United States
Environmental Protection
Agency

Environmental Research
Laboratory
Duluth MN 55804

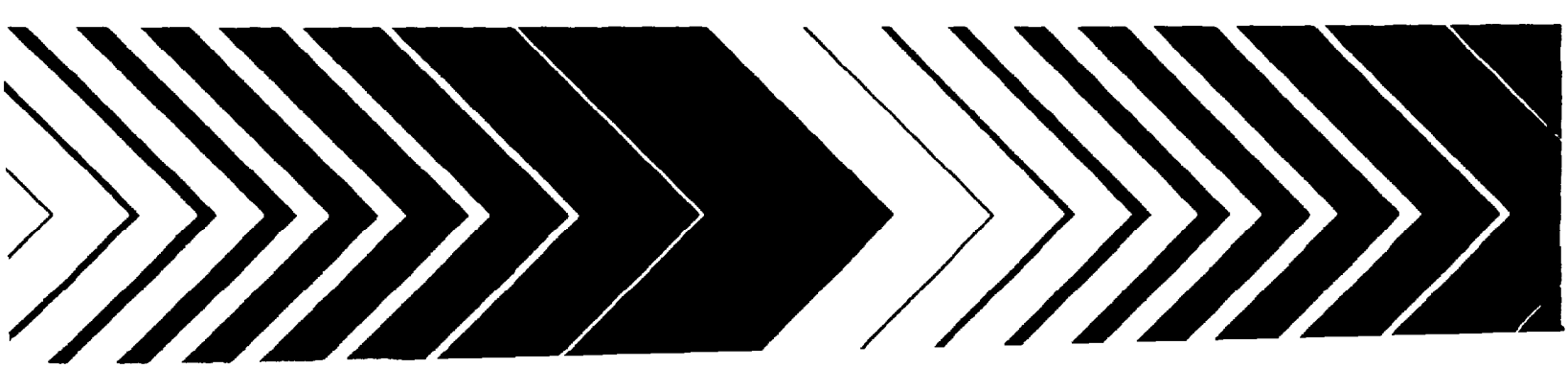
Permits Division
(EN-336)
Washington DC 20460

EPA/600/8-86/001
July 1986
OWEP 86 02

Research and Development

EPA

The Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Back River, Baltimore Harbor, Maryland



EPA/600/8-86/001
July 1986

The Validity of Effluent and Ambient Toxicity Tests for Predicting Biological Impact, Back River, Baltimore Harbor, Maryland

Edited by

Donald I. Mount, Ph.D.[1]
Alexis E. Steen[2]
Teresa Norberg-King[1]

[1]Environmental Research Laboratory
U.S. Environmental Protection Agency
6201 Congdon Blvd.
Duluth, Minnesota 55804

[2]EA Engineering Science, and Technology, Inc.
Hunt Valley/Loveton Center
15 Loveton Circle
Sparks, Maryland 21152

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, MN 55804

Disclaimer

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Foreword

The Complex Effluent Toxicity Testing Program was initiated to support the developing trend toward water quality-based toxicity control in the National Pollutant Discharge Elimination System (NPDES) permit program. It is designed to investigate, under actual discharge situations, the appropriateness and utility of "whole effluent toxicity" testing in the identification, analysis, and control of adverse water quality impact caused by the discharge of toxic effluents.

The four objectives of the Complex Effluent Testing Program are:

1. To investigate the validity of effluent toxicity tests in predicting adverse impact on receiving waters caused by the discharge of toxic effluents.

2. To determine appropriate testing procedures which will support regulatory agencies as they begin to establish water quality-based toxicity control programs.

3. To provide practical case examples of how such testing procedures can be applied in different toxic effluent discharge situations involving discharges to a variety of discharge situations.

4. To field test short-term chronic toxicity tests including the test organisms, *Ceriodaphnia dubia* and *Pimphales promelas*.

Until recently, NPDES permitting has focused on achieving technology-based control levels for toxic and conventional pollutants in which regulatory authorities set permit limits on the basis of national guidelines. Control levels reflected the best treatment technology available, considering technical and economic achievability. Such limits did not, nor were they designed to, protect water quality on a site-specific basis.

The NPDES permits program, in existence for over 10 years, has achieved the goal of implementing technology-based controls. With these controls largely in place, future controls for toxic pollutants will, of necessity, be based on site-specific water quality considerations.

Setting water quality-based controls for toxicity can be accomplished in two ways. The first is the pollutant-specific approach which involves setting limits for single chemicals, based on laboratory-derived no-effect levels. The second is the "whole effluent" approach which involves setting limits using effluent toxicity as a control parameter. There are advantages and disadvantages to both approaches.

The "whole effluent" approach eliminates the need to specify a limit for each of thousands of substances that may be found in an effluent. It also includes all interactions between constituents as well as biological availability.

To date, eight sites involving municipal and industrial dischargers have been investigated. They are, in order of investigation:

1. Scippo Creek, Circleville, Ohio
2. Ottawa River, Lima, Ohio
3. Five Mile Creek, Birmingham, Alabama
4. Skeleton Creek, Enid, Oklahoma
5. Naugatuck River, Waterbury, Connecticut
6. Back River, Baltimore Harbor, Maryland
7. Ohio River, Wheeling, West Virignia
8. Kanawha River, Charleston, West Virginia

This report presents the site study on Back River, Baltimore Harbor, Maryland, which was conducted in March 1984. The study site was an estuary of the Chesapeake Bay and receives discharges including a large POTW discharge.

This report presents the site study on Back River, Baltimore Harbor, Maryland, issuance or enforcement activities.

Rick Brandes
Permits Division

Nelson Thomas
ERL/Duluth

Project Officers
Complex Effluent Toxicity
Testing Program

Contents

Page

Foreword iii

List of Figures vii

List of Tables ix

Acknowledgments xii

List of Contributors xiii

Executive Summary xiv

Quality Assurance xv

1. Introduction 1-1

2. Study Design 2-1
   - 2.1 Toxicity Testing Study Design 2-1
   - 2.2 Hydrological Survey Study Design 2-1
   - 2.3 Biological Survey Study Design 2-1
   - 2.4 Integration of Laboratory and Field Efforts 2-1
   - 2.5 Research on Effluent Fractionation 2-1

3. Site Description 3-1

4. Toxicity of Effluents and Receiving Water 4-1
   - 4.1 Chemical and Physical Test Conditions 4-1
   - 4.2 Results of Fathead Minnow Growth Tests 4-1
   - 4.3 Results of *Ceriodaphnia* Reproduction Potential Tests 4-4
   - 4.4 Results of the Microtox® Tests 4-6
   - 4.5 Summary of Toxicity Data 4-7

5. Hydrological Studies of Patapsco River 5-1
   - 5.1 Dilution Analysis of the Patapsco POTW 5-1
   - 5.2 Evaluation of Hydrological Conditions of the Patapsco River ... 5-1

6. Hydrological Studies of Back River 6-1
   - 6.1 Dilution Analysis of the Back River POTW 6-1
   - 6.2 Hydrological Modeling of Back River 6-1
   - 6.3 Evaluation of Hydrological Conditions of the Back River and Middle River 6-1

7. Macrozooplankton/Ichthyoplankton of Back River and Middle River 7-1
   - 7.1 Community Structure 7-1
     - 7.1.1 Macrozooplankton 7-1
     - 7.1.2 Ichthyoplankton 7.1
   - 7.2 Differences Between Stations in Key Macrozooplankton Taxa 7-1
   - 7.3 Evaluation of the Macrozooplankton Community 7-3

8. Benthic Macroinvertebrates of Back River and Middle River 8-1
   - 8.1 Community Structure 8-1
   - 8.2 Spatial Trends in Key Taxa 8-1
   - 8.3 Evaluation of the Benthos Community 8-3

Contents (cont'd)

		Page
9.	Fish Community Survey	9-1
	9.1 Community Structure	9-1
	9.2 Fish Condition	9-1
	9.3 Evaluation of the Fish Community	9-3
10.	Comparison of Laboratory Toxicity Data and Receiving Water Biological Impact	10-1
11.	Effluent Fractionation Testing	11-1
	11.1 Fractionation Test Results	11-1
	11.1.1 *Ceriodaphnia* 48-Hour Acute Tests	11-1
	11.1.2 Microtox® Tests	11-2
	11.1.3 Chemical Analyses of Toxic Fractions	11-3
	11.2 Summary	11-3
References		R-1
Appendix A:	Toxicity Tests and Analytical Methods	A-1
	A.1 Sampling and Sample Preparation	A-1
	A.2 *Ceriodaphnia* Test Methods	A-1
	A.3 Fathead Minnow Test Method	A-2
	A.4 *Ceriodaphnia* Statistical Analysis	A-2
	A.5 Fathead Minnow Statistical Analysis	A-2
	A.6 Microtox® Testing Methods	A-2
Appendix B:	Hydrological Sampling and Analytical Methods	B-1
	B.1 Patapsco River Survey	B-1
	B.2 Back River and Middle River	B-1
Appendix C:	Biological Survey Sampling and Analytical Methods	C-1
	C.1 Plankton Survey	C-1
	C.2 Benthic Macroinvertebrate Survey	C-1
	C.3 Fish Survey	C-1
Appendix D:	Effluent Fractionation and Toxicity Testing Methods	D-1
	D.1 Sampling	D-1
	D.2 *Ceriodaphnia* Culture, Maintenance and Testing	D-1
	D.3 Microtox®	D-1
	D.4 Chemical Fractionation	D-1
Appendix E:	Toxicity Test Data	E-1
Appendix F:	Biological Data	F-1
Appendix G:	Support Chemical Fractionation Data	G-1

List of Figures

Number	Title	Page
3-1	Study area showing the two wastewater treatment plants and the biological sampling stations in Back River, Middle River, and Patapsco River	3-1
5-1	Surface dilution contours at the Patapsco POTW, 1103 through 1217 hours, 22 March 1984	5-1
5-2	Surface dilution contours of the Patpasco POTW, 1238 through 1417 hours, 22 March 1984. Also shown are locations of vertical sampling stations	5-1
6-1	Dye concentrations in the Back River as observed and predicted by the numerical model. Dye injection started at hour 62	6-2
6-2	Dye concentration in the Back River as predicted by the numerical model for simulated dye release beginning 1 March	6-2
7-1	Spatial trends of macrozooplankton community parameters, March 1984	7-2
8-1	Spatial trends of benthic community parameters	8-3
9-1	Spatial comparison of fish catches in Back River and Middle River on two days in March 1984	9-3
11-1	Schematic results of *Ceriodaphnia* acute tests on effluent fractions	11-2
11-2	Schematic results of Microtox® tests on effluent fractions	11-3
B-1	Map showing the Back River segmentation scheme and water sampling locations	B-2
D-1	Fractionation and testing procedure	D-2
G-1	Base/neutrals standard reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis	G-8
G-2	Surrogate spike standard reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis	G-9
G-3	Blank reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis	G-10
G-4	Reconstructed ion chromatogram for 3-day Patapsco POTW base/neutral fraction effluent analysis	G-11
G-5	Library search for possible compound from 3-day Patapsco POTW base/neutral fraction effluent analysis	G-12
G-6	Reconstruction ion chromatogram for 7-day Patapsco base/neutral fraction effluent analysis	G-13

List of Figures (cont'd)

Number	Title	Page
G-7	DFTPP reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis	G-14
G-8	DFTPP mass spectrum for 3-day and 7-day Patapsco base/neutral POTW fraction effluent analysis	G-15

List of Tables

Number	Title	Page
4-1	Seven-Day Percent Survival of Larval Fathead Minnows Exposed to Various Concentrations of Effluents in Reconstituted Water, Baltimore Harbor, Maryland	4-2
4-2	Mean Weight of Larval Fathead Minnows Exposed to Various Concentrations of Effluents in Reconstituted Water, Baltimore Harbor, Maryland	4-2
4-3	Seven-Day Percent Survival of Larval Fathead Minnows Exposed to Waters from Various Stream Stations for Ambient Toxicity, Baltimore Harbor, Maryland	4-3
4-4	Mean Weight of Larval Fathead Minnows Exposed to Waters from Various Stations for Ambient Toxicity, Baltimore Harbor, Maryland	4-3
4-5	Seven-Day Mean Percent Survival and Weight of Larval Fathead Minnows for Salinity Test at Baltimore Harbor, Maryland	4-4
4-6	Daily and Mean Salinity at Back River Stations, Baltimore Harbor, Maryland	4-4
4-7	Daily and Seven-Day Effluent Concentrations at Back River Stations, Baltimore Harbor, Maryland	4-5
4-8	Reproduction and Survival of *Ceriodaphnia dubia* for the Patapsco POTW Effluent and the Patapsco and Middle Rivers Ambient Stations, Baltimore Harbor, Maryland	4-5
4-9	Daily Survival and Mean Young Production of *Ceriodaphnia dubia* in Various Dilutions of Back River POTW Effluent, Baltimore Harbor, Maryland	4-5
4-10	Daily Survival and Mean Young Production of *Ceriodaphnia dubia* in Back River Ambient Station Water, Baltimore Harbor, Maryland	4-6
4-11	Daily Survival and Mean Number of Young Per Female in the Salinity Test, Baltimore Harbor, Maryland	4-6
4-12	EC50 Values for Microtox® Test for the Two POTW Effluents, Baltimore Harbor, Maryland	4-6
4-13	15-Minute Percent Light Reduction for 91 Percent Back River Ambient Samples, Baltimore Harbor, Maryland	4-7
5-1	Vertical Measurements of Dilution at Stations Surrounding Patapsco POTW Discharge, Baltimore Harbor, March 1984	5-2
6-1	Surface Water Quality Data for Back River and Middle River Stations from 9 March 1984 Through 16 March 1984	6-3
7-1	Abundance and Percent Composition of the Macrozooplankton Community of Back River and Middle River, 12 March 1984	7-1

List of Tables (cont'd)

