akron effluent after brood release explained why mortality of adults, not reduced reproduction, was the endpoint of importance in these chronic toxicity tests.

Routine water chemistry data for the effluent tests were collected for fathead minnows and *Ceriodaphnia*. Dissolved oxygen ranged from 4.6 to 9.5 mg/L. The range of pH was 7.3 to 7.8. Temperature was controlled and varied from 24.8 to 25.0°C. Conductivity varied from 774 to 929 μmhos. These values represent all effluent dilution and control tests.

Table 5-2. Effluent Toxicity Test Results for Akron POTW Using *Ceriodaphnia*, September 26–October 3, 1985

Exposure Concentration (% Effluent)	Mean Survival (%)	Mean Young/Female	95% Confidence Limits
Control[a]	100	34.6	28.4-40.8
1	90	35.8	29.4-42.4
3	100	28.1	22.9-33.3
10	100	30.5	20.2-40.8
30	40[b]	31.7	22.8-40.2
50[c]	0[b]	—[b]	—
75[c]	0[b]	—[b]	—
100	0[b]	—[b]	—
	NOEL = 10% effluent		
	AEC = 17.3% effluent		

[a]Station 3 water.
[b]Significantly lower ($P \leq 0.05$) than the control.
[c]Additional dilutions to typical series to ensure better resolution of toxic level.

Table 5-3. Effluent Toxicity Test Results Using the Snail *Aplexia* [a]

Effluent Exposure Concentration (% Effluent)	Mean Weight (mg)	Mean Survival (%)
Control[b]	0.296	100
1	NA	NA
3	NA	NA
10	0.264	100
30	0.251	90
50[d]	0.237	100
75[d]	0.215	100
100	0.208[c]	100
	NOEL > 100% effluent	
	AEC not calculated	

[a]Test methods are being developed by U.S. EPA Environmental Protection Research Laboratory-Duluth.
[b]Station 3 water.
[c]Significantly lower ($P \leq 0.05$) than the control.
[d]Additional dilutions to typical series to ensure better resolution of toxic level.

5.2.1 Test Species Sensitivity

Three aquatic organisms were tested as surrogates for the range of biological community response in the Cuyahoga River to the Akron POTW effluent. Of the three, *Ceriodaphnia* was the most sensitive. Fathead minnows were less sensitive and *Aplexia* were unaffected by exposure to the effluent. In this case, the fish population in the Cuyahoga River has been severely affected by an effluent that was not toxic to fathead minnow test organisms. This demonstrates the relation between test species and biological community sensitivity that was well documented in the Complex Effluent Toxicity Testing Program. A test organism should represent the sensitivity of a community under study, but which component of the community is not known *a priori*. "Careful analysis of our knowledge of toxicology, effluent decay, and relative sensitivity tells us that we cannot expect: 1) *Ceriodaphnia* toxicity to always resemble toxicity to benthic invertebrates; 2) fathead minnow toxicity to always resemble toxicity to fish; 3) fathead minnows and other fish to display the same relative sensitivity to different effluents" (29).

Ceriodaphnia, the most sensitive test organism, represents the sensitive part of the Cuyahoga's resident community, not necessarily the invertebrates or organisms closely associated with it physiologically. Further, there may be residents of the Cuyahoga River which are more sensitive than *Ceriodaphnia* to the Akron POTW effluent. When the effluent effect concentration of this representative sensitive species is exceeded an entire group of similarly sensitive or more sensitive species will be affected. Such an occurrence may affect an entire biological community, fish included.

5.2.2 Instream Effects Due To Effluent Toxicity

The results of effluent toxicity tests may be used to ascertain whether the Akron POTW discharge causes instream effects. To determine the effect of an effluent, the test NOEL concentrations are compared to instream effluent concentrations. The potential for adverse environmental impact is determined by (1):

$$IWC \leq NOEL \quad \text{(Equation 5-1)}$$

where

IWC = concentration of wastewater instream at a critical flow period (Instream Waste Concentration)

NOEL = the concentration of wastewater at which no toxic effect is observed (No Observed Effect Level)

If the NOEL is equal to or greater than the IWC, then no toxic effect is expected to occur instream. This relationship has been demonstrated at a number of NPDES discharge sites (see the Complex Effluent Toxicity Testing Program report series in the References).

Using Akron POTW discharge data and USGS flow data for the Cuyahoga River, the proportion of the river flow contributed by the effluent (IWC) was calculated (Table 5-4). The contribution was calculated for the river near the Akron POTW using available data on the mean flow from the Old Portage gauging station. Daily information on effluent discharge and river flow rates indicated that the Akron POTW effluent represented 12 to 48 percent of the Cuyahoga in 1984 and 6 to 56 percent in 1985 (19). The IWC values for the September 26 to October 3 test period are 38 to 54 percent (19). The NOEL value is 10 percent for the most sensitive species, *Ceriodaphnia* (Table 5-7). Therefore, the NOEL < IWC and a toxic impact would be expected in the river from the Akron POTW discharge.

Table 5-4. Akron POTW Effluent Contribution to Cuyahoga River Flow

Date	Akron POTW Effluent Flow[a] (m3/s)	River Flow Contribution[b] (% Effluent)
Sep 26 85	3.22	46
Sep 27 85	2.61	49
Sep 28 85	2.59	54
Sep 29 85	2.54	53
Sep 30 85	3.17	52
Oct 1 85	2.78	38
Oct 2 85	2.66	38
Mean	2.79	47

[a]From Monthly Operating Reports submitted by the City of Akron to Ohio EPA.
[b]Calculated using the river flow data from the USGS gauging station at Old Portage, Ohio (near Station 2 and upstream of the POTW discharge).
Note that the 7Q10 value at Old Portage gauge for this time period is 1.27 m^3/s.

Table 5-5. Acute Toxicity to *Ceriodaphnia* of Bypassed Wastewater From the Akron POTW[a]

Wastewater Concentration (%)	Survival (%)
Control[b]	90
3	100
6.2	90
12.5	100
25	0
50	0
NOEL = 12.5% wastewater	
LC50 = 17.7% wastewater	

[a]Sample collected September 26, 1985.
[b]Control and dilution water from Station 3.

Since complete mixing at the point of discharge is assumed to occur (Section 4.4.2), the NOEL of *Ceriodaphnia* is continuously exceeded instream from the point of discharge down to Station 13, a distance of about 40 km. Thus, a chronic toxic effect would be expected over this entire segment when there is no toxicity decay or dilution. This is confirmed by the fish and invertebrate population data (Chapter 2) and in the ambient toxicity data (Section 5.2).

5.2.3 Wastewater Bypassing Toxicity Results

Regulatory authorities have been concerned about the impact of wastewater bypassing at the Akron POTW. The influent was also known to be highly toxic (more toxic than the effluent) and thus could cause severe impact to the river. The volumes of total raw wastewater bypassed annually vary from 1.13 mil m^3 to 113 mil m^3 (300 to 3,000 mil gal) (Section 3.1.1). To estimate the toxicity of bypassed influent (raw wastewater), a bypass sample was collected during an event which occurred during the on-site testing. The sample was tested for acute toxicity due to the short release times typical of bypass events. Since *Ceriodaphnia* had a greater sensitivity to the Akron POTW effluent, they were used to test the toxicity of the bypass sample (Table 5-5). The LC50 of the raw sewage was 17.7 percent (using straight line interpolation). Complete mortality occurred after 24 hours in the 25 and 50 percent bypassed wastewater concentrations. There was no mortality in the 12.5 percent concentration of bypassed wastewater in 48 hours.

In terms of the volume discharged, the contribution of bypassing to the river flow and instream toxicity seems quite large. However, the actual contribution to instream

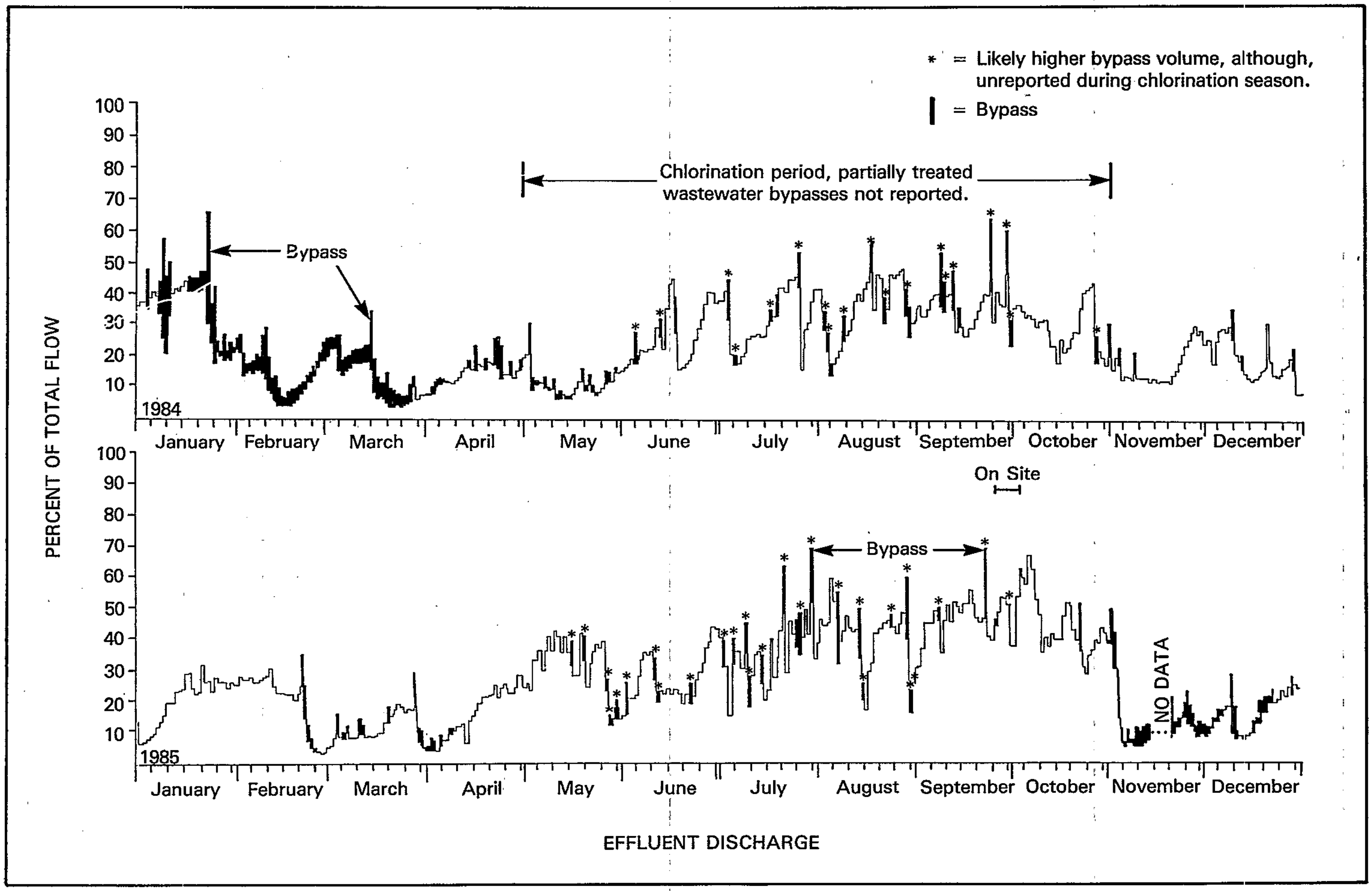


Figure 5-1. Percent Contribution of the Akron POTW Effluent and Bypass Flow to the Cuyahoga River

toxicity may be limited for several reasons. The duration of these events is often short, less than 12 hours (Figure 5-1) (19). In addition, bypassing has usually occurred during rainfall events when the volume of wastewater is increased beyond the capacity of the Akron POTW. Since the Cuyahoga River is a relatively swift-flowing river, bypassed wastewaters move quickly downstream, particularly during such rainfall events. It is estimated that the effluent has a flow time of less than 24 hours from the discharge point through the segment of river under study. It is important to note that the bypass sample did not affect the *Ceriodaphnia* until after 24 hours of exposure. Therefore, impacts of the bypassed wastewaters may be minimized by their quick passage and dilution downstream.