Number	Title	Page
7-2	Abundance and Percent Composition of the Macrozooplankton Community of Back River and Middle River, 16 March 1984	7-1
7-3	Composition of the Macrozooplankton Community of Back River and Middle River, 12 and 16 March 1984	7-2
8-1	Abundance of Benthic Macroinvertebrates Collected from Back River and Middle River, 19 March 1984	8-2
8-2	Composition of Benthic Community of Back River and Middle River, 19 March 1984	8-3
8-3	Shannon-Wiener Diversity Indices Associated Evenness and Redundancy Values, and Community Loss Index Calculated on Benthic Data from Back River and Middle River, 19 March 1984	8-4
9-1	Fish Catch and Water Quality Parameters, in Back River and Middle River, 7 March 1984	9-2
9-2	Fish Catch and Water Quality Parameters, in Back River and Middle River, 14 March 1984	9-2
9-3	Observations of Abnormalities by Species in Back River and Middle River, 7 March 1984	9-4
9-4	Observations of Abnormalities by Species in Back River and Middle River, 14 March 1984	9-4
11-1	LC50 Values Calculated by Moving Average Method, Based on *Ceriodaphnia dubia* 48-Hour Acute Tests	11-1
11-2	EC50 Values Based on Beckman Microtox® Acute Tests	11-3
11-3	Levels of Pesticides, Herbicides, and PCBs in 3-Day and 7-Day Composite Patapsco POTW Effluents	11-4
11-4	Levels of Base Neutral Compounds, Determined by GC-MS Analysis (EPA Method 625), for 3 Day and 7-Day Patapsco POTW Effluents	11-4
B-1	Dye Plume Mappings	B-3
D-1	Formulation for Moderately Hard Reconstituted Water and Final Water Quality Ranges	D-1
E-1	Routine Chemistry Data for the Ambient Tests, Baltimore Harbor, Maryland	E-1
E-2	Routine Chemistry Data for the Effluent Dilution and Salinity Test	E-1
E-3	Final Dissolved Oxygen Levels for *Ceriodaphnia dubia* Effluent, Ambient, and Salinity Tests, Baltimore Harbor, Maryland	E-2
F-1	Results of a X^2 Test Performed on the Number of Macrozooplankton Taxa, Back River, March 1984	F-1
F-2	Abundance of Macrozooplankton Collected from Back River and Middle River, 12 March 1984	F-1
F-3	Abundance of Macrozooplankton Collected from Back River and Middle River, 16 March 1984	F-2

List of Tables (cont'd)

Number	Title	Page
F-4	Analysis of Variance and Tukey's Studentized Range Test Results for *Eurytemora affinis*, Back River, March 1984	F-2
F-5	Water Quality Data from Back River and Middle River, 12 and 16 March 1984	F-3
F-6	Results of a X^2 Test Performed on the Number of Benthic Macroinvertebrate Taxa, Back River, March 1984	F-4
F-7	Water Quality Data from Back River and Middle River, 16 March 1984	F-4
F-8	Results of a X^2 Test Performed on the Number of Fish Taxa, Back River, March 1984	F-4
F-9	Trends in Abnormalities Observed Among Brown Bullheads Collected in Back River and Middle River, 7 March 1984	F-5
F-10	Trends in Abnormalities Observed Among Brown Bullheads Collected in Back River and Middle River, 14 March 1984	F-5
F-11	Trends in Abnormalities Observed Among White Perch Collected in Back River and Middle River, 7 March 1984	F-6
F-12	Trends in Abnormalities Observed Among Pumpkinseed and White Perch Collected in Back River and Middle River, 14 March 1984	F-5
F-13	List of Fish Species and Families Collected, Back River and Middle River, March 1984	F-7
G-1	*Ceriodaphnia dubia* Mortality in 48-Hour LC50 Tests on Back River and Patapsco POTW	G-2
G-2	Base/Neutral Standard Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis	G-3
G-3	Surrogate Spike Standard Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis	G-3
G-4	Blank Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis	G-4
G-5	Spike Blank Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis	G-4
G-6	Quantitation Report for 3-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis	G-5
G-7	Quantitation Report for 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis	G-6
G-8	Mass List for DFTPP Analysis on 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent	G-7

Acknowledgments

The assistance of Robert Donaghy and Robert Green from EPA Region III in conducting the onsite Microtox® tests was appreciated. The aid of Floyd Boettcher, Environmental Research Laboratory—Duluth, as field engineer is gratefully acknowledged.

List of Contributors

LABORATORY TOXICITY TESTS
Donald I. Mount[1], Scott E. Heinritz[1], and Jeffrey S. Denny[1]

HYDROLOGICAL SURVEY
Stephen R. Rives[2]

PLANKTON SURVEYS
Richard A. Connelly[2] and Michael T. Barbour[2]

BENTHIC MACROINVERTEBRATE SURVEY
Richard A. Connelly[2] and Michael T. Barbour[2]

FISH SURVEYS
Michael D. Schmitt[2]

COMPARISION OF LABORATORY TOXICITY DATA AND RECEIVING WATER BIOLOGICAL IMPACT
Donald A. Mount[1]

EFFLUENT FRACTIONATION TESTING
Wayne C. McCulloch[2] and Stephen E. Storms[2]

PRINCIPAL INVESTIGATOR
Donald I. Mount, Ph.D.[1]

[1]U.S. Environmental Protection Agency. Environmental Research Laboratory—Duluth, 6201 Congdon Blvd., Duluth, MN 55804.
[2]EA Engineering, Science, and Technology, Inc. Hunt Valley/Loveton Center, 15 Loveton Circle, Sparks, MD 21152.

Executive Summary

The toxicity of freshwater effluents discharged to brackish waters are difficult to assess because of the role of salinity. If high concentrations of effluents are of concern, then freshwater organisms are better since salinity will be low. If low concentrations are of concern then brackish water species are better for testing.

The purpose of this study was to measure the toxicity of effluents discharged to an estuary using freshwater test species and compare the predictions with the receiving water biological impact. In addition, ambient tests were done in conjunction with salinity tolerance tests to compare the agreement between the effluent toxicity tests and the ambient toxicity where salinity itself was not beyond acceptable ranges. Acceptable salinity was based on the concurrent salinity tests. A marine bacterium species was also tested in which the standard method requires salinity adjustment of the test solution so that salinity stress is not involved.

The main purpose for the study could not be pursued because the number of species in the estuary study was too small to use for comparisons. However, the effluent tests could be compared to ambient tests to see how well the effluent toxicity test predictions agreed with measured ambient toxicity.

The ambient and effluent toxicity data for daphnids agreed at all stations. Four of six stations were correctly predicted by the fathead effluent toxicity data but the Microtox® data for effluent and ambient toxicity did not agree. This may have been a result of decay of chlorine toxicity in the ambient samples. Salinity in the ambient samples had less effect than was predicted from the salinity tolerance tests.

Considering the confounding factors that existed, the agreement between effluent and ambient toxicity is considered good.

Quality Assurance

Coordination of the various studies was completed by the principal investigator preceding and during the onsite work. A reconnaissance trip was made to the site before the study and necessary details regarding transfer of samples, specific sampling sites, dates of collections, and measurements to be made on each sample were delineated. The mobile laboratory was established as the center for resolving problems and adjusting work schedules as delays or weather affected the completion of the study plans. The principal investigator was responsible for all Quality Assurance-related decisions onsite.

All instruments were calibrated by the methods specified by the manufacturers. For sampling and toxicity testing, the protocols described in the referenced published reports were followed. Where identical measurements were made in the field and laboratory, both instruments were cross-calibrated for consistency.

1. Introduction

One of the most difficult discharge situations occurs where freshwater effluents are discharged into saline water. Saltwater organisms are stressed by the freshwater effluent and freshwater organisms are stressed by the saline dilution water making an accurate measurement of impact difficult . Whether freshwater or brackish water organisms should be used for testing usually depends on the toxicity of the effluent. If the effluent is highly toxic the critical mixtures of dilution water and effluent will have salinities approaching those of the dilution water and brackish water species would be most appropriate. If, on the other hand, the effluent is of low toxicity, critical concentrations of effluent will be largely effluent and salinities will approach those of the effluent. In this case, freshwater organisms would be better test species.

The main approach intended in this study was to use freshwater test species for effluent tests and compare the results from those tests to the impact occurring in the estuary to see if the toxicity so measured was a valid estimate of effect for brackish water species. Ambient tests on freshwater species were to be used to the extent that salinity was within the tolerance of species. The specific tolerance of the lots of test species was to be determined simultaneously with the effluent and ambient tests.

Because in Microtox® testing, the test solution is adjusted to a suitable salinity, this test seemed to offer a "bridge" between the freshwater and brackish water species. Therefore, Microtox® testing was included as one of the toxicity tests.

This study site was the Back River and Patapsco River in Maryland. One publicly owned treatment works (POTW) was located on each river within the study area.

This report is organized into sections corresponding to the project tasks. Following an overview of the study design and a summary of the description of the site, the chapters are arranged into toxicity testing, hydrology, and ecological surveys. An integration of the laboratory and field studies is presented in Chapter 10. Special research study results are presented in Chapter 11 on effluent fractionation testing. All methods and other support data are included in the appendixes.

2. Study Design

The primary emphasis of this site study was the Back River POTW and the Back River estuary. Another POTW located on the Patapsco River, was also tested. Study components included 7-day *Ceriodaphnia dubia* toxicity tests, 7-day larval growth tests for fathead minnows and Microtox® using a luminescent marine bacterium, *Photobacteria phosphoreum*. Both effluents and ambient samples were tested. A hydrological survey of the Patapsco, Middle, and Back Rivers for time-of-travel of the effluent was completed and biological sampling of the macrozooplankton, ichthyoplankton, benthic macroinvertebrate and fish communities was done.

Difficulties were encountered in the field which prevented completion of all the tasks on the Patapsco River. A series of ambient stations for toxicity tests were established but a mechanical problem with the boat used for sampling made river bank sampling necessary. Further, the failure to get permission to sample from the bank at some places resulted in very inadequate station locations. The salinity at stations where sampling was conducted was too high to use freshwater organisms.

2.1 Toxicity Testing Study Design

Toxicity tests were performed on the two effluents to measure subchronic effects on the survival and growth of larval fathead minnows and survival and chronic reproductive effects on *Ceriodaphnia dubia* (Chapter 4). A wide range of effluent concentrations was used so that acute mortality as well as chronic effects could be measured. The objective of these tests was to estimate the minimum concentration of each effluent that would cause acute mortality or chronic effects. In addition, a salinity test was conducted to determine the salinity tolerance of the test organisms.

The Microtox® test was performed on effluent and ambient samples. The test is based on the ability of a toxicant to reduce the luminescence of a bacterium.

In addition to the effluent tests, ambient river stations were selected on Back River from above the discharge downstream to the confluence with the Chesapeake Bay. Samples were also collected in the Middle and Patapsco Rivers. Samples collected from these stations were used to measure ambient toxicity to *Ceriodaphnia dubia*, fathead minnows and Microtox®. These tests measured the loss of toxicity from the effluents after mixing, dilution from other inputs, degradation, and other losses such as sorbtion. These test results would also provide data for the prediction of ecological impact for comparison with the biological survey data, without having to know the effluent concentration.

2.2 Hydrological Survey Study Design

The hydrological measurements were conducted in the Patapsco River, Middle River, and Back River by dye studies at the two wastewater treatment plants (Chapters 5 and 6). By modeling downstream dilution contours for each effluent, the exposure concentrations at various stations could be established. Tide measurements were also made for the Back River.

2.3 Biological Survey Study Design

The field surveys included a quantitative assessment of the macrozooplankton, ichthyoplankton, benthic macroinvertebrates, and fish communities. Planktonic communities in lotic systems drift with the tides so they do not necessarily reflect exposure at the collection site whereas the benthic community is not nearly as mobile. Fish being quite mobile, also may be caught in locations where they may spend very little time.

Because an above normal incidence of tumors had been reported in fish from the study area, the fish captured in the survey were examined for gross abnormalities.

2.4 Integration of Laboratory and Field Efforts

The intent of the study was to compare the toxicity test predictions to biological response in the estuary. Due to an unusually cool period of weather preceding the site study which delayed the fish spawning, the number of species of ichthyoplankton was so small that only subjective comparisons could be made.

2.5 Research on Effluent Fractionation

The objective of the fractionation study was to identify the toxic components of the effluents through frac-

tionation, toxicity testing, and chemical analyses.

Particularly for POTW effluents as distinguished from industrial effluents, pretreatment is often the best way to reduce effluent toxicity thus the cause of the toxicity is needed to use this approach. The purpose was to develop methods for toxicity identification.

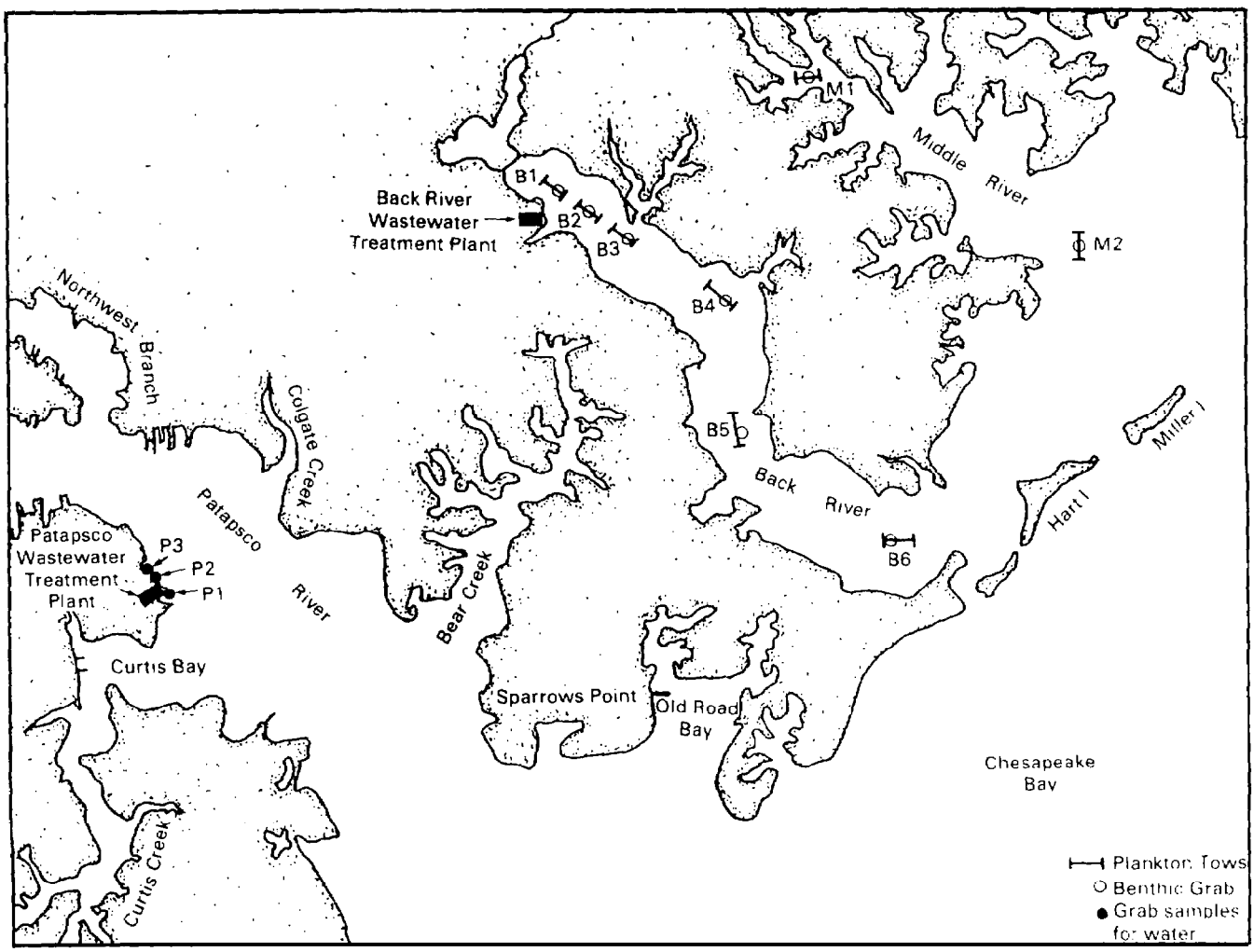
3. Site Description

Back River is tidally influenced and empties into the Chesapeake Bay 5.6 km north of the Patapsco River (Figure 3-1). The Back River POTW is the principal discharger and contributed approximately 79 percent of the river flow during the month of March 1984. The Back River POTW is located 10.3 km from the mouth of the Back River and receives waste from both industrial and residential sources. The design flow of Back River POTW is 100 mgd. A proportion of the effluent is shunted on demand to a nearby steel mill (which does not discharge to Back River) for cooling water. Therefore, discharge from the POTW to Back River may fluctuate considerably. During the study period of March 1984, the discharge from the POTW averaged between 67 and 209 mgd.

The study in Back River encompased 10.3 km and extended from the plant to the mouth of the river. Sampling stations were:

- Station B1—located at Sandy Point upstream of Bread and Butter Creek about 10.3 km from the river mouth. Water depth was 1.5 m during ebb

Figure 3-1. Study area showing the two wastewater treatment plants and the biological sampling stations in Back River, Middle River, and Patapsco River.



tide. Sediment was gray/black silt.

- Station B2—located at Norristown landfill and Cox Point about 9 km from the mouth of the river. Depth was 1 m during ebb tide. Sediment was black silt.
- Station B3—near Deep Creek about 7.9 km from the river mouth. Depth was 2 m during ebb tide. Sediment was gray/black silt.
- Station B4—upstream from Muddy Gut and surrounded by undeveloped land. Distance from the mouth of the river is 6.3 km. Water depth was 2 m during ebb tide. Sediment was gray/black silt with some detritus.
- Station B5—about 17 m to the right of channel marker N 10 (red), located approximately 3.4 km from the mouth. Depth was 3 m during ebb tide. Sediment was gray/black silt with some clay in the surface layer.
- Station B6—at the river mouth. Depth was 3 m during ebb tide. Sediment was gray silt with some sand
- Station M1—located in Middle River at the confluence with Dark Head Creek. Station M1 is 6.2 km from the mouth of Middle River. Water depth was 3 m during flood tide. Sediment was gray silt.
- Station M2—at the mouth of Middle River near channel marker R4. Water depth was 4 m during high slack tide. Sediment was black/brown silt with some sand and many clam shells.
- Station P1—located at the Patapsco POTW at the end of the dock. This location is in the Patapsco River near the entrance to Curtis Bay.
- Station P2—located at the Trans Maryland Terminal at the end of the dock.
- Station P3—located at the terminus of Chesapeake Avenue at the Patapsco River.

4. Toxicity of Effluents and Receiving Water

Toxicity tests were conducted on three species, a daphnid (*Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), and a bacterium (*Photobacteria phosphoreum*). Testing was conducted on the Patapsco and Back River POTW effluents and ambient stations from Middle, Back, and Patapsco Rivers. Where effluent concentration of the ambient test samples are known, the data from the effluent dilution tests and the ambient tests can be compared to see how well the effluent dilution test would predict toxicity occurring at the ambient stations. The ambient test data can be compared to the biological survey data to see how well the receiving water impact was predicted by the toxicity tests.

Because the study area was brackish water, a salinity test was completed on the two freshwater species to enable the effects of brackish water on toxicity to be estimated. Since the Microtox® test utilizes a marine bacterium, the standard protocol requires the sample to be adjusted for salinity, so a salinity test was not needed. However, the addition of salinity to the samples could possibly alter the toxicity measured.

The methods used for the three tests, as well as the details of the sampling, handling, and statistical analyses are given in Appendix A. Routine chemistry data is presented in Appendix E.

4.1 Chemical and Physical Test Conditions

The *Ceriodaphnia* were maintained in constant temperature cabinets at 25 ± 1°C. The mobile lab temperature ranged from 22-26°C, but because the fathead minnow test chambers were distributed over three shelf levels, the temperature varied due to air stratification. A reconstituted water control was located at every level and the control values were not pooled for statistical analysis. Because of this design, the control data for each level was used for comparison to the exposure concentrations for each respective level. The bacterial tests were all done at 15°C.

Tables E-1 and E-2 contain the chemistry data for initial pH, dissolved oxygen (DO), conductivity, and salinity plus the final DO values for the fathead minnow tests. The final DO values for the *Ceriodaphnia* tests are contained in Table E-3. Since the exposure concentrations were made for the *Ceriodaphnia* and fathead minnows as one sample, the initial values are the same for both species. The initial DO values are all near saturation. Temperatures of the effluent and ambient samples ranged from 5 to 12°C as they arrived at the mobile laboratory. After warming to test temperature (25°C), the samples had to be aerated to reduce super saturation. Although the final mean DO values for the fathead minnows are all above 5.0 mg/L, individual daily values fell as low as 2.3 mg/L. Most of these low values occurred on day two or day three of the test. Upon finding such values, the volume of test solution added daily was reduced from 2 to 1 L, which resulted in higher final DO values. Since this study was completed, other sites with water having a high BOD and the DO below 1.0 mg/L have been encountered. In this later study, fathead minnows had higher average weights than in previous studies (Mount and Norberg King, 1986). An assessment of this situation had led to the conclusion that the DO measurements taken by the oxygen probe do not reflect the micro-environmental conditions in which the fathead minnows are living. Fathead minnows were observed to move to a position near the surface of the water where, in all probability, the oxygen concentration is much higher than that measured by the probe. Such growth at such low measured DO concentrations would not be expected. Apparently, the behavior of the fish causing them to stay near the surface when DO is low, makes the test nearly independent of low DO effects.

The pH values changed little from initial to final; therefore, final pH readings were not made after the first two days. None of the initial pH values were less than or greater than 0.5 pH units of the culture pH values and thus did not warrant gradual transition of the test animals. The effluents were all fresh water, but in the ambient samples, particularly the Patapsco River ambient samples, salinity was high (8 ppt) and caused stress to the test animals.

4.2 Results of Fathead Minnow Growth Tests

No comparisons of Patapsco POTW effluent and/or toxicity test and Patapsco River ambient station

data will be made due to the high salinity values (8 ppt, Table E-1), which interfered with interpretation of the toxicity data. Samples were to be collected at designated deep-water areas; however, due to boat problems, the ambient stations were nearshore and the estimated effluent concentrations were not measured.

Tables 4-1 and 4-2 contain the fathead minnow survival and growth data for the Patapsco and Back River POTWs. Both effluents were diluted with reconstituted water, as the receiving water quality was influenced by the tide and may contain the discharged effluent which moves upstream and downstream in the tidal range. Survival and growth were different from the reconstituted-water control in the Patapsco POTW effluent only at 100 percent. In the Back River POTW effluent, survival was only affected at 100 percent, but growth was significantly reduced at 30 and 100 percent effluent. The 1 and 3 percent concentrations resulted in higher weight values than the controls, and weight at the 3 percent effluent was significantly higher ($P \leq 0.05$) than the control value. The calculated Acceptable Effluent Concentration (AEC) (geometric mean of 30 and 10 percent) is 17.3 percent. This value is subject to substantial error because of the interval between exposure concentrations in these tests, which followed a logarithmic dilution series.

Tables 4-3 and 4-4 contain the fathead minnow data for all ambient stations; the stations were compared to the appropriate reconstituted water control (Section 4-1 discusses control exposures). In the Back River ambient stations, only Station B1 had significantly lower survival ($P \leq 0.05$), and only Station B2 had significantly lower growth ($P \leq 0.05$). Significantly higher mortality ($P \leq 0.05$) occurred at all Patapsco ambient stations, although there was no inhibition of growth of those that survived.

Table 4-5 shows the effect of salinity (salinity test water was derived from high quality sea water diluted with reconstituted fresh water) on fathead minnows. In that salinity test, survival was significantly lower at concentrations of 16 ppt down to 4 ppt, whereas

Table 4-1. Seven-Day Percent Survival of Larval Fathead Minnows Exposed to Various Concentrations of Effluents in Reconstituted Water, Baltimore Harbor, Maryland

	Percent Effluent (v/v)					
	100	30	10	3	1	Control
Patapsco POTW						
Replicate A	0	100	100	80	100	100
Replicate B	0	100	100	100	90	90
Replicate C	0	90	90	100	90	100
Replicate D	0	90	80	100	100	100
Mean	0[a]	95	93	98	95	98
Back River POTW						
Replicate A	0	80	100	100	100	100
Replicate B	0	100	100	90	100	100
Replicate C	0	90	90	90	100	100
Replicate D	0	90	90	90	90	90
Mean	0[a]	90	95	93	98	98

[a] Significantly lower than the reconstituted water control ($P \leq 0.05$).

Table 4-2. Mean Weight (mg) of Larval Fathead Minnows Exposed to Various Concentrations of Effluents in Reconstituted Water, Baltimore Harbor, Maryland

	Percent Effluent (v/v)					
	100	30	10	3	1	Control
Patapsco POTW						
Replicate A	--	0.425	0.433	0.444	0.435	0.385
Replicate B	--	0.480	0.480	0.475	0.517	0.511
Replicate C	--	0.472	0.400	0.436	0.378	0.410
Replicate D	--	0.272	0.388	0.388	0.365	0.375
Weighted Mean	--[a]	0.414	0.428	0.435	0.423	0.418
SE	--	0.032	0.032	0.032	0.032	0.032
Back River POTW						
Replicate A	--	0.288	0.435	0.475	0.406	0.435
Replicate B	--	0.328	0.470	0.573	0.480	0.429
Replicate C	--	0.306	0.361	0.500	0.420	0.378
Replicate D	--	0.233	0.350	0.444	0.394	0.356
Weighted Mean	--[a]	0.290[a]	0.407	0.497	0.424	0.399
SE	--	0.024	0.023	0.024	0.023	0.023

[a] Significantly lower than the reconstituted-water control ($P \leq 0.05$).

Table 4-3. Seven-Day Percent Survival of Larval Fathead Minnows Exposed to Waters from Various Stream Stations for Ambient Toxicity, Baltimore Harbor, Maryland

Ambient Station	Stream Station					
Patapsco River	P1	P2	P3			
Replicate A	50	50	20			
Replicate B	40	20	70			
Replicate C	30	40	10			
Replicate D	30	20	10			
Mean	38[a]	33[a]	28[a]			
Back River	B1	B2	B3	B4	B5	B6
Replicate A	80	100	90	90	90	100
Replicate B	80	70	90	80	90	100
Replicate C	80	90	70	60	80	90
Replicate D	70	80	70	90	80	50
Mean	78[a]	85	80	80	85	85
Middle River	M1[b]	M2				
Replicate A	90	100				
Replicate B	90	100				
Replicate C	90	90				
Replicate D	80	90				
Mean	88[b]	95				

[a]Significantly lower than the reconstituted-water control for Back River effluent control. Table 4-1.
[b]Results shown cover a 6-day test period due to weather conditions.

growth was significantly lower only at concentrations of 12 and 16 ppt, and not at 8 ppt. Table E-1 shows the average salinity of the Patapsco ambient stations was around 8 ppt, which would suggest that the fathead minnow mortality could have been due to salinity levels totally. Since the average salinities at all Back River stations and Middle River stations (Table E-1) were 1.5 ppt or less, no adverse salinity effect should have occurred in those samples.

Table 4-7 gives the daily 7-day mean effluent concentrations in Back River as measured by the dye studies (Chapter 7). Mean effluent concentrations diminished from around 28 percent at Station B1 to 18 percent at Station B6, except for Station B4 where the mean was higher than at any other station. For Station B4, if the one daily high value of 74 percent is excluded, then the mean is 29 percent, very close to B1 and B2 values. The calculated AEC of the Back River POTW effluent was 17 percent. The effluent concentrations at Stations B3, B5, and B6 are only slightly higher than the AEC so measurable effects are unlikely and none were found. An effect was measured at Station B2, leaving only Stations B1 and B4 where effects were expected but not found. Given the possible error in calculating the AEC, the aging of the effluent after discharge and possible loss of toxicity, and the variable daily concentrations (as opposed to the constant exposures in the effluent test), one should consider the agreement reasonable.

Table 4-4. Mean Weight (mg) of Larval Fathead Minnows Exposed to Waters from Various Stations for Ambient Toxicity, Baltimore Harbor, Maryland

Ambient Station	Station					
Patapsco River	P1	P2	P3			
Replicate A	0.320	0.480	0.200			
Replicate B	0.513	0.650	0.564			
Replicate C	0.283	0.288	--			
Replicate D	0.283	0.325	0.250			
Weighted Mean	0.357	0.423	0.460			
SE	0.063	0.067	0.077			
Back River	B1	B2	B3	B4	B5	B6
Replicate A	0.394	0.291	0.378	0.411	0.478	0.305
Replicate B	0.350	0.350	0.344	0.419	0.417	0.375
Replicate C	0.369	0.288	0.307	0.358	0.431	0.417
Replicate D	0.300	0.306	0.236	0.317	0.419	0.520
Weighted Mean	0.355	0.306[a]	0.322	0.377	0.437	0.387
SE	0.026	0.025	0.025	0.025	0.025	0.025
Middle River	M1[b]	M2				
Replicate A	0.406	0.375				
Replicate B	0.483	0.585				
Replicate C	0.467	0.472				
Replicate D	0.400	0.383				
Weighted Mean	0.440	0.455				
SE	0.034	0.033				

[a]Significantly lower than the reconstituted-water control for Back River effluent control. Table 4-2.
[b]Results shown cover a 6-day test period due to weather conditions.