The type of injury to biota is not consistent with the expected effects from bypassing episodes. While instream organisms are exposed to pulses of very toxic raw wastewater for brief periods, these periods are probably not long enough to cause the toxic effects observed on the fish collected during the Ohio EPA biosurveys. Those fish exhibited a high incidence of lesions, tumors, and other abnormalities which are characteristic of long-term exposures. Also, this evaluation did not consider sediment toxicity from an accumulation of toxicants.

The instream effects from bypassed wastewater may be reduced by dilution with rainwater. If the toxicant source flow is not proportional to the flow increases associated with rainfall (e.g., an industrial vs. storm drain source for toxicants), the toxicant concentration will be reduced by dilution from the addition of stormwater regardless of whether the Akron POTW treatment is effective. This dilution assumes a continuous passive discharge in contrast to the potential for deliberate releases during rainfall (which has not been observed at Akron POTW). Further dilution of the toxicant will occur as the effluent mixes with the Cuyahoga River.

The role of bypassed wastewaters in causing a toxic effect on the Cuyahoga River is uncertain and deserves further study. On the basis of results from this study it appears that raw or partially treated sewage probably does contribute to instream impact. However, the relative importance of bypassed wastewater and the continuous, high-volume discharge of toxicants in the Akron POTW's effluent is uncertain. Steps are currently being taken at the Akron POTW to minimize bypassing by making process improvements.

5.2.4 Effluent Toxicity Variability

The Akron POTW's effluent was tested for chronic toxicity in August 1984 and May 1985 by ORD-Cincinnati and over a six-month period in 1985 by ERL-Duluth. ORD-Cincinnati tested three forms of the effluent (unchlorinated, chlorinated, and dechlorinated—achieved by holding time) twice. ERL-Duluth tested the chlorinated effluent before the site visit, while on-site, and twice following the site visit (Tables 3-2, 3-3, 5-1, 5-2, and 5-6).

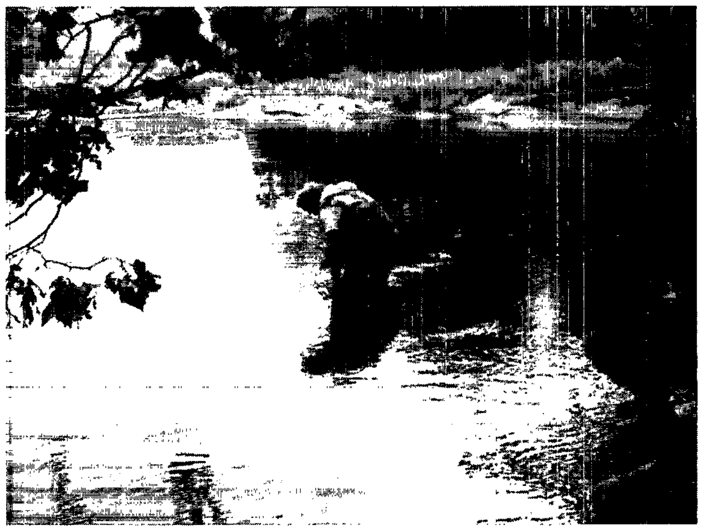
Table 5-6. Effluent Variability Test Results for *Ceriodaphnia* Exposed to Akron POTW Effluent, December 1985

Exposure Concentration	Mean Young/Female	95% Confidence Limits	7-Day Survival (%)
	December 2		
Control[a]	16.5	9.9-23.1	100
1	19.7	15.4-24.0	100
3	20.1	16.7-23.5	100
10	19.3	17.6-21.0	100
30	23.1	20.9-25.3	100
100	18.1	14.6-21.6	50[b]
	NOEL = 30% effluent		
	December 9		
Control[a]	13.7	10.2-17.2	100
1	15.6	9.4-21.8	70
3	15.3	11.2-19.4	100
10	18.3	13.3-23.2	90
30	21.5	17.8-25.2	100
100	17.1	13.7-20.3	0[b]
	NOEL = 30% effluent		

[a]Dilution water from Lester River, Duluth, MN.
[b]Value is significantly lower than the control at $P \leq 0.05$.

The results of these tests were used to initially assess the variability of the effluent's toxicity before the toxicity source investigation testing, described in Chapter 6, began.

Toxicity varied by one order of magnitude, from a NOEL of 10 percent to greater than 100 percent (essentially no measurable toxicity) (Table 5-7). Based on previous experience with effluent toxicity, this is not an unusually variable effluent. The cause of the variability is likely a combination of factors, including variable contributions by industrial dischargers and varying treatment efficiency.



Collecting an ambient water sample from the Cuyahoga River.

Table 5-7. Effluent Variability Characterized by No Observed Effect Levels (NOELs) From Toxicity Tests Using Fathead Minnows and *Ceriodaphnia*

	Fathead Minnows		*Ceriodaphnia*	
	Survival	Growth	Survival	Reproduction
EPA-Cincinnati				
Aug 1984	30	≥100	30	>100
May 1985	30	≥100	≥100	NA
ERL-Duluth				
Jul 1985	>100	>100	>100	>100
Jul 1985	NA	NA	30	10
Aug 1985	>100	>100	30	30
Sep 1985	>100	75	10	30
Dec 1985	—	—	30	>100
Dec 1985	—	—	30	>100

The variability data would be very important in a waste-load allocation and permit issuance. For example, the procedure for deriving water quality-based permit limits accounts for effluent variability. Procedures for such calculations are found in References 24 and 30. Data on the variability of effluent toxicity is also of importance in trying to determine the causative agents of the measured effluent toxicity.

5.3 Ambient River Toxicity Results

Ambient toxicity tests were conducted using fathead minnows, *Ceriodaphnia*, and *Aplexia* exposed to composite Cuyahoga River samples from 13 stations. The stations were located on a 44-km length of the Cuyahoga River from above the confluence with the Little Cuyahoga to downstream of Tinkers Creek (Figure 3-3). Station selection and locations are described in Section 4.3.

Routine water chemistry data were collected during the ambient tests for the fathead minnows and *Ceriodaphnia*. Dissolved oxygen ranged from 4.2 to 9.3 mg/L (final values below 5.5 mg/L were consistently obtained in the fathead minnow test chambers). The range of pH was 6.7 to 7.9. Temperature was controlled and varied from 24.9 to 25.4°C. Conductivity varied from 789 to 871 µmhos.

5.3.1 *Aplexia* Test Results

The trial chronic toxicity test using *Aplexia* did not indicate ambient toxicity (Table 5-10). Larval snail weight varied 0.261 to 0.400 and the higher weights were from stations downstream of the Akron POTW. Survival was 90 to 100 percent, except at Station 10 where it was 80 percent.

5.3.2 Fathead Minnow Test Results

The fathead minnows did not show any effects from ambient toxicity on their weight. Only at Station 8 was survival significantly lower ($P < 0.05$) than upstream of the Akron POTW at Station 1 (Table 5-8). Mean weights varied 0.297 to 0.413 mg. Mean survival varied 50 to 100 percent; however, with the exception of Station 8, survival was >70 percent.

5.3.3 *Ceriodaphnia* Test Results

Survival and young production of *Ceriodaphnia* were reduced at stations downstream of the Akron POTW (Table 5-9). Survival of adult *Ceriodaphnia* declined from

Fathead minnow larvae are being added to test chambers at initiation of an effluent toxicity test.

Table 5-8. Ambient Toxicity Test Results for the Cuyahoga River Using Fathead Minnows

Station	Mean Weight (mg)	Standard Error ±	Mean Survival (%)
1	0.391	0.029	100
2	0.355	0.031	90
3	0.403	0.030	95
POTW			
4	0.383	0.029	100
5	0.319	0.030	95
6	0.374	0.031	90
7	0.369	0.032	85
8	0.413	0.030	50[a]
9	0.381	0.034	70
10	0.297	0.032	85
11	0.329	0.032	80
12	0.317	0.033	80
13	0.329	0.030	90

[a]Significantly lower ($P \leq 0.05$) than Station 3.

100 percent at Stations 1 through 3, located upstream of the effluent discharge, to 44 percent or less at stations located downstream of the discharge.

Upstream of the point of discharge, no instream toxicity was observed. The Akron POTW discharge enters the river between Stations 3 and 4. At Station 4, the first sign of instream toxicity was observed when the mean survival was only 40 percent (remember that adult mortality is the parameter of concern). At Station 5, there was no survival. The reasons for the continued decline in *Ceriodaphnia* mortality between Stations 4 and 5 are not known. Possible explanations are that some aspect of the river's water chemistry briefly changed the equilibrium of the toxicant(s) responsible for the effluent toxicity or possibly the presence of nonpoint source dischargers or the influence of Yellow Creek. See Chapter 6 for information on the causes of toxicity.

The pattern of reduction in survival of *Ceriodaphnia* continues downstream with some fluctuations. This pattern is not unexpected because the flow of the Cuyahoga River is swift and the time of travel for the effluent between Stations 4 and 13 is less than 24 hours. Toxicity of the effluent is persistent at least for that period of time and distance on the Cuyahoga River.

The number of young produced by *Ceriodaphnia* exposed to Cuyahoga River water did not indicate any consistent pattern at downstream stations. There was no young production at Stations 5 and 13 due to adult *Ceriodaphnia* mortality, and low young production at Stations 1, 6, and 10 of from 13 to 16 per female. For the remaining stations, young production was >20 per female.

From the effluent toxicity test, the effect observed was adult mortality after a brood was produced, so the lack of a large effect on young production is not surprising. In contrast, survival was low at all stations downstream of the Akron POTW, and was significantly lower (P <0.05) than the control at Stations 5, 6, 7, 8, 9, 11, and 13. In the statistical analyses program used (27), mortalities occurring on day 7 do not affect statistical differences as much as early mortality. Most of the mortality occurred on days 5 and 6 in the effluent and ambient tests. The routine water chemistry data did not indicate changes in DO, pH, temperature, or conductivity. The test organisms that did survive at Stations 6 through 12 were extremely convulsive and would probably have rapidly died with additional test time (31).

Table 5-9. Ambient Toxicity Test Results for the Cuyahoga River Using *Ceriodaphnia*

Station	Mean Survival (%)	Mean Young/Female	95% Confidence Limits
1	100	16.1	9.4-22.8
2	100	20.1	13.8-26.5
3	100	21.5	17.0-26.0
POTW			
4	40	24.6	14.9-34.1
5	0[a]	—[a]	—
6	10[a]	15.1[a]	0.66-25.2
7	10[a]	39.4	23.7-51.3
8	33[a]	20.8	5.4-35.8
9	10[a]	21.0	11.0-32.9
10	44	13.6	10.9-16.4
11	12.5[a]	27.3	25.6-29.5
12	33[a]	23.3	15.3-31.2
13	0[a]	—[a]	—

[a]Significantly lower ≤ 0.05) than Station 3.

Table 5-10. Ambient Toxicity Test Results for the Cuyahoga River Using *Aplexia*[a]

Station	Mean Weight (mg)	Mean Survival (%)
1	0.261	100
2	0.276	100
3	0.296	100
POTW		
4	0.317	100
5	0.309	100
6	0.300	100
7	0.316	100
8	0.278	100
9	0.267	100
10	0.400	80
11	0.313	90
12	0.300	100
13	0.311	90

[a]Statistical analysis of these data was not conducted.

5.3.4 Landfill Drainage Test

An acute toxicity test was conducted on a sample from the landfill drainage stream located immediately downstream of the Akron POTW. When *Ceriodaphnia* were exposed to 100 percent sample over a 24-hour period, survival was 100 percent. Since there was no acute toxicity and the flow volume was intermittent and too small to cause chronic toxicity instream (far below 1% of the Cuyahoga River flow), further testing was not performed.