4.3 Results of *Ceriodaphnia* Reproduction Potential Tests

Table 4-8 contains the *Ceriodaphnia dubia* reproductive and survival data for the Patapsco POTW effluent and the Patapsco and Middle River ambient samples. The range of effluent concentrations initially selected of 1-100 percent for the Patapsco POTW effluent was too high, and additional test concentrations were set up which ranged from 3 percent as a high to 0.37 percent as a low. The 0.75 percent concentration was significantly lower ($P \leq 0.05$) than the control for both survival and reproduction. The calculated AEC is 0.53 percent (which is the geometric mean of 0.37 and 0.75).

Ceriodaphnia died quickly in all samples from the Patapsco River ambient stations (Table 4-8). Table E-1 reports the salinity of these stations to be about 8 ppt, which is enough to have caused the observed response. Reproduction and survival were normal in the Middle River samples.

The data for the Back River POTW effluent with cumulative survival for each day is shown in Table 4-9. Both survival and young production were significantly lower ($P \leq 0.05$) at 100, 30, and 10 percent concentrations, but not at 3 or 1 percent exposures. The calculated AEC is 5.5 percent.

Table 4-10 contains the reproductive and daily survival data for the Back River ambient stations. Survival was significantly ($P \leq 0.05$) lower at all stations, as was reproduction except at B6. No dilutions were made of these samples but some estimate of differences in relative toxicity can be obtained by looking at daily survival. Based on survival, Stations B2, B3, and B4 were most toxic; Stations B1 and B5 were similar to each other and less toxic than Stations B2, B3, and B4; and Station B6 was least toxic.

Reference to Table 4-11 shows that even at salinity levels of 0.25 ppt young production would be reduced, and at the salinities measured in these samples

Table 4-5. **Seven-Day Mean Percent Survival and Weight (mg) of Larval Fathead Minnows for Salinity Test at Baltimore Harbor, Maryland**

	Salinity Concentration (ppt)					
	16	12	8	4	2	Control
			Survival			
Replicate A	0	0	50	80	100	100
Replicate B	0	0	30	90	100	100
Replicate C	0	0	10	70	90	90
Replicate D	0	0	30	80	90	90
Mean	0[a]	0[a]	30[a]	80[a]	95	95
			Weight			
Replicate A	-	-	0.380	0.500	0.500	0.305
Replicate B	-	-	0.217	0.483	0.480	0.345
Replicate C	-	-	-	0.521	0.417	0.378
Replicate D	-	-	0.317	0.425	0.411	0.289
Weighted Mean	[a]	[a]	0.318	0.481	0.454	0.329
SE			0.040	0.023	0.021	0.021

[a] Significantly lower than the reconstituted water control ($P \leq 0.05$)

Table 4-6. **Daily and Mean Salinity (ppt) at Back River Stations, Baltimore Harbor, Maryland**

Station	9 Mar	10 Mar	11 Mar	12 Mar	13 Mar	14 Mar	15 Mar	Mean	SD
B1	0.9	1.0	1.0	1.3	1.1	1.0	1.0	1.0	0.13
B2	1.0	0.9	1.0	1.2	1.2	1.0	1.0	1.0	0.09
B3	1.1	1.2	1.1	1.1	0.9	0.9	1.0	1.1	0.22
B4	1.1	1.0	1.6	1.0	1.0	1.0	1.0	1.1	0.22
B5	1.2	1.3	2.1	1.5	1.5	1.2	1.5	1.3	0.48
B6	2.0	2.3	2.6	1.7	1.9	1.9	2.2	2.2	0.30

Source: Table B-1

Table 4-7. Daily and Seven-Day Mean Effluent Concentrations (%) at Back River Stations, Baltimore Harbor, Maryland

Station	9 Mar	10 Mar	11 Mar	12 Mar	13 Mar	14 Mar	15 Mar	Mean	SD
B1	43	7	55	3	70	2	17	28.0	27.6
B2	35	6	19	5	63	47	17	27.4	21.6
B3	9	10	23	15	39	33	34	23.3	12.3
B4	10	74	13	43	28	41	42	35.9	26.7
B5	16	50	14	9	12	21	16	19.7	13.9
B6	59	18	7	12	9	11	11	18.1	18.3

Table 4-8. Reproduction and Survival of *Ceriodaphnia dubia* for the Patapsco POTW Effluent and the Patapsco and Middle Rivers Ambient Stations, Baltimore Harbor, Maryland

Patapsco POTW (v/v) Percent Effluent Concentration	Mean Number of Young per Female	95% Confidence Interval	7 Day Percent Survival
100	0[a]	--	0[a]
30	0[a]	--	0[a]
10	0[a]	--	0[a]
3	0[a]	--	0[a]
1	0[a]	--	0[a]
Control[b]	26.8	22.8-30.7	90
3	0[a]	--	0[a]
1.5	0[a]	--	0[a]
0.75	16.3[a]	13.1-19.1	20[a]
0.37	27.5	24.3-30.7	100
Control[b]	24.0	21.8-26.3	80
	Ambient Samples		
Patapsco River			
P1	0[a]	--	0[a]
P2	0[a]	--	0[a]
P3	0[a]	--	0[a]
Middle River			
M1	29.2	27.6-30.8	100
M2	33.8	30.0-37.6	90
Control[b]	32.2	27.1-37.3	90

[a]Significantly lower than the reconstituted-water control ($P \leq 0.05$).
[b]Reconstituted-water controls.

Table 4-9. Daily Survival and Mean Young Production of *Ceriodaphnia dubia* in Various Dilutions of Back River POTW Effluent, Baltimore Harbor, Maryland

Back River Percent Effluent Concentration (v/v)	Cumulative Daily Survival (%)							Mean Number of Young per Female	95% Confidence Interval
	1	2	3	4	5	6	7		
100	0	0	0	0	0	0	0	0[a]	
30	30	0	0	0	0	0	0	0[a]	
10	100	100	100	0	0	0	0	0[a]	
3	100	100	100	90	90	90	90	31.9	28.2-35.7
1	100	100	100	100	100	100	90	33.6	29.0-38.2
Control	100	100	100	100	100	100	100	34.7	31.4-38.0

[a]Significantly lower than the reconstituted-water control ($P < 0.05$).

(Tables 4-6 and 6-1), which were from 1.0 to 2.2 ppt, mortality should have occurred around day 4 at 2.2 ppt and about day 6 or 7 for 1 ppt salinity. It is clear that mortality in Stations B1 through B5 occurred too soon to be only due to salinity, whereas at Station B6, mortality was delayed. This suggests that in either case, the salinity in the ambient sample was not correlated to toxicity in the same way it was in the salinity test.

As stated above, the AEC of the Back River POTW effluent was calculated to be 5.5 percent. Table 4-7 shows the mean effluent concentrations at each station. Mean effluent concentrations at Stations B1, B2, and B3 ranged from 23 to 28 percent. Table 4-9 shows that at 30 percent effluent, survival was zero percent at 2 days, and in the 10 percent effluent, zero percent survival at 4 days. Based on these comparisons, mortality at Stations B1, B2, B3, B4, and B5 occurred about as would be expected if it was due to effluent. The mortality at Station B6 occurred considerably later than it should have for effluent (or salinity) toxicity. Since the salinity measurement is nonspecific, one possibility is that what was being measured as salinity was, in fact, something else. Another possibility is that there was negative interaction between effluent and salinity.

4.4 Results of the Microtox® Tests

Table 4-12 contains the toxicity data from the Microtox® test for four days for both the Patapsco and Back River POTW effluents. The 9 March Back River sample was a prechlorination sample and the dramatic difference between its toxicity and the other samples suggests that chlorine may have been causing the toxicity. Because of this finding, the toxicity of pre- and post-chlorinated effluent was

Table 4-12. EC50 Values for Microtox® Tests for the Two POTW Effluents, Baltimore Harbor, Maryland

Effluent	Test Date	EC50 Value (% Effluent)
Back River POTW	9 Mar	>100[a]
	10 Mar	5.8
	11 Mar	1.5
	12 Mar	1.5
Patapsco POTW	9 Mar	1.5
	10 Mar	2.3
	11 Mar	10.2
	12 Mar	2.4

[a] Sample was collected before chlorination

Table 4-10. Daily Survival and Mean Young Production of *Ceriodaphnia dubia* in Back River Ambient Station Water, Baltimore Harbor, Maryland

Station	Cumulative Daily Survival (%) 1	2	3	4	5	6	7	Mean Number of Young per Female	95% Confidence Interval
B1	100	100	0	0	0	0	0	0[a]	--
B2	90	0	0	0	0	0	0	0[a]	
B3	100	10	0	0	0	0	0	0[a]	--
B4	100	0	0	0	0	0	0	0[a]	
B5	100	100	50	0	0	0	0	2.5[a]	--
B6	90	90	90	90	90	30	20[a]	38.0	30.6-45.6

[a] Significantly different from the reconstituted-water control ($P \leq 0.10$).

Table 4-11. Daily Survival and Mean Number of Young per Female in the Salinity Test, Baltimore Harbor, Maryland

Concentration (ppt)	Cumulative Daily Survival (%) 1	2	3	4	5	6	7	Mean Number of Young per Female	95% Confidence Interval
4	0	0	0	0	0	0	0	0	--
2	100	100	80	10	0	0	0	0	--
1	100	100	90	90	90	60	50[a]	7.9[a]	5.9-10.0
0.5	100	100	100	100	100	100	100	16.3[a]	13.8-18.8
0.25	100	100	100	100	100	100	100	14.8[a]	12.5-17.1
Control	90	90	90	90	90	90	90	32.2	26.9-37.4

[a] Significantly different from the reconstituted-water control ($P \leq 0.1$)

checked using 24-hour acute tests with *Ceriodaphnia,* and no difference was found.

Table 4-13 shows the percent light reduction for the Back River ambient stations. These samples were not toxic enough to measure an EC50. The mean values for light reduction show Stations B1 and B6 least toxic; Stations B2, B3, and B4 to be similar and most toxic; and Station B5 to be intermediate. This sequence is similar to the mortality pattern shown by the *Ceriodaphnia* chronic tests. The mean effluent concentrations (Table 4-7) that existed at the ambient stations were well above the EC50 values. Obviously, the effluent was less toxic in the ambient samples than was measured in the effluent tests. This may be due to the decay of chlorine toxicity.

Table 4-13. 15-Minute Percent Light Reduction for 91 Percent Back River Ambient Samples, Baltimore Harbor, Maryland

Station	Test Date				Mean
	9 Mar	10 Mar	11 Mar	12 Mar	
B1	16.3	14.1	12.8	13.7	14.2 (1.5)
B2	25.6	22.4	17.4	17.8	20.8 (3.9)
B3	24.4	16.5	25.6	30.1	24.2 (5.7)
B4	20.9	25.9	17.4	24.7	22.2 (3.9)
B5	15.7	17.1	15.1	16.4	16.1 (0.9)
B6	14.5	11.8	3.5	13.2	10.8 (5.0)

4.5 Summary of Toxicity Data

The low salinity present in the Back River did not appear to invalidate the tests with the freshwater species. The fathead minnows were tolerant enough of salinity that the effects could be ignored. Given a number of factors affecting the comparison of effluent and ambient toxicity data, expecially variable effluent concentrations in the ambient samples, the errors in estimating a threshold AEC, and decay of toxicity after discharge, the agreement appears good.

For *Ceriodaphnia,* although salinity should have masked the results, it did not seem to do so. At Stations B1 through B5 there was sufficient effluent to explain the toxicity and certainly the effects observed at Station B6 were not likely caused by salinity. The effluent present in the Station B6 sample was not as toxic as would be predicted from the effluent dilution tests.

The effluent and ambient Microtox® data do not agree. This could be explained by chlorine toxicity in the effluent decaying after discharge to Back River. However, chlorine did not seem to be the cause of toxicity with the *Ceriodaphnia.*

In general, the *Ceriodaphnia* and fathead minnow effluent and ambient tests agreed well. When a useful test to measure persistence of effluent toxicity becomes available, an even better agreement might be reached. These data do suggest that receiving waters with salinities within acceptable ranges and freshwater discharges can be evaluated with freshwater test organisms. The effluent toxicity tests, in this case, were reasonably reliable predictors of ambient toxicity. For much more saline estuaries, these freshwater organisms would not be useful.

5. *Hydrological Studies of Patapsco River*

5.1 Dilution Analysis of the Patapsco POTW

A water tracing dye was used to tag the effluent from the Patapsco POTW. By scaling the dye to the plant flow, effluent dilution can be calculated throughout the discharge plume, and the portion of effluent in water samples taken in the area can be estimated. Methods utilized in the dilution analysis of the Patapsco POTW are detailed in Appendix B. Plots of surface dilution are shown in Figures 5-1 and 5-2. Vertical profiles of dilution are given in Table 5-1, with their locations shown on Figure 5-2.

5.2 Evaluation of Hydrological Conditions of the Patapsco River

The flow regime in the Patapsco River is dominated by a three-layer, density-driven circulation pattern which was originally inferred from salinity and dye measurements, but which has been confirmed recently by long-term current measurements.

The hydrodynamic explanation for the circulation is that surface water in the Chesapeake Bay is typically fresher, and bottom water in the Bay is typically saltier, than water at the same depths in the Patapsco River. As a result, there is an inflow of surface water from the Bay, overriding the Patapsco River surface water and an inflow of bottom water from the Bay underriding the Patapsco River bottom water. These two inflows are then balanced by an outflow at middepth. The surface layer is the thinnest of the three layers, approximately 2 m thick. The middle layer is typically 6-8 m thick and the bottom layer 3-5 m thick.