5.3.5 Comparison of Ambient Toxicity to *Ceriodaphnia* with River Survey Data

Examination of *Ceriodaphnia* survival data in conjunction with the biosurvey data from Ohio EPA (Section 2.2) indicates a similar pattern near the Akron POTW. The number of benthic taxa and benthic diversity values declines from maximum levels of about 50 upstream of the Akron POTW to minimum levels of about 20 at RK 60 through 40 in 1984 (Figure 5-2). The Invertebrate Community Index data illustrate the same trend of a decline from good water quality beginning downstream of the confluence with the Little Cuyahoga (upstream of the Akron POTW) to poor quality near RK 55 downstream of the Akron POTW (Figure 5-3). Recovery toward upstream levels was better in the 1986 survey than in the 1984 survey. More noticeably, the fish community Index of Well-Being declines rapidly from good to very poor status just downstream of the Akron POTW (Figure 5-3). Data from 1984 to 1986 show this decline, although the magnitude is smaller in 1985 and 1986. The fish index never improves to the level found upstream of the Akron POTW.

5.4 Summary

The principal conclusion from this data is that the Akron POTW's effluent is a major cause of the toxic impact to the Cuyahoga River. Of the three organisms tested, *Ceriodaphnia* is most sensitive to the effluent and ambient samples. The *Ceriodaphnia* effluent toxicity data and river flow data indicate that the concentrations of effluent in the river are sufficient to cause an adverse biological effect.

No evidence was obtained to show that bypassing events greatly influence or markedly changed the pattern of ambient toxicity observed. In studies of effluent and ambient toxicity at other sites, wastewater bypassing did affect ambient toxicity (32). The good survival of adult *Ceriodaphnia* at Stations 1, 2, and 3 suggests that upstream of the Akron POTW the water quality is good.

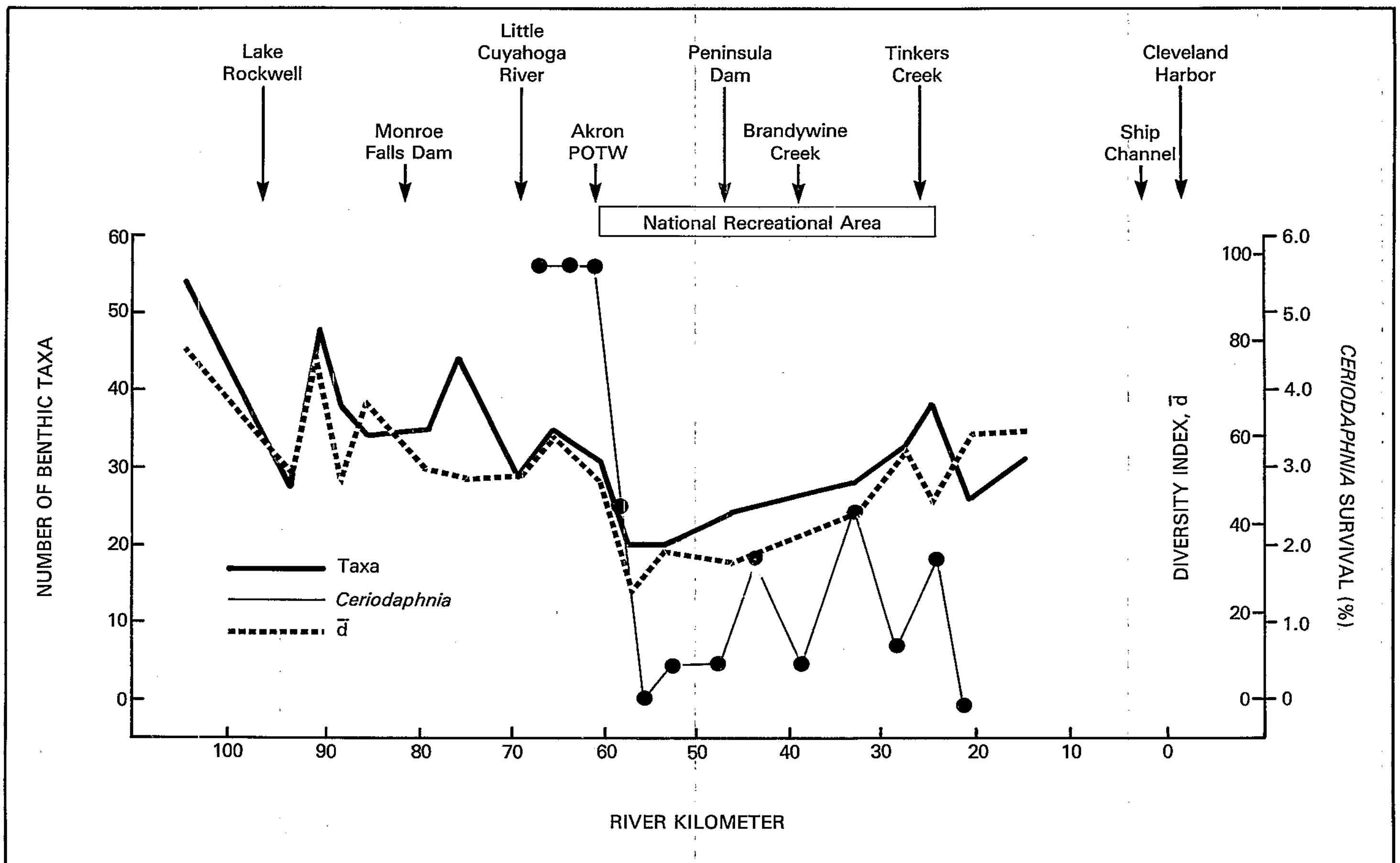


Figure 5-2. Profile of the Number of Benthic Taxa, Benthic Diversity, and *Ceriodaphnia* Survival in the Cuyahoga River, Ohio

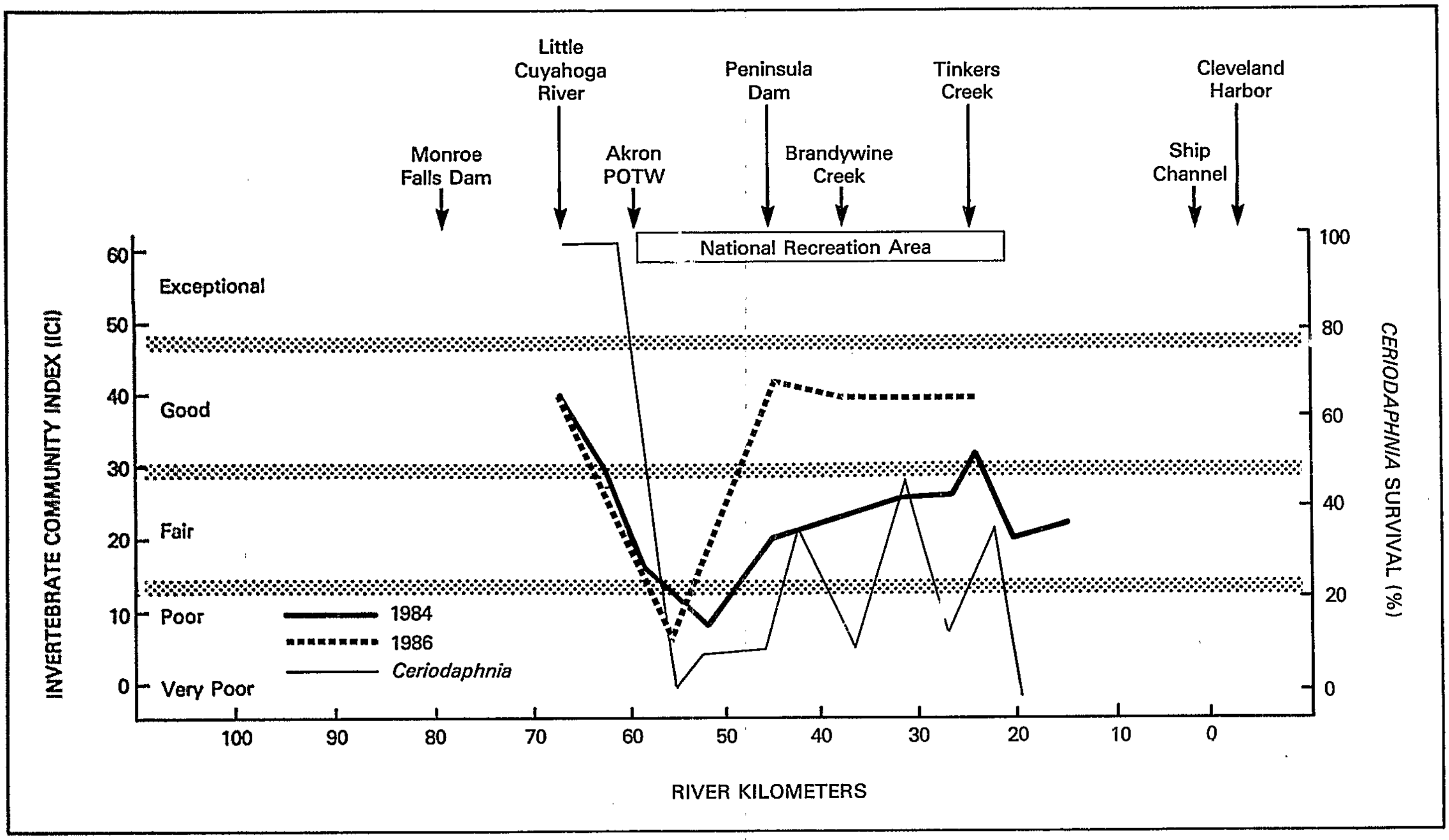


Figure 5-3. Profile of the Cuyahoga Macroinvertebrate Community (17) and *Ceriodaphnia* Survival

This is supported by Ohio EPA's fish and invertebrate surveys data. Although there are other dischargers to the Cuyahoga River and several tributaries, the *Ceriodaphnia* survival and young production data did not show a pattern which would indicate other important sources of toxicity (Figure 5-5).

Analysis of the various types of information yields the following conclusions:

1. The Akron POTW effluent causes the impact to the biota observed in the Ohio EPA surveys. This is clearly seen in Figures 5-3 and 5-4, where the ambient toxicity data is plotted against the fish and invertebrate biosurvey data.
2. Whatever the role of bypassing in causing an instream toxic effect, the toxicity of the continuously discharged effluent is sufficient to cause an instream effect.
3. No other important sources of toxicity were observed within the study area. There are point-source discharges on tributaries up- and downstream of the Akron POTW, as well as nonpoint source dischargers. However, the Akron POTW effluent dominates the Cuyahoga in the reach where adverse biological community impacts were observed.
4. The Akron POTW must reduce the toxicity of its effluent before improvement in the Cuyahoga's resident biota will occur.
5. Investigation into the source of the effluent's toxicity should take place to account for any observed variability in the toxicity.

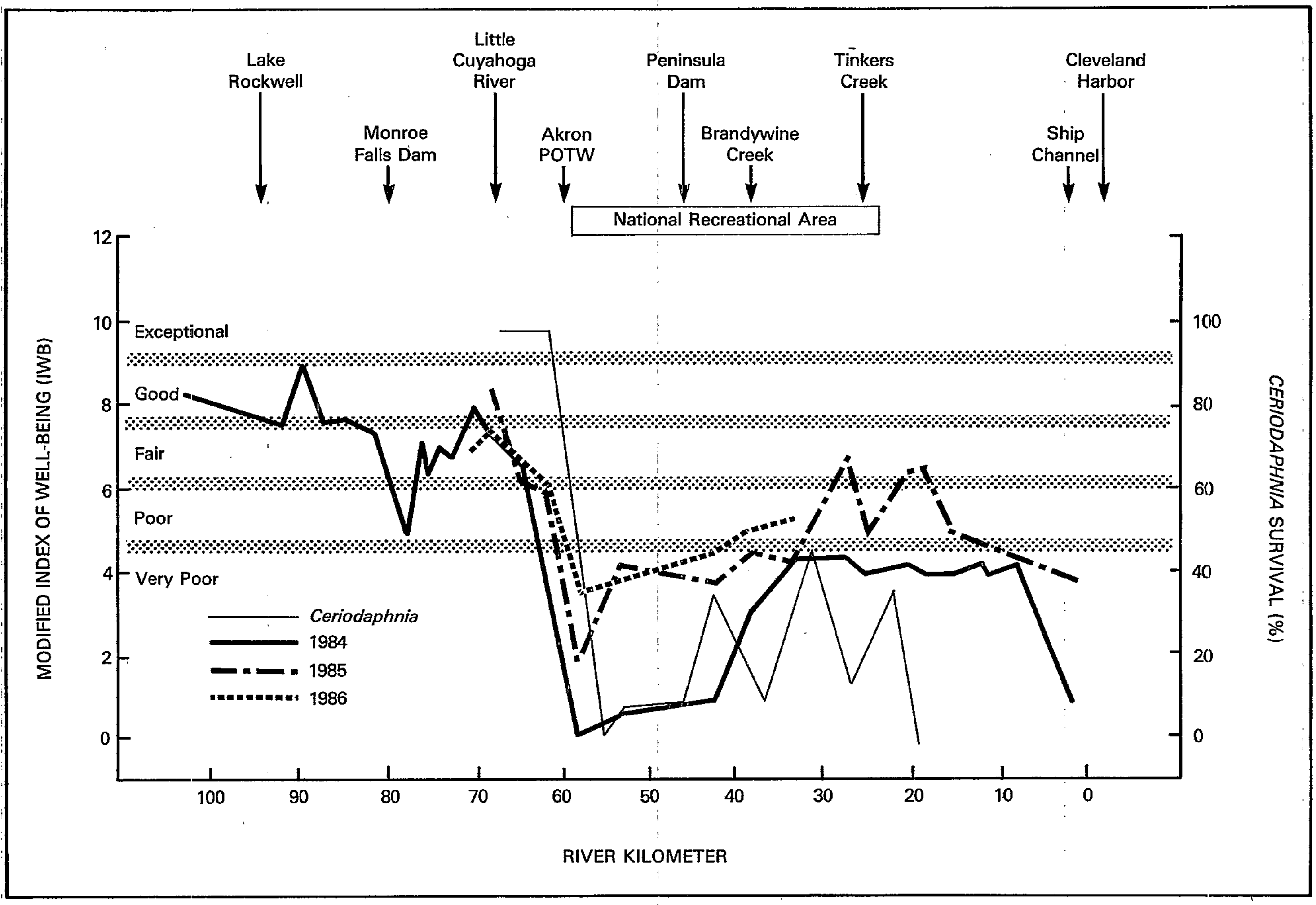


Figure 5-4. Profile of the Cuyahoga River Fish Community Using a Modified Index of Well-Being (17) and *Ceriodaphnia* Survival

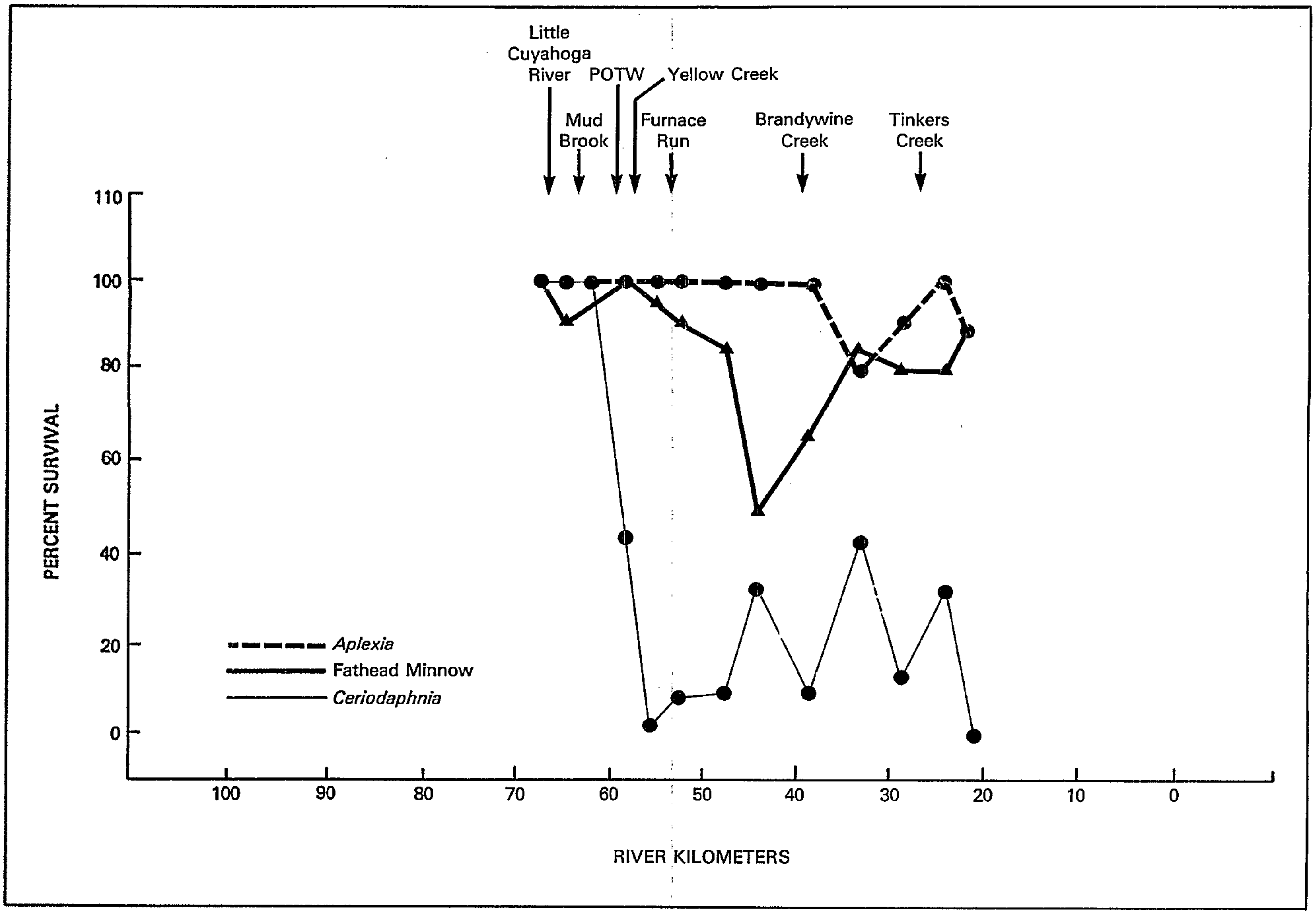


Figure 5-5. Survival of Fathead Minnows, *Ceriodaphnia*, and *Aplexia* Exposed to Ambient Water From the Cuyahoga River

6. Toxicity Source Investigation

6.1 Introduction

From the results of analyses presented in this document, the discharge from the Akron POTW was identified as a source of toxicants to the Cuyahoga River. The next tier in pollution control is to reduce the level of toxicity. U.S. EPA recommends that sources which discharge unacceptable levels of toxicity be required to perform a Toxicity Reduction Evaluation (TRE). The initial steps in a TRE are presented in Mount and Anderson-Carnahan (31) and include determining what component(s) of the effluent are contributing to the toxicity. Chemical and toxicological information on the effluent is needed to answer these questions:

- What is the variability associated with the compound(s) causing the toxicity in the POTW effluent?
- What compound(s) are causing that toxicity?
- Is the same chemical always responsible for the toxicity?

This information is used to determine what measures are needed to control the effluent toxicity to acceptable levels by helping to select treatment methods for toxicant removal or to identify toxicants which can be prevented from entering the wastestream.

In practice, a POTW discharging a toxic effluent may first desire to investigate toxicant origins within their collection system. That investigation may be accomplished by testing wastewater from specific locations in the collection system to identify important contributors of toxicants. Toxic inputs may be controlled through implementation of a pretreatment program. In this case, the Akron POTW effluent was tested for toxicity and fractionated to chemically isolate toxicants in an attempt to identify important compounds and to determine appropriate treatments which would reduce effluent toxicity.

In the first phase of a TRE, investigators conduct toxicity tests to screen and characterize the chemical and physical properties of the effluent and suspected toxicants. "A parallel series of relatively simple, low cost analyses" and sample treatment are conducted, each of which is designed "to remove or render biologically unavailable a specific group of toxicants such as oxidants, metals, nonpolar organics and metal chelates" (33). After these sample treatments (for example, aeration or filtration) toxicity tests are conducted to determine their effectivenes in reducing toxicity.

The second phase of the TRE process is the Toxicity Source Investigation (TSI) which may be conducted in two ways: using bench-scale treatability studies to further examine the success of various treatments on the toxicity without identifying the toxicant; or identifying the toxicant through the chemical and toxicological examination of sequential effluent fractions intended to isolate toxicants. This second option was selected for the Akron POTW. This type of analysis is still being developed and detailed procedures are to be published in 1988 by U.S EPA in a manual titled "Phase II - Toxicant Identification/Source Investigation and Control Manual."

Information concerning the waste treatment processes and the effluent composition at the Akron POTW was accumulated during this study. The effluent toxicity test results showed variable toxicity (Table 5-7), although this is not unusual for a POTW. Hence the TSI process paid particular attention to changes in the effluent characteristics over time. The ambient test results demonstrated that toxic effects occurred in the river, and that survival decreased with distance downstream from the discharger (Tables 5-8 and 5-9). The lowest survival of fathead minnows occurred when exposed to Station 8 and Station 9 waters. Significantly lower survival of *Ceriodaphnia* occurred when exposed to water from Station 5 and further downstream, with the exception of Stations 10 and 12. This persistent effect indicated that the toxicant(s) were not rapidly degraded, and was consistent with the observed impacts to the biological community of the Cuyahoga River downstream of the Akron POTW.

6.2 General Procedures for the Toxicity Source Investigation

6.2.1 Sample Type and Sampling Frequency

Samples were initially collected by compositing effluent over a 24-hour period. As effluent toxicity declined, the later samples, primarily those collected on and after April 22, 1986, were grab samples to capture more toxic effluent by avoiding the averaging effect of composite samples. The samples were placed on ice and shipped to ERL-Duluth via overnight carrier for testing. This sample collection effort began in January 1986 and continued through September 1986. The sampling schedule was initially set to obtain three samples each month on consecutive days. The schedule was modified slightly when grab samples were collected so that from two to four samples monthly were tested. Aliquots from each sample were taken for the various test procedures.

6.2.2 Test Species

Laboratory toxicity tests use aquatic species as detectors of toxicity. Consequently, it is crucial for a sensitive species to be used as the detector. Toxicity testing data presented in Chapters 3 and 5 indicated that *Ceriodaphnia* was the most sensitive of the three aquatic organisms tested. Further, *Ceriodaphnia* are adaptable to fractionation testing. ERL-Duluth maintains cultures of *Ceriodaphnia* for testing. Use of this species would provide continuity with previous test results using *Ceriodaphnia*.

6.3 Phase I Test Procedures

The testing procedures were developed during this effort and have since been modified. The most advanced description of these procedures is in Mount and Anderson-Carnahan (33).

6.3.1 Physical Treatment

The three samples collected in January 1986 were subjected to air stripping for removal of volatile compounds and then filtered to remove suspended particulate material. Procedures for these sample preparations are described in Mount and Anderson-Carnahan (33). The air stripping procedures included adjusting the pH so that acidic, neutral, and basic conditions were created. The altered pH levels were maintained during the aeration process. After aeration, the pH was returned to original levels before performing toxicity testing.

Test results were examined to determine whether toxicity was reduced and if so, by how much in each of the three pH conditions. If the toxicity is reduced similarly in the three aerated samples, but not removed, this would indicate that the toxicity is partially due to volatile compounds which are unaffected by pH. If toxicity is reduced and dissimilar between the aerated samples, then the general type of compound(s) contributing to the toxicity is revealed (e.g., greater toxicity reduction in the basic aerated sample indicates that a basic volatile compound was contributing more to the toxicity).