Figure 5-1. **Surface dilution contours at the Patapsco POTW, 1103 through 1217 hours, 22 March 1984. Contours are derived from data taken on horizontal transects of plume area at high tide.**

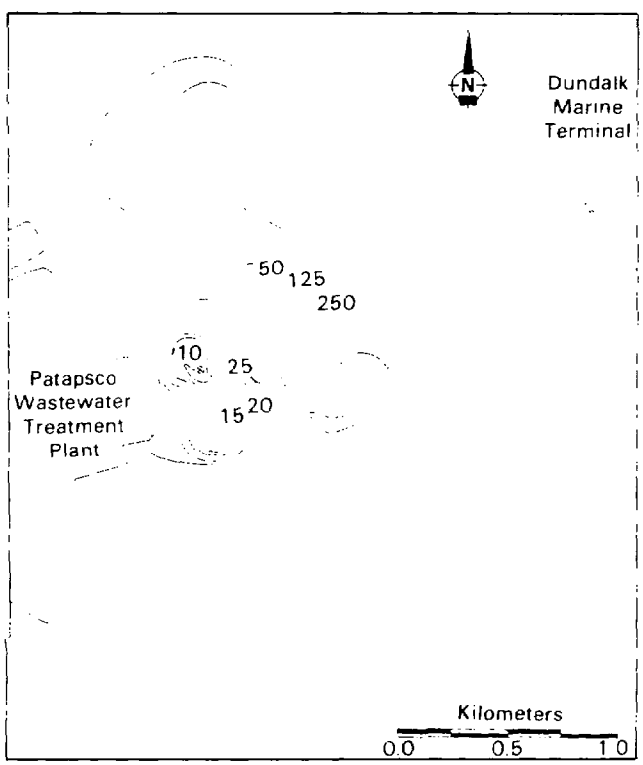


Figure 5-2. **Surface dilution contours of the Patapsco POTW, 1238 through 1417 hours, 22 March 1984. Also shown are locations of vertical sampling stations. Contours are derived from data taken on horizontal transects of plume area at ebb tide.**

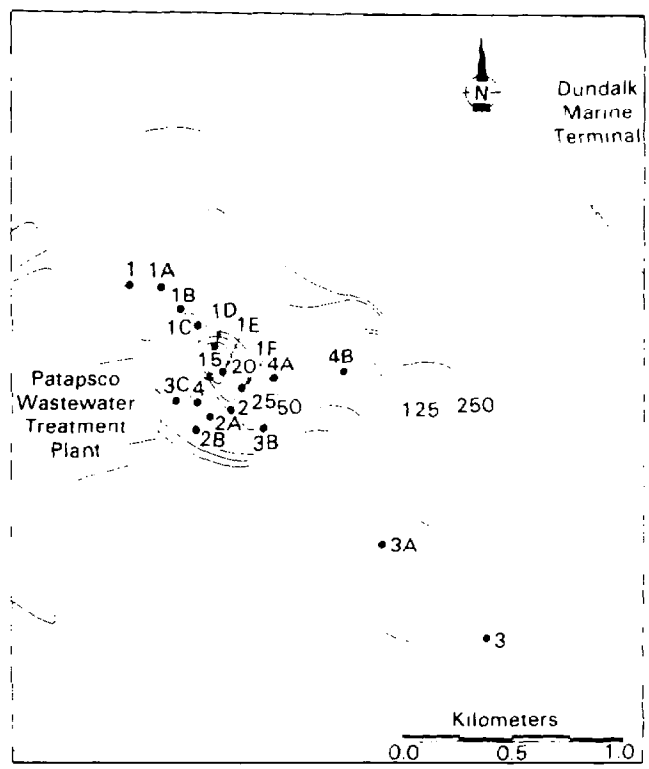


Table 5-1. Vertical Measurements of Dilution[a] at Stations Surrounding Patapsco POTW Discharge, Baltimore Harbor, March 1984

	Station (time)								
Depth (m)	1 (1135)	1A (1149)	1B (1201)	1C (1211)	1D (1221)	1E (1230)	1F (1249)	2 (1300)	2A (1311)
Surface	471	566	472	27	26	26	32	29	26
1	471	472	708	32	31	24	35	29	26
2	353	472	354	57	38	32	38	32	29
3	353	472	472	83	54	48	48	114	29
4	236	472	283	114	65	57	57	114	41
5	236	237	283	131	70	96	76	202	
6	476	237				144	--		
7	1428								
8	--								
9	-								
10	--								
11	--								

	2B (1319)	3 (1341)	3A (1351)	3B (1403)	3C (1417)	4 (1434)	4A (1443)	4B (1452)
Surface	48	202	--	41	101	108	27	81
1	45	202	1412	41	105	94	30	76
2	45	218	2825	48	101	101	29	78
3	51	202	1412	45	283	101	38	78
4	-	202	1412	54	473	189	54	89
5	--	236	1412	306	203	109	76	98
6		357	710				83	89
7								98
8								259
9								720
10								1443
11								-

Note: See Figure 5-2 for station locations.
[a]Dilution is defined as the ratio of the discharge concentration to the concentration measured in the field.

For short periods of time (less than 10 days), the three-layer circulation can be overshadowed by a wind-driven circulation in which either the surface layer follows the wind with a counter flow at depth or a large wind-induced set up/down in the Bay forces water into or out of the Patapsco River at all depths.

Periods of high freshwater runoff can also generate the usual two-layer estuarine flow, but the effect is limited to the upper reaches of the Patapsco River and its branches.

Residence times for Baltimore Harbor can be as short as 3 days during strong wind events or as long as 20 days when wind and density forcing are weak. More typically, residence time is between 8 and 10 days when the three-layer circulation is dominant.

Velocities in each of the three layers average between 3 and 5 cm/sec and typical outflow in the middle layer ranges between 200 and 300 m^3/sec. This is a substantial flushing rate and explains why residence times are so much shorter than would be the case for simple tidal and river flushing.

The outfall from the Patapsco POTW discharges at a depth of approximately 6 m which places it in the middle, outflowing layer. Although the initial plume is buoyant, turbulent mixing in the near field will cause the plume density rapidly to approach that of the ambient water and much of the effluent will remain in the middle layer and be transported bayward at the above-mentioned velocities. That part of the plume which reaches the surface layer will be initially transported upstream until vertical mixing incorporates it into the middle layer and it is flushed out. Without a more comprehensive and detailed study, it is not possible to quantify the average distribution of effluent dilution.

6. *Hydrological Studies of Back River*

6.1 Dilution Analysis of the Back River POTW

Water samples were collected in Back River and Middle River during the period 8-16 March 1984. Analysis of these samples required an estimation of the fraction of the water sample which had passed through the Back River POTW, and, to quantify this estimate, the plant effluent was "tagged" with a water tracing dye.

Two problems arose with the dye tracing technique. First, to tag all the treated water in the river would have required injecting the dye for a longer period of time than was economically feasible. Second, due to the high chlorine residuals in the plant flow, the dye injection point had to be moved into the river downstream of the outfall. Methods utilized in the dilution analysis of the Back River POTW are presented in Appendix B.2.

To address the first problem, a one-dimensional hydrodynamic mathematical model (Hunter, 1975) was applied to Back River and calibrated to simulate a longer dye release, and the measured dye dilutions were then adjusted by the ratio of the concentrations predicted by the simulated longer release to the actual modeled release at the location of the water sample.

Because of the second problem, dye distribution near the outfall can be expected to be very different from what it would have been had the dye been injected in the plant. The cross-sectional averaging inherent in the one-dimensional model will mitigate the disparity somewhat, but the accuracy of the results will be poorer at locations near the source.

6.2 Hydrological Modeling of Back River

Figure 6-1 shows model predictions for dye concentrations at three locations in Back River (Transects 5, 8, and 11; Figure B-1) versus elapsed time referenced to the start of integration (0100 hours, 5 March 1984).

For this computer model run, the dye injection was started on 7 March to simulate the field study. Agreement is quite good at the mouth and, except for the measurements on 15 March, is reasonable at the other locations. It is not known why the 15 March values are so high.

The calibrated model was then run again with a simulated dye injection beginning on 1 March to allow the simulated dye levels in the river to more nearly reach equilibrium levels at which all effluent present would have been tagged. As could be expected, the model dye concentrations are higher (Figure 6-2) at equilibrium than the previous model run.

To estimate what the dye concentrations in the water samples would have been had the dye injection into the river begun six days earlier (1 March), the ratio dye concentrations from each of the two computer runs was multiplied by the octanol measured concentrations in the samples. These ratios are a function of location and time. These predicted dye concentration ratios were then used to calculate the percent POTW effluent at each of the stations during the period 9-16 March, based on the steady state model with dye levels close to equilibrium levels (Table 6-1).

6.3 Evaluation of Hydrological Conditions of the Back River and Middle River

It takes about two weeks for a contaminant introduced on a continuous basis at the head of the Back River to reach equilibrium levels throughout the river. The model runs also show that, when the contaminant source is turned off upstream, the lower sections of the river are not affected for approximately 3 days.

Because the river is so shallow, tidal elevation at the mouth is an important factor in driving an interchange of water between the river and the bay. Large fluctuations over periods of a few days are capable of flushing the river in a relatively short time, and estimations of river flushing rates must be understood in this context.

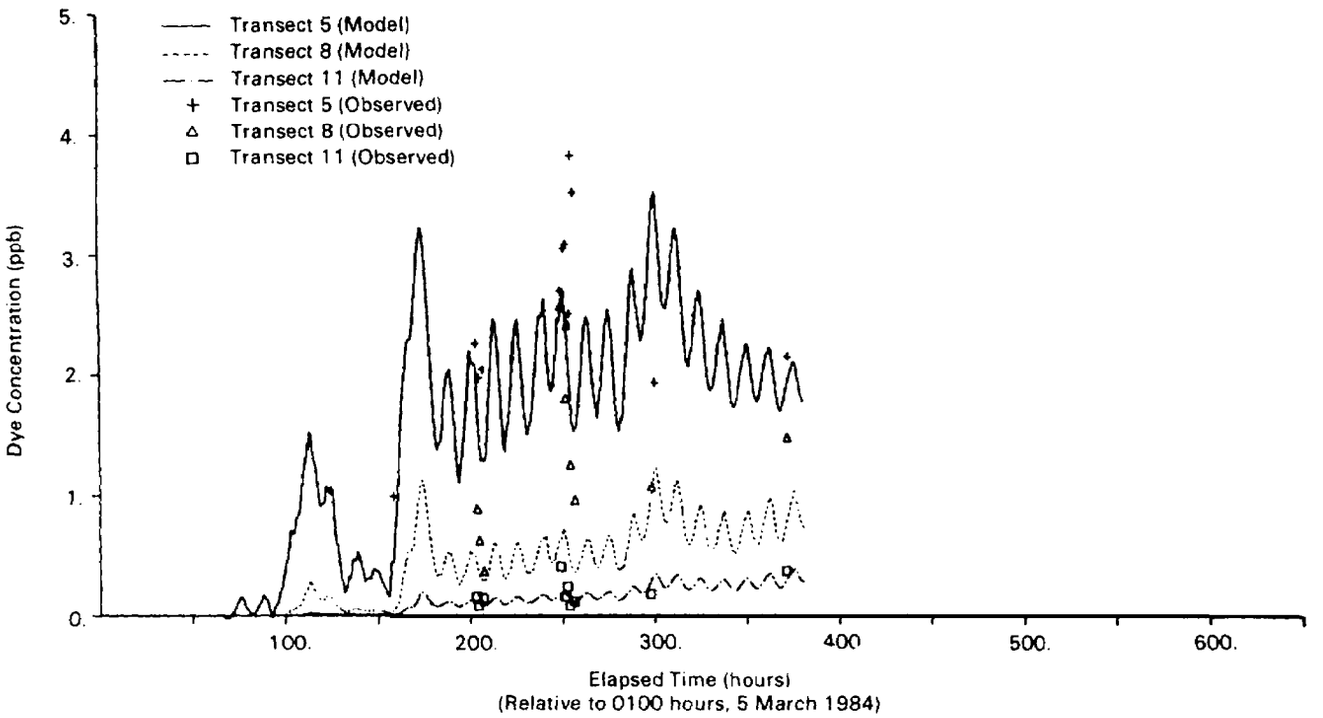


Figure 6-1. **Dye concentrations in the Back River as observed and predicted by the numerical model. Dye injection started at hour 62.**

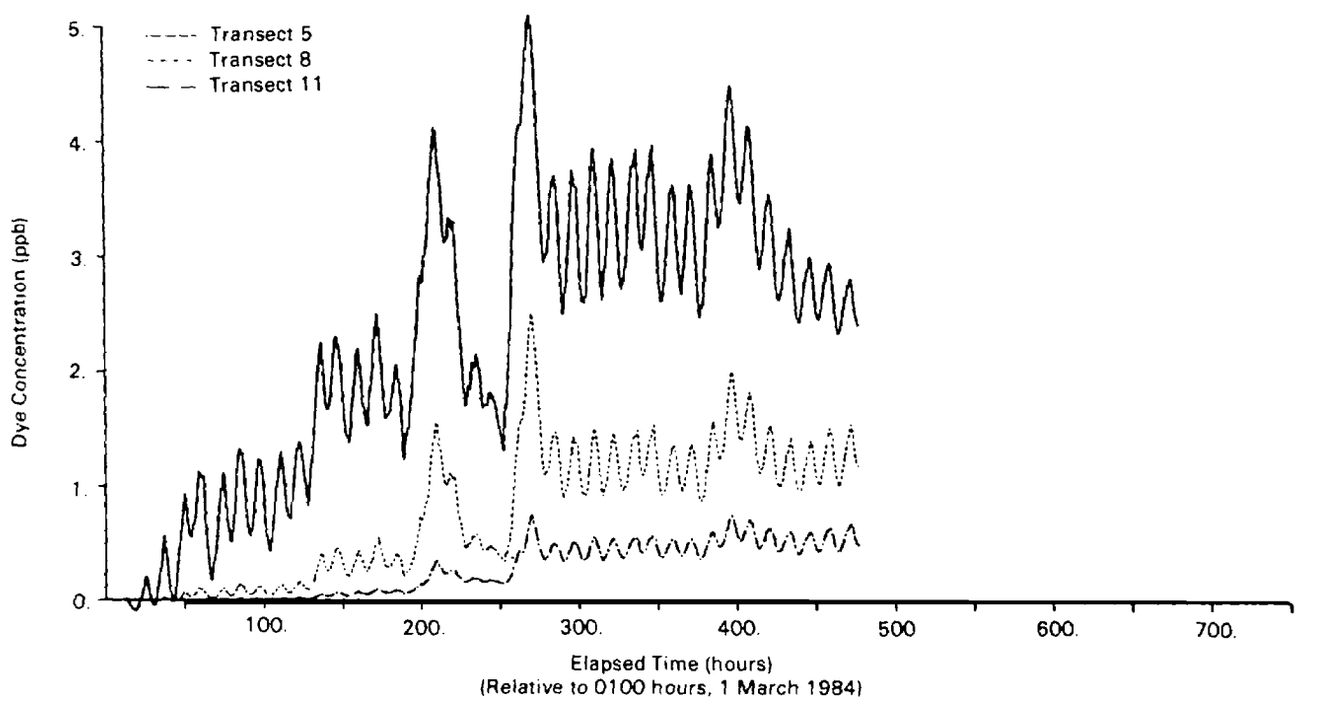


Figure 6-2. **Dye concentration in the Back River as predicted by the numerical model for simulated dye release beginning 1 March.**

Table 6-1. Surface Water Quality Data for Back River and Middle River Stations from 9 March 1984 Through 16 March 1984

Date	Station	Time	Temperature (C)	pH	Dissolved Oxygen (mg/L)	Conductivity (μmhos)	Salinity (ppt)	Ammonia (mg/L)	Percent Effluent[a]
9 Mar	B1	0712	1.2	8.2	14.5	1,205	0.9	5.51	43.0
	B2	0724	1.9	7.8	14.5	1,373	1.0	7.50	34.9
	B3	0732	2.1	8.1	15.3	1,584	1.1	8.17	8.5
	B4	0740	2.3	7.9	14.4	1,557	1.1	6.10	10.4
	B5	0752	1.9	8.4	15.3	1,730	1.2	5.51	15.7
	B6	0805	1.8	9.0	15.4	2,780	2.0	1.78	58.5
	M1	1010	1.5	7.7	13.3	1,950	1.4	0.09	--
10 Mar	B1	0917	1.7	7.9	14.5	1,457	1.0	7.09	6.8
	B2	0910	3.5	7.1	11.7	1,219	0.9	8.85	5.7
	B3	0857	1.3	8.1	14.4	1,618	1.2	6.45	10.0
	B4	0845	1.8	7.4	10.2	1,459	1.0	7.83	74.0
	B5	0830	1.3	7.9	13.3	1,837	1.3	5.97	50.1
	B6	0805	0.9	8.9	15.0	3,080	2.3	1.46	17.6
	M1	0700	1.3	8.4	12.4	2,590	1.9	0.15	
	M2	0730	1.0	7.5	13.2	2,680	1.9	0.15	--
11 Mar	B1	0913	3.8	7.3	12.4	1,390	1.0	8.60	54.5
	B2	0900	3.6	7.2	11.5	1,380	1.0	8.60	18.8
	B3	0855	2.1	8.0	14.2	1,550	1.1	6.29	22.6
	B4	0843	1.9	8.6	15.4	2,230	1.6	4.08	13.4
	B5	0830	1.5	8.9	16.7	2,800	2.1	2.43	13.9
	B6	0815	1.5	8.4	14.7	3,510	2.6	0.47	7.2
	M1	0738	1.8	7.7	13.4	2,250	1.6	0.10	--
	M2	0751	1.4	7.7	13.2	3,160	2.3	0.08	
12 Mar	B1	0844	1.4	8.1	14.3	1,749	1.3	5.89	3.4
	B2	0853	2.1	7.9	14.0	1,616	1.2	6.29	5.0
	B3	0859	3.1	7.3	12.0	1,588	1.1	8.25	14.5
	B4	0909	2.8	7.2	10.2	1,360	1.0	8.77	42.7
	B5	0917	1.8	8.3	14.2	2,100	1.5	4.90	8.9
	B6	0930	2.0	8.6	15.0	2,310	1.7	3.93	11.6
	M1	1022	1.8	7.8	12.9	2,370	1.7	0.07	
	M2	1005	1.4	7.8	13.4	2,490	1.8	0.11	
13 Mar	B1	1147	2.5	7.3	12.2	1,549	1.1	10.4	70.0
	B2	1135	3.4	7.1	11.4	1,595	1.1	11.1	53.4
	B3	1125	3.3	7.0	9.0	1,315	0.9	9.15	39.0
	B4	1115	2.8	7.0	13.7	1,464	1.0	7.09	28.2
	B5	1105	2.0	8.4	15.2	2,070	1.5	4.60	11.8
	B6	1055	2.0	8.7	15.5	2,620	1.9	2.41	9.2
	M1	1000	2.2	7.6	13.1	2,370	1.7	0.07	
	M2	1020	1.6	7.7	13.6	2,360	1.7	0.10	--
14 Mar	B1	1030	2.8	7.3	12.1	1,406	1.0	0.61	2.3
	B2	1040	4.2	7.1	10.2	1,454	1.0	8.25	47.3
	B3	1048	5.7	6.9	9.5	1,268	0.9	8.77	32.6
	B4	1058	4.6	7.1	9.3	1,350	1.0	9.20	41.2
	B5	1108	3.1	8.6	16.4	1,733	1.2	5.51	20.5
	B6	1122	2.4	8.8	14.5	2,650	1.9	3.26	11.4
	M1	1220	2.6	7.7	13.1	2,360	1.7	0.07	--
	M2	1236	2.0	7.9	12.7	2,870	2.1	0.14	--
15 Mar	B1	1334	6.7	7.4	11.1	1,483	1.0	5.51	16.7
	B2	1322	7.4	7.0	10.1	1,350	1.0	6.85	16.6
	B3	1311	7.1	6.9	8.3	1,412	1.0	7.47	33.7
	B4	1304	6.9	7.0	8.5	1,424	1.0	7.09	42.3
	B5	1247	4.9	8.8	16.3	2,110	1.5	4.52	16.2
	B6	1230	4.5	9.0	16.0	2,960	2.2	2.34	10.9
	M1	1155	3.4	7.7	12.3	2,400	1.7	0.05	--
	M2	1208	3.3	8.1	13.1	2,770	2.0	0.05	--

Table 6-1. (Continued)

Date	Station	Time	Temperature (C)	pH	Dissolved Oxygen (mg L)	Conductivity (μmhos)	Salinity (ppt)	Ammonia (mg L)	Percent Effluent[a]
16 Mar	B1	1310	10.2	6.8	7.0	1,198	0.8	7.75	66.7
	B2	1319	8.7	6.9	5.7	1,331	0.9	8.08	30.4
	B3	1325	9.8	6.8	6.3	1,250	0.9	8.17	33.3
	B4	1335	10.3	6.8	5.8	1,288	0.9	8.00	49.3
	B5	1355	6.2	8.8	16.3	2,830	2.1	2.78	11.5
	B6	1405	5.2	8.9	15.8	3,650	2.7	1.28	7.0
	M1	1430	5.5	7.9	12.6	2,400	1.7	0.07	
	M2	1440	5.4	8.4	13.5	2,920	2.1	0.05	--

[a]Percent effluent is based on the assumption that the dye was well mixed into the average plant flow (81 mgd from 6 through 16 March). Values further from the source are probably more accurate.

7. Macrozooplankton/Ichthyoplankton of Back River and Middle River

7.1 Community Structure

7.1.1 Macrozooplankton

The zooplankton communities in Back River and Middle River were overwhelmingly dominated by the estuarine copepod *Eurytemora affinis*. Most of the specimens were large, overwintering adults, the majority being gravid females. They constituted 99.9 percent of all taxa taken at each river during both sampling dates (Tables 7-1 and 7-2). *Eurytemora affinis* was also the dominant zooplankton species found during a study of the tidal rivers, including Middle River (EA 1981). The amphipod *Monoculodes edwardsi* was the second most abundant taxa in Back River and the cladoceran *Daphnia* was second in abundance in Middle River.

7.1.2 Ichthyoplankton

No ichthyoplankton (fish larvae or eggs) were taken during the two days of sampling. Gravid white perch were collected by trawl in Back River and Middle River during this period. None of the specimens collected were ripe which indicates that spawning probably had not yet occurred. Water temperatures during the trawl collections ranged from 2.4 to 3.4°C at the mouth of Back River where the highest numbers of white perch were collected during the two sampling occasions. According to Dovel (1971), most white perch spawning occurs between 8 and 15°C in upper Chesapeake Bay. Yellow perch, another early spring spawner, were collected in low numbers only in Middle River, but not enough specimens of a mature size were taken to indicate spawning condition.

Table 7-1. Abundance and Percent Composition of the Macrozooplankton Community of Back River and Middle River, 12 March 1984

Taxa	Density (No./m³)	Percent Composition
Back River		
Eurytemora affinis	363.9	99.97
Monoculodes edwardsi	0.086	0.024
Daphnia	0.005	0.001
Chaoborus	0.004	0.001
Gammarus	0.004	0.001
Ostracoda	0.001	<0.001
Neomysis americana	0.001	<0.001
Hemiptera	0.001	<0.001
Nematoda	0.001	<0.001
Middle River		
Eurytemora affinis	749.6	99.99
Daphnia	0.061	0.008
Monoculodes edwardsi	0.038	0.005

7.2 Differences Between Stations in Key Macrozooplankton Taxa

A total of 16 macrozooplankton taxa were collected during the two sampling dates. The number of taxa

Table 7-2. Abundance and Percent Composition of the Macrozooplankton Community of Back River and Middle River, 16 March 1984

Taxa	Density (No./m³)	Percent Composition
Back River		
Eurytemora affinis	301.6	99.98
Monoculodes edwardsi	0.038	0.013
Ceriodaphnia	0.009	0.003
Daphnia	0.009	0.003
Gammarus	0.006	0.002
Leptocheirus plumulosus	0.002	0.001
Ostracoda	0.001	<0.001
Chironomidae pupae	0.001	<0.001
Diptera pupae	0.001	<0.001
Chaoborus larvae	0.001	<0.001
Eubosmina	0.001	<0.001
Neomyusis americana	0.001	<0.001
Middle River		
Eurytemora affinis	818.2	99.92
Daphnia	0.630	0.077
Monoculodes edwardsi	0.022	0.003
Eubosmina	0.012	0.001
Collembola	0.008	0.001
Chaoborus	0.006	0.001
Diptera pupae	0.004	<0.001
Alinyraccuma proximoculi	0.004	<0.001

per station was low, ranging from three to eight in Back River and from five to six in Middle River (Table 7-3) Combining the number of taxa from the two collections indicated no significant differences in number of taxa among stations (P = 0.05) (Table F-2). *E. affinis* was the only taxon taken at all stations. *Monoculodes edwardsi* was taken at seven of the eight stations sampled. The other taxa were uncommon, and occurred at low densities at one to five stations.

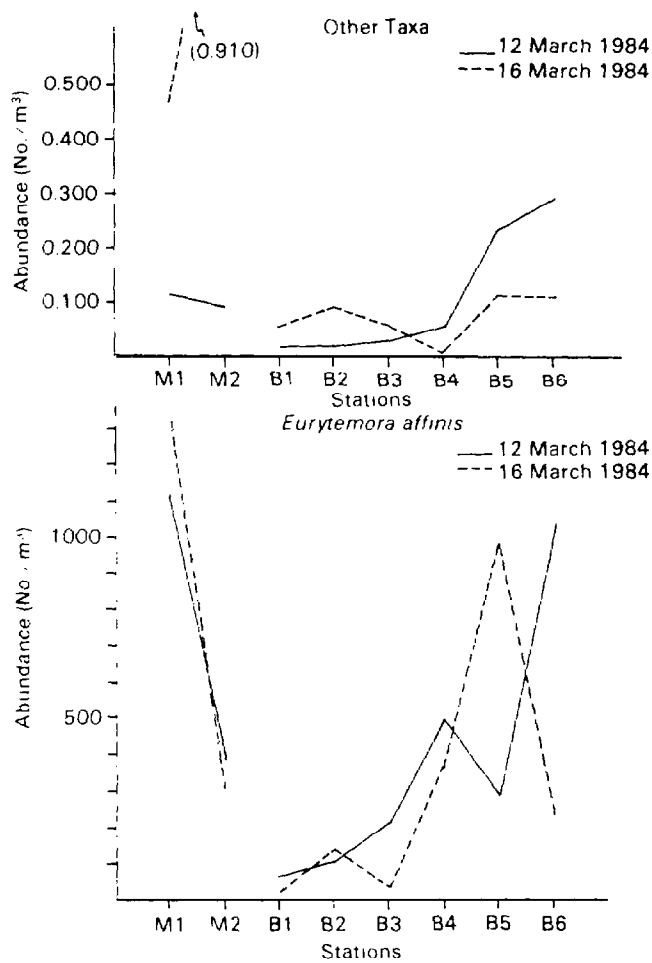
Abundance per station for *E. affinis* ranged from a mean density of 19/m^3 at Station B1 near the Back River POTW (Figure 3-1) to 1,321/m^3 at Station M1 in Middle River (Tables F-2 and F-3). Results of a 2-way ANOVA indicated both a significant (P = 0.0001) station and date effect for transformed densities of *E. affinis* (Table F-4). A significant interaction term suggested some inconsistency in abundance trends between the two collection dates. However, results of the Tukey's multiple comparison test (Sokal and Rohlf, 1981) showed abundances at the reference station (M1) and the lower Back River stations (B4, B5, and B6) to be higher than those at the upper Back River stations (B1, B2, and B3). The densities of all other taxa combined ranged from 0.008/m^3 at Station B4 to 0.910/m^3 at Station M2. All plankton collections were made on flood tide with the exception of Stations M1 and M2 which were sampled at ebb tide on 16 March. The difference in tidal collections at the Middle River stations may have influenced the abundance of other zooplankton taxa, but did not affect the density of *E. affinis* (Figure 3-1). The general trend in abundance in Back River was an increase in density from upriver to downriver for *E. affinis* and the other taxa (Figure 7-1). This distribution is probably the result of the salinity regime in this area which ranged from 0.6 ppt upriver to 2.1 ppt near the mouth of Back River (Table F-5). *Eurytemora* is an estuarine copepod which is typically most abundant between 1 and 10 ppt salinity (Cronin et al., 1962). The distribution of *E. affinis* in Back River is comparable to the results of a study by Heinle and Flemer (1975) on the Patuxent River. During February and March they collected the highest density of *E. affinis* adults at a salinity of 2.9-5.4 ppt, respectively, and a much lower density to no specimens at salinities less than 1.2 ppt. In Middle River, *E. affinis* was much more abundant upriver at Station M1. The salinity was similar at Station M1 (1.3-1.4 ppt) and Station M2 (1.5-2.1 ppt).