Filtering was the second physical treatment for the Akron POTW effluent. Samples were filtered with glass-fiber filters so that adsorption of dissolved organics would be reduced. Toxic compounds may be adsorbed on suspended material. The filtered samples were used in toxicity tests to determine the effect of the removal of suspended particulates (i.e., filterable solids). If toxicity is removed by filtering, then filtration may be used as an effluent treatment to reduce toxicity.

6.3.2 EDTA-Chelation Test

The ethylenediaminetetraacetate ligand (EDTA) is used to observe whether EDTA-chelatable cations (possibly metals) are causing toxicity. Direct analyses for such metals are less useful since much of the measured concentration may be biologically unavailable in the effluent and does not contribute to the observed toxicity. In the EDTA-chelation procedure, the results of toxicity tests on whole effluent samples spiked with EDTA are compared to results of tests on samples without the addition of EDTA If toxicity is removed with the addition of EDTA then toxicity may be caused by a metal.

6.3.3 Oxidant Reduction Test

Sodium thiosulfate is used as a reducing agent to determine whether oxidants are contributing to the toxicity of the effluent. In sufficient concentration, sodium thiosulfate is toxic to *Ceriodaphnia* and this toxicity is a function of the concentration of electrophiles in the sample. If sufficient electrophiles are present in the effluent to consume the thiosulfate, then toxic effects will not be observed. Therefore, varying amounts of sodium thiosulfate are added to determine the toxic concentration and, once known, the predetermined concentrations are added to the effluent. The results of toxicity tests on whole effluent samples with sodium thiosulfate added are compared to results of tests without the addition.

Chlorine is a typical oxidant that is extensively used for disinfecting wastewaters. The information from this test is also used to aid in the search for the toxicant and to initially identify an effective treatment for the removal of toxicity.

6.3.4 Whole Effluent Acute Toxicity Test Procedures

Typical effluent static acute toxicity tests were conducted to obtain 48-hour LC50s using a concentration series that progressed at 0.5 concentration intervals: 0, 6, 12, 25, 50, and 100 percent effluent. If greater resolution was desired for the higher concentration range, then a 75 percent concentration was substituted for the six percent concentration. The testing methods for *Ceriodaphnia* are generally those from Peltier and Weber (34). The LC50 values were calculated using graphical interpolation.

The results of these static toxicity tests were used to judge the toxicity of the effluent in comparison to the various solid phase extraction fractions and to the results of the EDTA-chelation and/or oxidant reduction tests.

6.4 Phase II Test Procedures

On receipt of the whole effluent sample, an aliquot of the sample was immediately tested for acute toxicity. In general, aliquots of whole effluent samples were subjected to the solid phase extraction (SPE) test to investigate toxicity due to nonpolar organics. These extracted samples were sometimes subjected to the EDTA-Chelation test to investigate toxicity due to cations, and/or the Oxidant Reduction test to investigate toxicity due to oxidants.

6.4.1 Solid Phase Extraction (SPE) Test Procedures

The purpose of the SPE procedure is to simplify the identification of nonpolar organic toxicants. The effluent is separated into fractions containing smaller numbers of compounds than in the whole effluent. This procedure was capable of removing toxicity and therefore was the predominant procedure used after the initial January 1986 samples. Effluent sample material partitioned on a C-18 (octadecylsilane bonded silica gel solid phase extraction column [J.T. Baker Chemical Co., Phillipsburg, New Jersey]) was sequentially extracted from the column using 25, 50, 75, 80, 85, 90, 95, and 100 percent methanol solutions in water for the SPE test. Methanol concentrations are increased in successive elution volumes so that the polarity decreases for each fraction of effluent material that is eluted with each volume of methanol and water.

Effluent fractions from the SPE test, as performed here, represent concentrations of the effluent which may be up to five times more concentrated than the original sample (whole effluent) depending on the efficiency of sorption onto the C-18 column and elution into the methanol-water fraction. Consequently, if toxicity is not observed from exposure to such concentrated samples, then the components in that fraction are assumed to not cause toxicity in the whole effluent. If toxicity is observed from exposure to such concentrated samples, then toxicity may contribute to the toxicity of the whole effluent. However, the observed toxicity may not be of sufficient strength to cause a toxic effect in the whole effluent (a dilution of up to five times the concentration in the SPE fraction).

6.4.2 Acute Toxicity Fractionation Tests

Acute toxicity tests were conducted using effluent sample material that had been eluted from the C-18 column during the pass-through of each methanol-water fraction. Toxic effects were determined to have occurred when the mortalities of *Ceriodaphnia* exposed to the methanol fractions were greater than in the effluent control.

To determine whether a toxic effect in one of the concentrated SPE test fractions causes toxicity in the whole effluent, definitive toxicity tests should be conducted on the effluent fraction(s) of interest. Concurrent chemical analysis should be performed to determine the actual concentrations of a particular toxicant in the fraction of concern. To determine the toxicant concentration and exposure concentration, the compound(s) believed to be causing the toxicity must be identified and the recovery efficiency of that compound from the C-18 column into the methanol fraction must be known.

In this effort at the Akron POTW, the toxic compound(s) was never identified. Consequently, definitive toxicity tests and associated chemical analyses were not conducted.

6.4.3 EDTA-Chelation of SPE Fractions

After June 1985 the EDTA-chelation test was conducted on some of the methanol fractions from the SPE test. This was done to ensure that the metals in the effluent were not causing toxicity in the methanol fractions. When EDTA was added to specific SPE fractions, toxicity tests were again performed, but only to indicate the presence of a toxic effect (i.e., greater *Ceriodaphnia* mortalities than in the effluent controls).

6.4.4 Chemical Analysis of Metals

Effluent samples from January, April, July, and August were analyzed for the presence of metals. The concentrations of cadmium, chromium, copper and zinc were determined using standard U.S. EPA methods for atomic absorption spectroscopy.

6.4.5 Chemical Analysis of Organics

Extracts of methanol fractions of whole effluent from the four August 1985 samples were injected into a gas chromatograph with an Ion Trap Mass Detector to examine the classes of compounds in the SPE fractions and to search for suspect toxicants. The methanol fractions were first diluted with water to reduce the concentration of methanol, extracted with hexane, concentrated (about 10 times), and then injected into the gas chromatograph. The analytical methods met the performance criteria for quality control listed in Section 8.2 of U.S. EPA Method 625.

The chromatograms from each sample were examined to locate peaks either common to the toxic fractions or of sufficient concentration to yield valuable spectra. Spectra identification information from the U.S. EPA/NIH Mass Spectral Database was used to identify common peaks.

6.5 Phase I Data and Results

6.5.1 Physical Treatment

Whole effluent samples with different pH values were subjected to air stripping. The samples were returned to their original pH and then tested for toxicity to *Ceriodaphnia*. This treatment was used to remove volatile compounds. No effect on the toxicity of the Akron POTW effluent was found as a result of air stripping.

Whole effluent samples were filtered to remove suspended particulates and, similarly this treatment did not affect the toxicity to *Ceriodaphnia* when compared to untreated effluent.

Results of these two physical treatments indicate that the toxicants were nonvolatile compounds and they were not associated with solids in the effluent.

6.5.2 Whole Effluent Toxicity

Twenty-nine effluent samples (composite and grab) were collected over the course of this study and static acute toxicity tests were conducted using *Ceriodaphnia* to determine 48-hour LC50 values. The LC50s varied from 38 to >100 percent effluent for samples collected from Janaury 13 to September 26, 1986 (Table 6-1). Over

Table 6-1. Whole Effluent Acute Toxicity Test Results for *Ceriodaphnia* Exposed to Akron POTW Effluent, 1986

Sample Date/Type[a]	48-hour LC50 (% Effluent)	Zinc Concentration (μg/L)	EDTA-Chelation Toxicity Test Result	Oxidant Reduction Test Result
January 13, C	82	136		
January 14, C	>100			
January 15, C	>100			
February 7, C	>100			No effect
February 8, C	79			No effect
February 9, C	38			No effect
March 28, C	77			
March 29, C	81			
March 30, C	67			
April 22, 1G	50	274		
2G	82			
3G	100			
4G	67			
1G[b]	<25		Toxicity removed	
June 16, C	>100		No effect	
June 26, 1G	>100		No effect	
2G	>100		No effect	
June 27, 1G	>100		No effect	
2G	>100		No effect	
July 9, 1G	>100		No effect	
2G	>100		No effect	
July 10, 1G	>100		No effect	
2G	72	146	(c)	
August 6, C	>100	58	No effect	
August 14, C	>100	66	No effect	
August 18, C	>100	57	No effect	
August 26, C	>100	26	No effect	
September 18, C	>100		No effect	
September 22, C	>100		No effect	

[a]C = composite, G = grab sample
[b]The first grab sample for April 22 was retested after 30 days.
[c]Toxicity reduced in 80 and 95 percent, but unaffected in 85 percent methanol fraction.

half of the LC50 values were >100 percent effluent (17 of the 29 samples). The effluent grab samples collected in June and July had LC50 values >100 percent effluent, except one of the July 10 samples which had an LC50 of 72 percent effluent.

The majority of the LC50 values for the winter and spring samples collected through April 22, 1986 indicated that the effluent was acutely toxic. In contrast, whole effluent samples collected after that date had no acutely toxic effects (except one).

6.5.3 EDTA-Chelation

The EDTA-chelation test was performed on 14 whole effluent samples from June through July. None of these effluents had shown acutely toxic effects on *Ceriodaphnia*, and the addition of EDTA had no effect on the observed toxicity, with two exceptions (Table 6-1). The retested sample from April 22 was acutely toxic at <25 percent effluent, and the addition of EDTA removed the toxicity. The whole effluent grab sample from July 10 was acutely toxic at an LC50 of 72 percent and the addition of EDTA reduced the effluent toxicity. This result indicates that metals were causing the toxic effects on this particular sample.

6.5.4 Oxidant Reduction

The oxidant reduction test was performed on three samples collected in February 1986. There was no reduction of toxicity from the addition of sodium thiosulfate to the effluent (Table 6-1). The lack of toxicity reduction indicates that oxidants are not the cause of toxicity in the effluent samples. A typical oxidant present is chlorine used for disinfection. The chlorination period at the Akron POTW is from May 1 to October 31, so that the sample tested did not contain chlorine.

6.6 Phase II Data and Results

6.6.1 SPE Fractionation

For each of the samples obtained from the Akron POTW, the SPE toxicity tests were conducted in conjunction with effluent toxicity tests to determine 48-hour LC50s of the whole effluent. The results of the SPE tests indicated that, even in cases where the LC50s were >100 percent effluent, there was toxicity in at least one of the eight methanol fractions (Tables 6-1 and 6-2). And conversely, while the March 29 sample had an LC50 of 81 percent effluent, the SPE fractions did not indicate toxicity. This sample was not tested for the EDTA-chelation or Oxi-

Table 6-2. Solid Phase Extraction Toxicity Test Results[a]

	% Methanol							
Date/Type[b]	25	50	75	80	85	90	95	100
January 13, C	-	-			T[c]			T[c]
January 14, C	-	-						T[c]
January 15, C	-	-			T[c]			
February 8, C					T[c]			
February 9, C					T[c]			
March 28, C	-	-			T[c]			
March 29, C	-	-						
March 30, C	-	-			T[c]			
April 22, 1G	-	-			T[c]			T[c]
2G	-	-			T[c]			T[c]
3G	-	-			T[c]			T[c]
4G	-	-			T[c]			T[c]
July 10, 2G				T[c]	T[c]		T[c]	
August 6, G					T[c]			T[c]
August 14, C					T[c]			
August 18, C					T[c]			
August 26, C					T[c]			

[a] - denotes not tested
[b] C = composite, G = grab sample
[c] Toxicity found in that fraction

dant Reduction effects to further determine if the whole effluent toxicity was due to either metals or oxidants, respectively.

The SPE fraction which predominantly contained toxic components was the 85 percent methanol fraction. This result indicated that it was the more nonpolar organic compounds which caused toxicity and that such compounds were consistently present in the effluent. Further, the 100 percent methanol fraction, containing the most nonpolar compounds, resulted in toxic effects to *Ceriodaphnia* in four of the samples. Toxic effects in the 10 percent methanol fraction were only observed when the toxic effects in the 85 percent methanol fraction were great (when mortality was ensuing rapidly). This suggests that there is more than one toxic, nonpolar compound in the effluent, and that while their polarities differ they may have a common industrial source.

6.6.2 EDTA-Chelation

Further examination of the July 10, 1986 sample indicated that toxic effects were observed in the 80, 85, and 95 percent methanol fractions. The EDTA-chelation test results for the 80 and 95 percent fractions indicated that the toxicity was again reduced with the use of EDTA. So, in contrast to the 85 and 100 percent methanol fractions, the toxic effects exhibited in the 80 and 95 percent fractions were contributed by metal compounds. Further, the EDTA test on the 85 percent fraction indicated that toxicity was not reduced when EDTA was added.

These results illustrate that there are at least two types of toxicants present in the Akron POTW effluent—nonpolar organics and metals. The nonpolar organic toxicity appears to be due to a compound that is eluted off the C-18 columns using 85 and 100 percent methanol solutions. The 85 percent methanol fraction demonstrated the greatest and most consistent toxicity, while the 100 percent methanol fraction was less consistent. In addition, the loss of toxic effects by the addition of EDTA demonstrated that at least one of the toxic components was chelatable.

6.6.3 Organic Chemicals

From the examination of the GC/MS chromatogram of the April 22 grab samples, six compounds were found in relatively high concentrations. Three of those were identified using computerized compound libraries. The compounds were 1-methyl naphthalene, 2-methyl naphthalene, and 3,5-bis (1,1-dimethylethyl) phenol. Original concentrations in whole effluent for these three compounds in the SPE fractions were estimated to be in the mg/L range. Based on quantitative structure activity relationship estimates and toxicity data, these concentration levels are too low to be suspected of causing toxicity (35,36). Plans to purify other toxic SPE fractions, especially the 85 percent methanol fraction, to further examine the predominant source of toxicity could not be completed since effluent samples after April 22 were not acutely toxic to *Ceriodaphnia*.

6.6.4 Metals

Zinc had been identified earlier as a potential toxicant because of the very high concentrations in the influent (over 600 mg/L). The high influent concentrations partially arose from the use of zinc orthophosphate in the municipal water treatment plant at concentrations of 200 to 300 mg/L and from metal plating facilities discharging to the Akron POTW (37). Therefore, zinc concentrations were monitored in some of the samples for the whole effluent acute toxicity tests. Concentrations of zinc ranged from 136 to 274 mg/L for three samples collected in January, April, and July. These concentrations are similar to the effluent concentrations of approximately 200 mg/L zinc (37). Lower effluent zinc concentrations were obtained in the four August samples, 26 to 66 mg/L of zinc, but do not correspond to the high influent levels during August (mean concentration of 846 mg/L zinc). Apparently, treatment was effective in removing a large portion of the zinc present in the influent. Concentrations of zinc found to be acutely toxic range from 32 to 76 mg/L (38, 25), so that even the low concentrations of zinc in the effluent may have been acutely toxic. However, since the bioavailability of zinc and the bioavailable portion in the effluent was not known, the actual toxic concentration is unknown.

The concentrations of cadmium, chromium, and copper were also measured from effluent samples collected in April, July, and August 1986. The concentrations were at or below the water quality criteria for protection of aquatic life (hardnesses of up to 200 mg/L $CaCO_3$). These metals were judged not to be contributors to the observed effluent toxicity in April. During July and August the effluent was not toxic.

6.7 Wastewater Bypassing Concerns

Wastewater bypassing had been identified as a potential source of the toxicants causing the adverse impacts observed in the Cuyahoga River when this case study began. The frequency and duration of bypassing was examined and indicated that most events were of less than 12 hours duration and were often the consequence of rainfall events when the Akron POTW capacity was exceeded (19). Travel time through the study area was estimated to be less than 24 hours, but acute toxicity to *Ceriodaphnia* did not occur until after 24 hours of exposure in the one sample of bypassed wastewater tested. The information gained in this effort does not confirm initial suspicions that bypassing of untreated or partially treated wastewaters is the cause of adverse conditions in the Cuyahoga River. Wastewater bypassing, however, could be a contributing factor.

6.8 Summary

The tools used to conduct the TSI were refined and expanded during this study, and are the basis for the procedures described in the U.S. EPA manual for TREs by Mount and Anderson-Carnahan (31). In this case study, effluent toxicity decreased markedly before toxicity characterization procedures were able to identify causative toxicants.

7. Conclusions and Application of Case Study Data to Setting Permit Limits

7.1 Introduction

The Akron POTW was studied through a cooperative effort between various Federal and state agencies. The purpose of the work was to determine if the Akron POTW discharge was a source of contaminants/toxicants to the Cuyahoga River and to document the process of discovery and research using water quality-based procedures for the control of toxics. These conclusions and recommendations are based on the research conducted at the Akron POTW only and are not meant to influence the pollution control and abatement activities of Ohio EPA or the City of Akron. The results of the toxicity testing are summarized in Table 7-1 and then used in the development of sample permit limits for the Akron POTW.

7.2 Toxicity Testing Needs to Reduce Uncertainty Levels

U.S. EPA (24) provided guidance for the degree of whole effluent toxicity testing needed to determine whether to 1) continue toxicity data generation, 2) begin permit limit derivation, or 3) place the discharger in a low priority permitting category. This guidance includes approximating the uncertainty associated with toxicity test data due to effluent variablity, test species sensitivity, and acute-to-chronic ratios (ACR). U.S. EPA (24) provides uncertainty factor values for each, from 1 to 1,000 and the more information available the lower the factor value. The goal is to obtain sufficient information to lower the level of uncertainty so that

$$\frac{\text{Toxic Response}}{\text{IWC}} > \text{level of uncertainty} \qquad \text{(Equation 7-1)}$$

where

Toxic response is measured either by LC50s for acute toxicity or by NOELs for chronic toxicity.

IWC = instream waste concentration; Q effluent/ Q river + Q effluent

Level of Uncertainty (UF) = product of the three uncertainty factors

Table 7-1. Toxicity Testing Summary for the Akron POTW

Test Dates	Investigators	Test Species	Toxicity Range[a] (% Effluent)	Sample Type	Number of Tests
Aug 1984	EPA-Cincinnati[b]	Fathead minnow	30-≥100 NOEC	Effluent	2
Aug 1984	EPA-Cincinnati[b]	*Ceriodaphnia*	30 NOEC	Effluent	2
May 1985	EPA-Cincinnati[b]	Fathead minnow	30 NOEC	Effluent	1
May 1985	EPA-Cincinnati[b]	*Ceriodaphnia*	≥100 NOEC	Effluent	1
Jul 1985	ERL-Duluth	*Ceriodaphnia*	20-100	Ambient	4
Jul-Aug 1985	ERL-Duluth	*Ceriodaphnia*	30->100 NOEL	Effluent	3
Jul-Aug 1985	ERL-Duluth	Fathead minnow	>100 NOEL	Effluent	2
Sep-Oct 1985	ERL-Duluth	*Ceriodaphnia*	10 NOEL	Effluent	1
Sep-Oct 1985	ERL-Duluth	Fathead minnow	75 NOEL	Effluent	1
Sep-Oct 1985	ERL-Duluth	*Aplexia*	>100 NOEL	Effluent	1
Sep-Oct 1985	ERL-Duluth	*Ceriodaphnia*	0-100	Ambient	13
Sep-Oct 1985	ERL-Duluth	Fathead minnow	50-100	Ambient	13
Sep-Oct 1985	ERL-Duluth	*Ceriodaphnia*	80-100	Ambient	13
Dec 1985	ERL-Duluth	*Ceriodaphnia*	30 NOEL	Effluent	2
Jan 1986	ERL-Duluth	*Ceriodaphnia*	82->100 LC50	Effluent	3
Feb 1986	ERL-Duluth	*Ceriodaphnia*	38->100 LC50	Effluent	3
Mar 1986	ERL-Duluth	*Ceriodaphnia*	67-81 LC50	Effluent	3
Apr 1986	ERL-Duluth	*Ceriodaphnia*	50-100 LC50	Effluent	4[c]
Jun 1986	ERL-Duluth	*Ceriodaphnia*	>100 LC50	Effluent	5
Jul 1986	ERL-Duluth	*Ceriodaphnia*	>100 LC50	Effluent	4
Aug 1986	ERL-Duluth	*Ceriodaphnia*	>100 LC50	Effluent	4
Sep 1986	ERL-Duluth	*Ceriodaphnia*	>100 LC50	Effluent	2

[a]*Survival data only.*

[b]*Tests were conducted on unchlorinated, chlorinated, and dechlorinated effluent. Data used were those from the latter two categories.*

[c]*Test data from the 30-day toxicity decay test conducted on the April 22 sample are not included.*

Using the lowest LC50 value of 38 percent effluent and flows corresponding to the 7Q10 and mean Akron POTW flow (Table 5-4), Equation 7-1 was solved for the necessary level of uncertainty. The left side of the equation has a value of 0.55, and for this to be greater than the level of uncertainty would require that each of the uncertainty factors have a value less than 1, which is not possible.

$$\frac{0.38}{\left[\dfrac{2.79\ m^3/s}{1.27\ m^3/s + 2.79\ m^3/s}\right]} = 0.55$$

Initial values for the uncertainty factors are: 10 to 100 for effluent variability, 10 for species sensitivity, and 10 for the acute-to-chronic ratio (2). While short-term effluent variability was briefly examined in Chapter 6 (Table 6-1) by using grab samples in acute toxicity tests, U.S EPA recommended that such testing be conducted monthly for at least one year. Similarly, long-term effluent variability tests were not conducted in sufficient number to address this source of uncertainty. The uncertainty factor for effluent variability when the variability in effluent toxicity is less than an order of magnitude is 10 (24) (Table 7-1).

Three species were used for chronic toxicity testing during the on-site visit (Chapter 5) and *Ceriodaphnia* was identified as the most sensitive species. While monthly effluent samples were not tested at the frequency recommended by U.S. EPA (24), it may be argued that sufficient testing was conducted before the reconnaissance trip, during the reconnaissance trip, and during the site visit to have adequately addressed species sensitivity. Therefore, the uncertainty factor for species sensitivity is 1. Chronic toxicity tests are to be conducted using three species for comparison to the acute toxicity test data generated for the species sensitivity factor. The frequency of recommended testing for the determination is from 144 to 216 tests per year. This level of testing was not met; however, ambient toxicity testing was conducted using three species to measure actual instream toxicity so that an ACR is not needed (24). Consequently, the uncertainty factor for ACR is 1.

The minimum level of uncertainty based on the above discussion is equal to 10 X 1 X 1, or 10. This value is greater than 0.55 from Equation 7-1. In such cases, further testing cannot reduce the level of uncertainty to satisfy Equation 7-1, so the data generated from definitive toxicity testing can be used to set permit limits (24). When effluent dilution in the receiving water at critical low flows (or design flows) is ≤ 100 to 1, then definitive data generation will always be recommended. Note that the above discussion was presented for illustrative purposes as part of the permit derivation process since toxicity data are not necessary to set permit limits.

7.3 Derivation of Sample Permit Limits

The concepts developed and presented in the Technical Support Document for Water Quality-based Toxics Control (24) and the Permit Writer's Guide (30) will be discussed as appropriate to the work conducted at the Akron POTW for the derivation of sample permit limits. The derivation of sample limits is presented for educational purposes only and does not represent an official U.S. EPA regulatory action. The most sensitive species to the Akron POTW effluent and ambient waters was *Ceriodaphnia* and results from those toxicity tests will be used for calculating the sample limits.