Table 7-3. Composition of the Macrozooplankton Community of Back River and Middle River, 12 and 16 March 1984

	Station							
Taxa	B1	B2	B3	B4	B5	B6	M1	M2
Nematoda					X			
Eubosmina					O		O	O
Daphnia				X	XO	XO	XO	XO
Ceriodaphnia	O	O						
Ostracoda	X	O						
E affinis	XO	XO	XO	XO	XO	XO	XO	XO
N americana					X	O		
A proximoculi							O	
L plumulosus						O		
Gammarus		O			XO	O		
M edwardsi	O	XO	XO	XO	XO	XO		XO
Diptera pupae		O					O	
Chironomidae pupae		O						
Chaoborus	X	XO	X					O
Hemiptera					X			
Collembola							O	
Total	5	8	3	3	8	6	6	5

Note X 12 March 1984
O 16 March 1984

Figure 7-1. Spatial trends of macrozooplankton community parameters, March 1984.



7.3 Evaluation of the Macrozooplankton Community

The zooplankton communities in Back River and Middle River (reference area) were both characterized by low diversity (number of taxa) and dominance by the estuarine copepod *E. affinis* at all stations. Similar values for maximum abundance occurred in both river systems, indicating no discernable response in the Back River community to enrichment from the Back River POTW. The density of *E. affinis* in Back River increased from upriver to downriver in response to increasing salinity levels. The freshwater input from the wastewater treatment plant could be contributing to the restriction of high density populations of *E. affinis* to the lower reaches of Back River.

8. Benthic Macroinvertebrates of Back River and Middle River

Benthic macroinvertebrates were collected on 19 March 1984 at six stations in Back River and two stations in Middle River (reference area). The objectives of the study were to determine the composition and abundance of the benthic fauna in order to assess the response of the community to the discharge of the Back River POTW.

The substrate type was fairly uniform from station to station consisting mainly of fine black or gray silt with small amounts of detritus and occasional shell fragments, especially in Middle River. Middle River was characterized by similar temperature levels and low salinity at both stations. Temperature was highest upriver in Back River near the POTW and decreased downriver. Salinity was lowest upriver, increasing to levels downriver which were similar to Middle River.

8.1 Community Structure

Twenty-four taxa of benthic macroinvertebrates were collected in Back and Middle Rivers. Seven taxa comprised a cumulative 90.3 percent of the total benthos (Table 8-1). Three oligochaete taxa constituted 56.6 percent of the fauna followed by the pelecypod *Rangia cuneata* (12.2 percent), the amphipod *Leptocheirus plumulosus* (10.2 percent), the polychaete *Scolecolepides viridis* (7.5 percent), and Ostracoda (3.8 percent). *R. cuneata* was taken only at Station M2 but at high densities. The number of taxa at Stations B1, B3, and B4 were significantly lower ($P = 0.05$) than the expected number of taxa (F-6).

8.2 Spatial Trends in Key Taxa

The oligochaete worms were the most widespread and abundant group, and the only group found at all stations (Table 8-2). Immature tubificid oligochaetes without capilliform chaetae was the most abundant taxa, comprising 24.7 percent of the total benthos. Most of these individuals were probably in the *Limnodrilus* group, the highest percentage probably being *L. hoffmeisteri*. *Tubificoides heterochaetus* (19.2 percent) was the second most abundant taxa followed by *L. hoffmeisteri* (12.6 percent).

The number of taxa at each station ranged from 2 at Station B4 to 13 at Station M2 (Figure 8-1). Station M2 near the mouth of Middle River (Figure 3-2) had numerous specimens of the pelecypods *Rangia cuneata* and *Mytilopsis leucophaeta*. Some pelecypods were also present at Station B6 in Back River which had the next highest number of taxa (12). The presence of these species and their empty shells provides habitat which attracts more taxa. These locations also had the highest salinity levels (2.7 ppt at Station M2; 3.5 ppt at Station B6) (Table F-7) of any stations sampled, which accounted for the presence of more estuarine taxa in these areas. Only two oligochaete taxa were present at the least diverse station, Station B4 in Back River. The stations upriver of Station B4 also had few taxa (3-5) and these communities were also dominated by oligochaete worms.

The trends in abundance distribution of the benthos were influenced by a few and sometimes different dominant taxa. The communities at Stations B2 through B5 had similarly low abundance, ranging from the lowest density of 1,304/m^2 at Station B4 to 1,677/m^2 at Station B5 (Table 8-1). These stations were all dominated by oligochaetes in the *Limnodrilus* group, especially *L. hoffmeisteri*. The highest abundance was at Station B6 (5,977/m^2) which had a much different and more diverse community than the upstream stations.

Station B6 was dominated by the estuarine oligochaete *T. heterochaetus* (4,286/m^2), and less importantly by the polychaete *Scolecolepides virides* (846/m^2) and Ostracoda (459/m^2). Station B1, nearest to the Back River POTW, also had high abundance (4,271/m^2) but it had a less diverse habitat, dominated by primarily freshwater oligochaetes, *L. hoffmeisteri* and *L. cervix*, both tolerant species common in areas with a high degree of organic enrichment (Stimson et al., 1982). The two Middle River stations (M1 and M2) had fairly high abundance (3,741/m^2 and 4,300/m^2, respectively) and more diverse communities than most Back River stations (except B6). Station M1 was dominated by *L. plumulosus* (2,451/m^2) and Station M2 was dominated by *R. cuneata* (2,967/m^2).

A community loss index was calculated, based on total number of taxa, to assess differences between a reference station (M1) and all other stations sampled (Table 8-3). Stations M2 and B6 were most similar to the reference station. Station dissimilarity to the reference station was greatest at Stations B1 and B4, especially at Station B4, since only two taxa were collected. Since relatively few taxa were taken at

Table 8-1. Abundance (No./m²) of Benthic Macroinvertebrates Collected from Back River and Middle River, 19 March 1984

Station	M1		M2		B1		B2	
Species	Number Indivs.	Pct Comp.	Number Indivs.	Pct. Comp.	Number Indivs	Pct Comp.	Number Indivs.	Pct Comp.
Imm. Tub. w/o Cap. Chaet	0 00	0 00	28.67	0.67	2809.33	65.77	602.00	42 00
Tubificoides heterochaet	129 00	3 45	186 33	4 33	0.00	0 00	0 00	0.00
Limnodrilus hoffmeisteri	0 00	0 00	0.00	0 00	802.67	18.79	659.33	46.00
Rangia cuneata	0.00	0 00	2967.00	69.00	0 00	0.00	0 00	0.00
Leptocheirus plumulosus	2451 00	65 52	0.00	0.00	0.00	0.00	0 00	0.00
Scolecolepides viridis	473.00	12 64	473.00	11.00	0.00	0.00	0 00	0 00
Ostracoda	358.33	9 58	28.67	0.67	0.00	0 00	0 00	0.00
Limnodrilus cervix	0.00	0 00	0.00	0.00	645.00	15.10	129 00	9.00
Clinotanypus L.	114.67	3.07	100.33	2.33	0 00	0 00	0 00	0.00
Mytilopsis leucophaeta	0.00	0.00	258.00	6.00	0.00	0 00	0 00	0 00
Corophium lacustre	71.67	1.92	0.00	0.00	14 33	0 34	0 00	0.00
Coelotanypus L.	0.00	0.00	0.00	0 00	0 00	0 00	0 00	0 00
Pelecypoda	14.33	0.38	0.00	0 00	0.00	0 00	0 00	0.00
Nematoda	86.00	2.30	28.67	0 67	0.00	0.00	0 00	0.00
Cyathura polita	0.00	0 00	100.33	2.33	0.00	0.00	0 00	0.00
Procladius L.	28.67	0 77	43.00	1 00	0.00	0 00	0.00	0.00
Monoculodes edwardsi	14 33	0.38	57.33	1.33	0.00	0 00	0.00	0.00
Heteromastus filiformis	0 00	0 00	0.00	0.00	0.00	0.00	0.00	0 00
Chironomidae P.	0.00	0 00	0.00	0.00	0.00	0.00	28 67	2 00
Nemertea	0.00	0 00	14.33	0.33	0.00	0.00	0 00	0 00
Rhithropanopeus harrisii	0.00	0 00	14.33	0 33	0.00	0 00	0 00	0 00
Acarina	0.00	0.00	0.00	0.00	0 00	0 00	14.33	1 00
Chironomus L.	0.00	0.00	0.00	0.00	0 00	0 00	0.00	0 00
Macoma mitchilli	0.00	0.00	0.00	0.00	0 00	0 00	0.00	0 00
Station Total	3741.00		4300 00		4271.33		1433 33	

B3		B4		B5		B6			
Number Indivs	Pct Comp	Number Indivs	Pct. Comp.	Number Indivs	Pct. Comp.	Number Indivs.	Pct Comp	Number Total	Pct. Comp
845.67	53 64	845 67	64.84	860 00	51 28	14.33	0 24	750.71	24.73
0 00	0.00	0.00	0.00	57 33	3.42	4285.67	71.70	582.29	19.19
659 33	41 82	458.67	35.16	487.33	29 06	0.00	0.00	383.42	12.63
0 00	0.00	0.00	0.00	0 00	0 00	0.00	0.00	370.88	12.22
0.00	0.00	0.00	0 00	14 33	0.85	14.33	0.24	309.96	10.21
0.00	0.00	0 00	0.00	28.67	1.71	845.67	14.15	227.54	7.50
0.00	0.00	0 00	0 00	71.67	4.27	458.67	7.67	114 67	3.78
0.00	0.00	0 00	0 00	0.00	0.00	0.00	0.00	96 75	3.19
0.00	0.00	0 00	0 00	14.33	0 85	143.33	2.40	46 58	1.53
0.00	0.00	0 00	0 00	0.00	0.00	0.00	0.00	32.25	1.06
71.67	4.55	0 00	0 00	0.00	0.00	0.00	0.00	19 71	0.65
0.00	0.00	0 00	0 00	143.33	8.55	0.00	0 00	17.92	0 59
0.00	0.00	0 00	0.00	0.00	0.00	114 67	1.92	16.13	0 53
0.00	0.00	0 00	0.00	0.00	0.00	0 00	0.00	14 33	0.47
0.00	0.00	0 00	0.00	0 00	0.00	0 00	0 00	12 54	0 41
0.00	0.00	0 00	0.00	0.00	0.00	28.67	0.48	12 54	0 41
0 00	0.00	0 00	0.00	0.00	0 00	14.33	0 24	10.75	0.35
0 00	0 00	0.00	0.00	0 00	0 00	28.67	0 48	3 58	0.12
0 00	0 00	0.00	0.00	0 00	0 00	0.00	0.00	3 58	0.12
0 00	0 00	0 00	0.00	0 00	0 00	0.00	0.00	1 79	0.06
0 00	0.00	0.00	0.00	0 00	0 00	0.00	0.00	1.79	0.06
0 00	0 00	0.00	0.00	0.00	0 00	0.00	0.00	1.79	0.06
0 00	0 00	0.00	0 00	0 00	0 00	14.33	0.24	1.79	0.06
0.00	0.00	0 00	0 00	0 00	0.00	14.33	0.24	1.79	0.06
1576 67		1304 33		1677 00		5977.00		3035.88	

Table 8-2. Composition of Benthic Community of Back River and Middle River, 19 March 1984

Species	Station							
	M1	M2	B1	B2	B3	B4	B5	B6
Nemertea		X						
Nematoda	X	X						
Limnodrilus cervix		X	X					
Limnodrilus hoffmeisteri			X	X	X	X	X	
Imm. tub. w/o cap. chaetae		X	X	X	X	X	X	X
Tubificoides heterochaetus	X	X					X	X
Heteromastus filiformis								X
Scolecolepides viridis	X	X					X	X
Ostracoda	X	X					X	X
Cyathura polita		X						
Leptocheirus plumulosus	X						X	X
Corophium lacustre	X		X		X			
Monoculodes edwardsi	X	X						X
Rhithropanopeus harrisii		X						
Acarina				X				
Chironomidae pupae				X				
Procladius larvae	X	X						X
Clinotanypus larvae	X	X					X	X
Coelotanypus larvae							X	
Chironomus larvae								X
Pelecypoda	X							X
Mytilopsis leucophaeta		X						
Rangia cuneata		X						
Macoma mitchilli								X
Total number of taxa	10	13	4	5	3	2	8	12

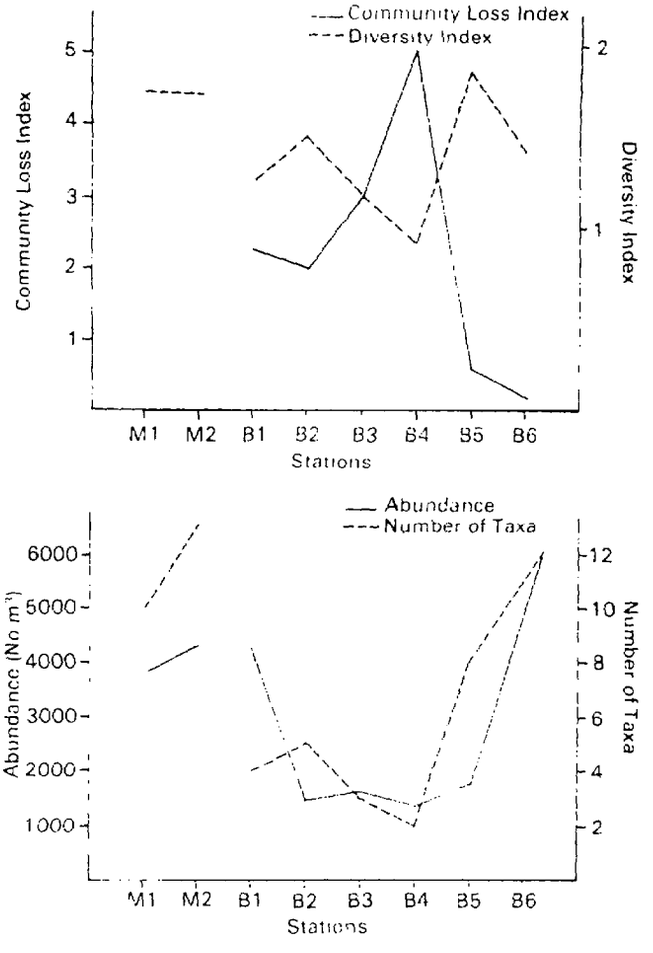
even the reference station, a difference of one or two taxa made a dramatic difference in the index values. These small differences in numbers of taxa probably reflect patchiness in these communities which were responsible for the wide range of values.