7.3.1 Exposure Criteria

Exposure criteria were developed for acute and chronic exposures as the next step in the permit limit derivation process. For these two exposures, the recommended criteria were defined as: criterion maximum concentration (CMC) for acute exposures, and a criterion continuous concentration (CCC) for chronic exposures (24, 30). These exposure criteria are for completely mixed discharges when measuring whole effluent toxic effects. A completely mixed discharge is one that mixes rapidly with the entire receiving water flow. The CMC and CCC are recommended targets to limit effluent toxicity and are defined below.

$$CMC \leq 0.3\ TU_a, \text{ and} \qquad \text{(Equation 7-2)}$$

$$CCC \leq 1.0\ TU_c \qquad \text{(Equation 7-3)}$$

where

$$TU = \frac{100}{\text{LC50 or NOEL}}$$

7.3.2 Wasteload Allocation

The purpose of the wasteload allocation (WLA) is to set values for acceptable effluent concentrations. U.S. EPA (30) defines wasteload allocation as "the portion of a receiving water's total maximum daily pollutant load that is allocated to one of its existing or future point sources of pollution." State definitions of mixing zones and design flow specifications will greatly influence the WLA process through model selection and will determine where the permit limits are to be met.

Several types of models have been developed for the WLA process and selection is dependent upon the available data and the type of discharge. A steady-state wasteload allocation model is acceptable to determine exposure limits and effluent quality levels which will meet discharge criteria. U.S. EPA (24) regards this model type as appropriate for effluent-dominated streams like the Cuyahoga River. The discharge from the Akron POTW is sufficiently large relative to the flow of the Cuyahoga so that the effluent often composes more than 20 percent of the river flow. This procedure also assumes that there are no upstream concentrations of pollutants or toxicants (30). This is in agreement with the ambient toxicity data for the Cuyahoga upstream of the Akron POTW discharge, where *Ceriodaphnia* survival was 100 percent.

Since the Akron POTW is being treated as a single discharger, the allocation process is quite simple. There are no other dischargers for which allowable toxicant concentrations must be apportioned.

For situations in which the effluent is rapidly and completely mixed, U.S. EPA (24) states that two wasteload allocations should be conducted to meet the CMC and CCC at specific exposure durations and frequency. From visual observations of dye dilution at the Akron POTW discharge (Chapter 4), the effluent was seen to be rapidly mixed and for this discussion will be regarded as completely mixed. However, U.S. EPA (24) states that when mixing zones have been specified, the wasteload allocation cannot be based on the assumption that an effluent is completely mixed at the point of discharge. The State of Ohio standards allow the Ohio EPA Director to define the size of a mixing zone for a receiving watercourse up to one-third of the cross-sectional area of that watercourse with a length up to five times the width of the river at the point of discharge (Ohio Water Quality Standards. Chapter 3745-1 of Ohio Annotated Code). Within this mixing zone acutely toxic conditions are to be prevented (24).

Receiving water flow information is needed in the form of design flows for calculating the acceptable effluent concentrations (24). If design flows are not specified by the regulatory agency for calculation of the WLA, then hydrologically-based design flows can be used to calculate acceptable effluent loads. It is the policy of Ohio EPA to allow use of the whole design flow in WLA calculations, without accounting for the mixing zone, where the receiving water and effluent mix rapidly (e.g., for the Cuyahoga River) (39). Such design flows for stressed systems are recommended to be the 1Q10 for calculating the CMC and the 7Q10 for calculating the CCC (30). Note that the Ohio EPA does not use 1Q10 design flows for permitting; however, for consistency with U.S. EPA general recommendations (24,30) 1Q10 design flows will be used in this example. U.S. EPA (30) states that since the "acceptable effluent toxicity is a function of dilution and the ambient criteria, the available dilution will drive the WLA and thus the permit limit. The key to this procedure is to determine the dilution factor." Acceptable instream toxicity values to meet the ambient criteria are determined by U.S. EPA (24,30) as

$$\text{WLA} \leq \text{dilution factor X CMC or CCC} \quad \text{(Equation 7-4)}$$

where

the dilution factor $= Q_e + Q_s / Q_e$ and

Q_s = design flow for the receiving water (Cuyahoga River)

Q_e = design flow for the effluent (Akron POTW)

The equation above is appropriate for situations where the source water and receiving water are not the same. The source of the Akron drinking water and much of the subsequent Akron POTW influent is the Lake Rockwell reservoir, located upstream of the Akron POTW.

The 7Q10 value is 1.27 m^3/s and the 1Q10 value is 0.87 m^3/s for the Cuyahoga River (21). The "effluent flow which could cause the greatest impact" is recommended to be used by U.S. EPA (30). The Akron POTW has its NPDES permit limits for conventional pollutants based on an effluent flow of 3.94 m^3/s. In contrast to the worst-case conditions which are examined using the design flows, the mean effluent discharge was 2.79 m^3/s during the site visit when chronic toxicity tests were conducted (Table 5-7). In addition, acute toxicity data were generated for the TSI during 1986 when the mean discharge was 3.22 m^3/s (Table 6-1). When not accounting for the mixing zone, a dilution factor of 1.22 for the CMC and a dilution factor of 1.32 for the CCC would be used for the calculations of acceptable effluent toxicity. With the mixing zone, the available receiving water for dilution is reduced and the dilutions are 1.07 for the CMC and 1.11 for the CCC. Further calculations are based on the dilutions without the mixing zone following Ohio EPA policy, and solutions have been rounded.

Dilution With No Mixing Zone

$$\text{CMC:} \quad \frac{3.94\ m^3/s + 0.87\ m^3/s}{3.94\ m^3/s} = 1.22$$

$$\text{CCC:} \quad \frac{3.94\ m^3/s + 1.27\ m^3/s}{3.94\ m^3/s} = 1.32$$

Dilution With the Ohio Mixing Zone

$$\text{CMC:} \quad \frac{3.94\ m^3/s + 1/3\ (0.87\ m^3/s)}{3.94\ m^3/s} = 1.07$$

CCC: $\dfrac{3.94\ m^3/s + 1/3\ (1.27\ m^3/s)}{3.94\ m^3/s} = 1.11$

The solution for Equation 7-4 for WLA_a is <0.366 TU_a and for WLA_c is <1.32 TU_c.

WLA_a: $1.22 \times 0.3\ TU_a = 0.366$

WLA_c: $1.32 \times 1.0\ TU_c = 1.32$

The WLA_a is converted to a chronic measure by multiplying by the ACR value. Using a discharger-specific ACR is the first opportunity to use toxicity data in the calculation of permit limits.

7.3.3 Permit Limit Calculations

The chronic and acute wasteload calculations correspond to two criteria which are used as the basis for determining long-term averages (LTAs). An LTA is a mean effluent value that will result in an acceptable record of compliance with water quality standards and it is based, in part, on past effluent performance information such as variablity. U.S. EPA has set probability estimates for these levels at 99 percent which means that statistically, there will be one time in a hundred that the level will be exceeded. The one-hour LTA corresponds to acute exposures and the four-day LTA corresponds to chronic exposures.

$$\text{1-hour LTA} = e^{(\mu + 0.5\,\sigma 2)} \qquad \text{(Equation 7-5)}$$

where

$\mu = \ln(WLA_a) - z\sigma$

$\sigma = \sqrt{\ln((CV^2 + 1)}$

z = 2.326 for the 99th percentile occurrence probability

CV = coefficient of variation

$$\text{4-day LTA} = e^{(\mu + 0.5\,\sigma^2)} \qquad \text{(Equation 7-6)}$$

where

$$\mu = \ln(WLA_c) - z\sqrt{\ln\left[1 + \frac{(e^{s^2} - 1)}{4}\right]}$$

Equations 7-5 and 7-6 were solved for two conditions: values provided by U.S. EPA (30) for the coefficient of variation (CV) of 0.6, and an ACR value of 10 versus calculated values based on data from this study. The CV term in Equation 7-5 is the second opportunity for toxicity data to be used in the permit calculations. Remember, toxicity data are not necessary to calculate a permit limit.

Using U.S. EPA values for the CV and the ACR, the acute LTA was 1.17 TU_c and the chronic LTA was 0.77 TU_c.

$$\text{1-hour LTA} = e^{(0.009 + 0.5 \times 0.307)} = 1.17\ TU_c$$

where

$\mu = \ln(0.366 \times 10) - 2.326 \times 0.554 = 0.009$

$\sigma = \sqrt{\ln[(0.6)^2 + 1]} = 0.554$

$$\text{4-day LTA} = e^{(-0.404 + 0.5 \times 0.307)} = 0.77\ TU_c$$

where

$$\mu = \ln(1.32) - 2.326\sqrt{\ln\left[1 + \left(\frac{e^{0.037} - 1}{4}\right)\right]} = -0.404$$

The four-day value is next converted into units representative of a daily value since "effluent toxicity tends to be more variable than streamflow in the directions of concern" (30). The four-day LTA conversion is accomplished by

$$LTA_{conv} = e^{(\mu + 0.5\,\sigma^2)} \qquad \text{(Equation 7-7)}$$

where

$$\mu = \text{4-day LTA's } \mu\ (0.5 \times \sigma^2) + 0.5 \times \ln\left[1\left(+\frac{e^{\sigma^2} - 1}{4}\right)\right]$$

$$LTA_{conv} = e^{(-0.514 + 0.5 \times 0.307)} = 0.70\ TU_c$$

where

$$\mu = -0.404 - (0.5 \times 0.307) + 0.5 \times \ln\left[1 + \left(\frac{e^{0.037} - 1}{4}\right)\right] = -0.514$$

So the chronic LTA becomes 0.70 TU_c. The lower of the two LTAs (1.17 TU_c versus 0.70 TU_c) is selected to further develop the permit limits. The most restrictive LTA in this example is the chronic value of 0.70 TU_c which was used to determine the maximum daily permit limit and the average monthly permit limit. U.S. EPA set probability estimates for meeting the permit limits at 95 percent.

$$\text{Maximum Daily Limit} = e^{(\mu + z\sigma)} \qquad \text{(Equation 7-8)}$$

where

z = 1.645 for the 95th percentile occurrence probability

$\mu = \ln \text{LTA} - 0.5\sigma^2$

$\sigma^2 = \ln(CV^2 + 1)$

$$\text{Average Monthly Limit} = e^{(\mu_n + z\sigma_n)} \qquad \text{Equation 7-9)}$$

where

$$\mu_n = \mu + \frac{(\sigma^2 - \sigma_n^2)}{2}$$

$$\sigma_n^2 = \ln\left[1 + \left(\frac{e^{\sigma^2} - 1}{n}\right)\right]$$

n = number of monthly effluent samples

The maximum daily limit result using the LTA derived from the U.S. EPA values for CV and ACR indicated that the maximum daily limit is 1.25 TU_c. The average monthly limit using the same LTA indicated that, for the typical case of four effluent samples per month, the chronic exposure limit is 0.91 TU_c.

$$\text{Maximum Daily Limit} = e^{(-0.510 + 1.645 \times 0.554)} = 1.49\ TU_c$$

where

$$\mu = \ln 0.70 - 0.5 \times 0.307 = -0.510$$

$$\text{Average Monthly Limit} = e^{(-0.400 + 1.645 \times 0.293)} = 1.08\ TU_c$$

where

$$\mu = -0.510 + \left(\frac{0.307 - 0.086}{2}\right) = -0.400$$

$$\sigma^2 = \ln\left[1 + \left(\frac{e^{0.307} - 1}{4}\right)\right] = 0.086$$

Translated into percent effluent values, these results require that the mean daily limit of toxicity is >67 percent effluent (NOEL) and that the mean monthly limit of toxicity is >92 percent effluent (NOEL). Permit compliance should ensure that acute toxicity does not occur within the mixing zone and that chronic toxicity does not occur beyond the mixing zone. During the on-site chronic toxicity testing, ambient water from Station 4 located downstream of the mixing zone resulted in *Ceriodaphnia* survival of 40 percent (Table 5-9). The ambient toxicity data indicates that the Akron POTW is not in compliance with these sample permit limits especially since the ambient sample was not collected at the design low flow, so that there was more available dilution than at 7Q10 or 1Q10 flows.

Permit limit calculations can be performed without using assumed values for the CV or ACR when sufficient data has been collected. The CV of the acute LC50 data is 0.2 and the CV of the chronic NOEL data is 0.7 for *Ceriodaphnia* survival (Tables 5-7 and 6-1). The chronic CV was determined using the *Ceriodaphnia* survival data only. For simplicity of calculation, and since neither the sampling for the acute and chronic testing was conducted simultaneously nor was the purpose of the testing for permitting, a mean CV of 0.45 was used. Similarly, for the determination of the ACR, the ratio of mean values for the acute (LC50s) and chronic (NOELs for survival) data was used. The ACR is 4 using this procedure. With these two new values Equations 7-4 et seq. can be reevaluated. The acute LTA is 0.59 TU_c and the chronic LTA is 0.86 TU_c.

$$\text{1-hour LTA} = e^{(-0.618 + 0.5 \times 0.1844)} = 0.59\ TU_c$$

where

$$\mu = \ln(0.366 \times 4) - 2.326 \times 0.4294 = -0.618$$

$$\sigma = \sqrt{\ln[(0.45)^2 + 1]} = 0.4294$$

$$\text{4-day LTA} = e^{(-0.239 + 0.5 \times 0.1844)} = 0.86\ TU_c$$

where

$$\mu = \ln(1.32) - 2.326\sqrt{\ln\left[1 + \left(\frac{e^{01844} - 1}{4}\right)\right]} = -0.239$$

This four-day LTA value is converted using Equation 7-7 to obtain a daily value for comparison to the acute LTA. When the chronic LTA is converted, the 0.86 TU_c becomes 0.81 TU_c.

$$LTA_{conv} = e^{(-0.306 + 0.5 \times 0.1844)} = 0.81$$

where

$$\mu = -0.239 - (0.5 \times 0.1844) + 0.5 \times \ln\left[1 + \left(\frac{e^{0.1844} - 1}{4}\right)\right] = -0.306$$

The limiting LTA of 0.59 TU_c was derived from the acute LTA using site-specific data, in contrast to the previous limit calculations which were controlled by the chronic LTA. When the limiting site-specific LTA value was used in the limit derivation process, the maximum daily limit is 1.09 TU_c and the average monthly limit is 0.83 TU_c.

$$\text{Maximum daily limit} = e^{(-0.619 + 1.645 \times 0.4294)} = 1.09\ TU_c$$

where

$$\mu = \ln 0.59 - 0.5 \times 0.1844 = -0.619$$

$$\text{Average monthly limit} = e^{(-0.551 + 1.645 \times 0.222)} = 0.83\ TU_c$$

where

$$\mu = -0.619 + \left(\frac{0.1844 - 0.0494}{2}\right) = -0.551$$

$$\sigma^2 = \ln\left[1 + \left(\frac{e^{0.1844} - 1}{4}\right)\right] = -0.0494$$

Translated into percent effluent values, these results require that the mean daily limit of toxicity is >91 percent effluent (NOEL) and that the mean monthly limit of toxicity is >100 percent effluent (NOEL). As mentioned previously, *Ceriodaphnia* survival was 40 percent at Station 4 which is located downstream of the discharge and beyond the boundary of the mixing zone. Therefore, the

Akron POTW is also not in compliance with the maximum daily or average monthly limit calculated with Akron POTW-specific data.

The abrupt loss of acute toxicity in June 1986 was quite welcome, but did prevent further investigation of the source and identification of the toxicant(s). The last four months of acute toxicity testing for the TSI resulted in *Ceriodaphnia* LC50 values of >100 percent effluent. If these tests had been conducted for monitoring purposes, the results would have been used to determine compliance. However, LC50 values are not directly comparable with permit limits which are expressed in NOELs and must be converted to a chronic measure using the ACR. The converted LC50 represents a NOEL of 10 percent effluent using the U.S. EPA value of 10 for the ACR and a NOEL of 25 percent effluent using the Akron-specific ACR of 4.

Comparing these two NOEL values to the permit limits indicates that the Akron POTW is not in compliance since the limits require NOELs >67 percent effluent. However, an LC50 value above 100 percent cannot be determined with present test procedures which use whole effluent. In order to meet the sample permit limits, the LC50 values would need to be >670 to 1,000 percent effluent. Procedures to concentrate complex effluents, which would not introduce artifacts into the original effluent, are now being developed. U.S. EPA (30) has acknowledged that the detection limit is curently 100 percent effluent, and has recommended monitoring tests (acute or chronic be selected so that results would be within detection limits). Because of the low dilution available to the Akron POTW effluent, the sample maximum daily limit and average monthly limit values require NOEL values which are beyond the level of detection using acute tests. Chronic toxicity tests, which directly yield a NOEL, would be appropriate for compliance monitoring.

7.4 Conclusions

Water quality-based control procedures were used at the Akron POTW to indicate the presence of toxicants in the effluent, determine the ambient toxicity, seek methods to reduce toxicity levels, and then to calculate sample permit limits. Examination of the results of this case study illustrates that:

1. Tier 1 screening indicated that the Akron POTW was a priority candidate for toxicity testing.

2. Tier 2 definitive data generation indicated that there was ambient toxicity and variable whole effluent toxicity downstream of the Akron POTW which abruptly ended in the late spring of 1986.

3. Uncertainty factor calculations indicated that it was not possible for the Akron POTW to satisfy Equation 7-1, so that further testing to reduce the uncertainty factors was not necessary and calculation of permit limits may be made based on available data.

4. *Ceriodaphnia* was the most sensitive test organism.

5. Sample permit limits were derived for two cases: one with values for CV and ACR set by U.S. EPA, and one with site-specific data from the Akron POTW for these parameters. Site-specific values are more appropriate, but can only be used when data are available (in this case research data was used). The limits derived using U.S. EPA's values were based on chronic LTA performance levels while the limits derived using the Akron-specific data were based on acute LTA performance levels.

6. Calculated sample permit limits require NOELs >67 percent effluent. In order for the Akron POTW to comply with these limits, their effluent cannot show any measurable acute toxicity.

The Akron POTW has improved its treatment facilities and processes. New sludge handling facilities have been constructed and have begun operation for the composting of this wastewater treatment byproduct. This reduces the need for sludge storage at the Akron POTW and the volume of material which may contain toxicants for recirculation through the clarifiers. The frequency of bypassing untreated or partially treated wastewater has also been reduced as a result of new treatment systems.

In conjunction with the toxicity testing conducted by U.S. EPA, biological surveys have been conducted annually by Ohio EPA since 1984. Results of those and other surveys indicate that fish and invertebrate communities in the Cuyahoga River have been gradually improving, but an impacted zone still remains downstream of the Akron POTW even after effluent acute toxicity disappeared. The disappearance of the effluent acute toxicity is not known to be permanent since toxicity testing of the effluent was not performed after September 1986. Results from future effluent toxicity testing and surveys of the biota in the Cuyahoga River will detemine whether the toxicity disappearance has been sustained and the effect of its absence on the biota. The control of effluent toxicity does not eliminate the potential for other sources of toxicants to cause adverse biological effects. These areas were beyond the scope of this study. Traditional measures of water quality indicate that the river should be capable of supporting healthy and abundant populations representative of a warm water river. Ohio EPA is continuing the chemical and biological monitoring surveys in the Cuyahoga River to document any improvements. U.S. EPA will also continue monitoring the toxicity of the effluent.

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Glossary

acute - involving a stimulus severe enough to rapidly induce a response; in toxicity tests, a response observed in 96 hours or less typically is considered an acute one. An acute effect is not always measured in terms of lethality; it can measure a variety of effects. Note that acute means **short**, not mortality.

acute-chronic ratio (ACR) - the ratio of the acute toxicity (expressed as an LC50) of an effluent or a toxicant to its chronic toxicity (expressed as an NOEL). Used as a factor for estimating chronic toxicity on the basis of acute toxicity data.

additivity - the characteristic property of a mixture of toxicants which exhibits a cumulative toxic effect equal to the arithmetic sum of the effects of the individual toxicants.

ambient toxicity - toxicity manifested by a sample collected from an aquatic receiving system.

anoxic - without oxygen.

antagonism - the characteristic property of a mixture of toxicants which exhibits a less than additive cumulative toxic effect.

bioavailability - the property of a toxicant that governs its effect on exposed organisms. A reduced bioavailability would have a reduced toxic effect.

chronic - involving a stimulus that lingers or continues for a relatively long period of time, often 1/10 the life span or more. Chronic should be considered a relative term depending on the life span of an organism. A chronic effect can be lethality, growth, reduced reproduction, etc. Chronic means **long**.

conservative pollutant - a pollutant that is persistent and not subject to decay or transformation.

continuous stimulation model - a fate and transport model that uses timeseries input data to predict receiving water quality concentrations in the same chronological order as the input variables.

Criteria Continuous Concentration (CCC) - the U.S. EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable effect.

Criteria Maximum Concentration (CMC) - the U.S. EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed for a brief period of time without causing mortality.

critical life stage - the period of time in an organism's life span when it is the most susceptible to adverse effect caused by exposure to toxicants, usually during early development (egg, embryo, larvae). Chronic toxicity tests are often run on critical life stages to replace long duration, life cycle tests since the toxic effect occur during the critical life stage.

design flow - the critical flow used for steady-state wasteload allocation modeling.

diversity - the number and abundances of species in a specified location.

duration - the period of time over which the instream concentration is averaged for comparison with criteria concentrations. This specification limits the duration of concentrations above the criteria.

effluent biomonitoring - the measurement of the biological effects of effluents (such as toxicity, biostimulation, and bioaccumulation).

LC50 - the toxicant concentration killing 50 percent of exposed organisms at a specific time of observation.

lognormal probabilistic dilution model - a dilution model that calculate the probability distribution of receiving water quality concentrations from the lognormal probability distributions of the input variables.

magnitude - how much of a pollutant (or pollutant parameter such as toxicity), expressed as a concentration or toxic unit, is allowable.

No Observed Effect Level (NOEL) - the highest measured continuous concentration of an effluent or a toxicant which causes no observed effect on a test organism.

1Q10 - the discharge at the 10-year recurrence interval taken from the frequency curve at annual values of the lowest mean daily discharge.

permit averaging period - the duration of time over which a permit limit is calculated—day(s), week, or month.

persistence - that property of a toxicant or an effluent which is a measurement of the duration of its effect. A persistent toxicant or toxicity maintains effect after mixing, degrading slowly. A nonpersistent toxicant or toxicity may have a quickly reduced effect after mixing, as degradation processes such as volatilization, photolysis, etc. transform the chemical.

plug flow sampling - a monitoring procedure that follows the same slug of wastewater throughout its transport in the receiving water. Water quality samples are collected at receiving water stations, tributary inflows, and point source discharges only when a dye slug or tracer passes that point.

probability - a number expressing the likelihood of occurrence of a specific event, such as the ratio of the number of outcomes that will produce a given event to the total number of possible outcomes.

7Q10 - the discharge at the 10-year recurrence interval taken from a frequency curve of annual values of the lowest mean discharge for seven consecutive days.

steady-state model - a fate and transport model that uses constant value of input variables to predict constant values of receiving water quality concentrations.

sublethal - involving stimulus below the level that causes death.

synergism - the characteristic property of a mixture of toxicants which exhibits a greater than additive cumulative toxic effect.

total maximum daily load (TMDL) - the total allowable pollutant load to a receiving water such that any additional loading will produce a violation of water quality standards.

toxic unit acute (TU_a) - the reciprocal of the effluent dilution that causes 50 percent of the test organisms to die by the end of the acute exposure period.

toxic unit chronic (TU_a) - the reciprocal of the effluent dilution that causes no unacceptable effect on the test organisms by the end of the chronic exposure period.

uncertainty factors - factors used in the adjustment of toxicity data to account for unknown variations. Where toxicity is measured on only one test species, other species may exhibit more sensitivity to that effluent. An uncertainty factor would adjust measured toxicity upward and downward to cover the sensitivity range of other, potentially more or less sensitive species.

wasteload allocation (WLA) - the portion of a receiving water's total maximum daily pollutant load that is allocated to one of its existing or future point sources of pollution.

whole effluent toxicity - the aggregate toxic effect of an effluent measured dirctly with a toxicity test.