An index of diversity based on information theory was calculated to examine the community at each station (Table 8-3). In comparison with the community loss index which considers only the number of species, the diversity index considers the way individuals are distributed among species. Overall, diversity was low at all stations due to the lack of abundance of many taxa and dominance of a few taxa at most stations. Generally, diversity was greatest in Middle River at Stations M1 (1.7725) and M2 (1.7614) (the reference stations), and Station B5 (1.8942) in Back River, which supports the trends indicated by the other data analyses. Station B6, which had the highest number of taxa, had relatively low diversity as indicated by the index (1.4443) due to the numerical dominance of *T. heterochaetus*. Stations B1 through B4 had low diversity and were dominated by oligochaetes.

8.3 Evaluation of the Benthos Community

The benthic communities at the reference stations in Middle River were fairly similar to each other in

Figure 8-1. Spatial trends of benthic community parameters.



respect to abundance, number of taxa, and diversity. These stations were most similar to the stations (B5 and B6) at the downriver portion of Back River. Much of these similarities may be attributable to the similar salinity regime in these areas. The community in the upriver portion of the Back River was much different, being characterized by low numbers of taxa and dominance by one group, the oligochaete worms. This was especially evident at Stations B1 and B2 immediately up and downriver, respectively, of the Back River POTW effluent where the oligochaetes *L. hoffmeisteri* and *L. cervix* were the dominant fauna. These species are often the dominant organisms in degraded freshwater and oligohaline environments.

Table 8-3. Shannon-Wiener Diversity Indices ($\bar{d}$), Associated Evenness and Redundance Values, and Community Loss Index Calculated on Benthic Data from Back River and Middle River, 19 March 1984

Station	Diversity[a]	Evenness[b]	Redundance[b]	Number of Species	Number of Individuals	Community Loss Index[c]
B1	1.2902	0.6451	0.355	4	12,814	2.2500
B2	1.5330	0.6602	0.3416	5	4,300	2.0000
B3	1.2107	0.7639	0.2370	3	4,730	3.0000
B4	0.9355	0.9355	0.0647	2	3,913	5.0000
B5	1.8942	0.6314	0.3710	8	5,031	0.6250
B6	1.4443	0.4029	0.5987	12	17,931	0.1667
M1	1.7725	0.5336	0.4681	10	11,233	--
M2	1.7614	0.4760	0.5260	13	12,900	0.2308

[a] Calculated on a log base 2.
[b] Sum of evenness and redundance pairs is equal to 1.
[c] Calculated using Station 1 as reference station. (Courtemanch 1983)

9. *Fish Community Survey*

9.1 Community Structure

The fish community of Back River differed from that in the Middle River reference area, although water quality characteristics measured were comparable between areas for two sampling dates (Tables 9-1 and 9-2). In Back River on both sampling dates brown bullhead predominated in catches and was distinctly more abundant at Station B4 near the middle of the river. Toward the mouth of Back River, white perch increased in abundance as brown bullhead numbers declined, resulting in somewhat larger total catches downstream compared to upstream stations. In contrast, Middle River catches were dominated by pumkinseed, particularly at the upstream station. White perch were also collected in Middle River, but unlike catches in Back River, were most abundant upstream. The number of taxa was low at all stations and differences ($P \leq 0.05$) were not determined among stations (Table F-8).

Back River and Middle River fish catches also differed in the variety of species present and in the number of fish collected per station. Trends in these parameters are shown in Figure 9-1 in which station data are scaled spatially by distance from the mouth of each river. Although relatively few species were collected in either river, slightly more were collected in the Middle River reference area on a per-trawl basis. The disparity was greatest on 7 March when six species were collected at Station M1 compared to a maximum of three at each of two stations in Back River. When station catches are combined by date, the disparity remains; seven and six species were collected at Stations M1 and M2, respectively, compared to 3, 2, 1, 4, 3, and 4 species at Stations B1 through B6, respectively.

The trends in total catch-per-trawl were strikingly similar to the two sampling dates (Figure 9-1) which lends confidence to the observed patterns. The largest catch at any station was made at Station B4 in Back River. Excluding these very large catches, opposing trends in abundance are evident in the two rivers; catches increased toward the mouth of Back River but increased toward the headwaters of Middle River. However, the average catch size in Back River and Middle River was virtually identical: 53 and 55 fish per tow, respectively, on 7 March and 64 and 60 fish per tow on 14 March.

9.2 Fish Condition

Twenty-seven types of anomalous conditions were observed among all fish examined from Back River and Middle River (Tables 9-3 and 9-4). Most abnormalities were derived from examination of the external surface of specimens. The variety of abnormalities observed per species was a function of the number of specimens examined grossly, and no single species appeared to display an unusually high variety of abnormalities.

As described in the previous section, the fish communities of Back River and Middle River were largely comprised of different species, which limits inter-area comparison of the incidence of anomalies. Brown bullhead catfish were collected almost exclusively in Back River, while pumpkinseed sunfish were largely restricted to Middle River. Only white perch were relatively abundant in each river.

Fifteen different conditions of abnormalities observed among brown bullheads in Back River on the two survey dates were recorded. Hemorrhaging of fins and the lower jaw area also was observed on virtually all specimens, apparently more severely among older fish and those collected upstream in Back River (Tables F-9 and F-10). Although this condition was the most obvious abnormality recorded, its ubiquitous occurrence precluded a *meaningful percent occurrence* tally. In addition, hemorrhaging was suspected to have been induced by the trauma of collection by trawling; the use of set nets would be more appropriate for an investigation of this abnormality.

Trends in the incidence of abnormalities among brown bullhead in Back River are difficult to discern. Only a few conditions were recorded for more than one specimen or at more than one station. Therefore, to enhance upstream/downstream differences, the data were combined for Stations B1, B2, and B3 and for Stations B4, B5, and B6. Fin erosion occurred most frequently and displayed a consistent trend on the two survey dates. It was most prevalent among specimens collected upstream, and specifically at Stations B2 and B3. Another fin anomaly, regenerated rays, was observed six times over the two dates and only among upstream specimens. Other conditions observed less frequently on both dates but which showed a higher incidence upstream include healed/

Table 9-1. Fish Catch and Water Quality Parameters, in Back River and Middle River, 7 March 1984

	Station							
Species	B1	B2	B3	B4	B5	B6	M1	M2
Brown bullhead catfish	6	12	17	126	69	1	1	2
Gizzard shad	1	1		1			1	2
Spotfin shiner	1						1	
White perch					10	74	27	7
Channel catfish						1		
Pumpkinseed sunfish							57	5
Yellow perch							3	3
Number of fish	8	13	17	127	79	76	90	19
Number of species	3	2	1	2	2	3	6	5

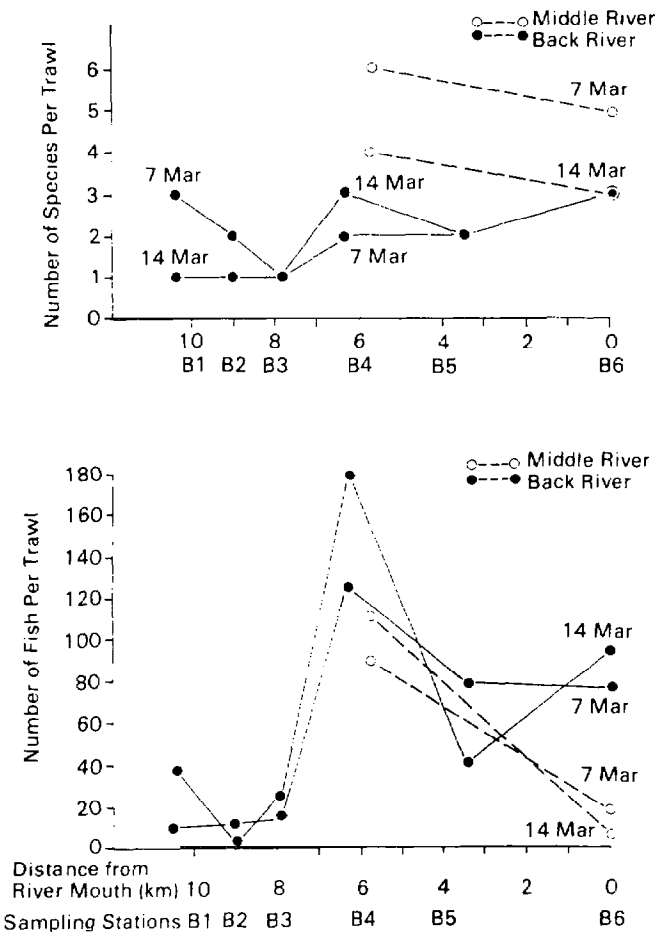
	Station							
Water Quality	B1	B2	B3	B4	B5	B6	M1	M2
Depth (m)	1.0	2.0	1.5	2.0	2.0	3.0	2.5	3.0
Temperature (C)	5.1	5.0	5.6	4.4	4.0	3.4	4.6	3.3
Dissolved oxgen (mg/L @ 25°C)	9.4	10.8	8.0	15.9	17.8	13.1	12.1	12.8
Conductivity (μmhos/cm)	1,316	1,546	1,388	2,070	2,520	4,350	2,920	2,160
pH	7.3	7.5	7.2	8.6	9.0	8.0	7.8	7.6
Hour	0925	1015	1100	1134	1242	1418	1603	1712

Table 9-2. Fish Catch and Water Quality Parameters in Back River and Middle River, 14 March 1984

	Station							
Species	B1	B2	B3	B4	B5	B6	M1	M2
Brown bullhead catfish	39	2	25	179	39			
Pumpkinseed sunfish				1	1	3	89	4
Threespine stickleback				1				
Channel catfish						1	8	
Yellow perch							5	3
White perch						91	10	
Blueback herring								1
Number of fish	39	2	25	181	40	95	112	8
Number of species	1	1	1	3	2	3	4	3

	Station							
Water Quality	B1	B2	B3	B4	B5	B6	M1	M2
Depth (m)	1.0	1.0	1.5	2.0	2.0	2.5	2.2	3.0
Temperature (C)	5.6	6.3	6.3	4.7	3.4	2.4	2.4	1.9
Dissolved oxgen (mg/L @ 25°C)	11.2	10.0	9.0	15.6	15.7	15.8	12.5	14.1
Conductivity (μmhos/cm)	1,693	1,520	1,380	1,583	2,570	3,220	2,670	2,840
pH	6.9	7.3	7.6	8.2	8.2	8.4	7.2	7.4
Hour	1621	1558	1515	1427	1312	1216	1002	1117

Figure 9-1. **Spatial comparison of fish catches in Back River and Middle River on two days in March 1984.**



healing scars and nodules/tumors. By contrast, blind eye was recorded only downstream on both dates.

Unlike brown bullheads, which lacked macroparasites, white perch and pumpkinseed were notable for the incidence of gill parasites, suspected to be *Ergasilus*, and for leeches, usually found on the fins (Tables F-11 and F-12). The incidence of *Ergasilus* was substantial in white perch from both rivers, with the rate in Middle River (Station M1) consistently higher. Over both dates, the incidence in Back River and Middle River was 34 and 51 percent, respectively. The spatial trend for leeches was similar and the overall rates for the two rivers was 2.5 and 9.1 percent, respectively. Gill raker erosion and blind eye were recorded less frequently on both dates, with the first more prevalent in Back River and the second more prevalent in Middle River.

The data for pumpkinseed sunfish provide some evidence to support the trends in the incidence of parasites among white perch (Table F-12). Although only four specimens were examined from Back River, all were free of abnormalities whereas a similar number collected at Station M2, at the mouth of Middle River, exhibited parasites and other conditions. The finding of a relatively high incidence of fin erosion (6 percent) and regenerated fin rays (14-25 percent) among upper Middle River pumpkinseed sunfish is interesting in that these two abnormalities occurred most frequently among brown bullheads collected from upper Back River.

These observations of fish condition show that the incidence of fin erosion, regenerated fin rays, and two other abnormalities is higher among brown bullheads in upper Back River compared to specimens from downriver stations. The first two abnormalities, however, were also frequently observed among pumpkinseed sunfish in the Middle River reference area. Prominent abnormalities among pumpkinseed and white perch were infestation with *Ergasilus* and leeches. The incidence of these parasites was higher in specimens from Middle River. Unfortunately, the limited distributions of bullheads and pumpkinseed largely precluded a more detailed inter-river comparison of fish condition.

9.3 Evaluation of the Fish Community

The present study demonstrated a sharp contrast between the fish communities of Back River and Middle River. Back River contained fewer species on the average and was dominated by brown bullheads Middle River was dominated by pumpkinseeds and white perch. The Middle River fauna are more representative of late winter-early spring trawl catches in the upper western embayments of Chesapeake Bay. In previous studies conducted in waters near the present study area during late February and early March of 1979 and 1980, when water temperatures were comparable to those of the present study (2.0-8.5°C), EA (1980, 1981, and unpublished data) collected no more than six brown bullheads in 10-minute trawls. Sampling in the 1979 and 1980 studies included areas of offshore of Middle River, within adjacent creeks of Seneca, Dundee and Saltpeter Creeks, and the Gunpowder River; and very often no brown bullheads were collected. The Bush River and Gunpowder River which are located near the Middle and Back Rivers were sampled intensively by EA (1974) in 1972 and 1973 with the collection of as many as 28 specimens per tow (in upper Bush River), but again, most trawls resulted in no catch or contained only a few bullheads. By contrast, white perch and yellow perch were usually dominant with frequent occurrences of pumpkinseed, tessellated darter, and spotfin shiner. The large catches of pumpkinseeds in upper Middle River in the present study were rather unique, but were similar to catches made previously in upper Dundee Creek (EA 1980).

Table 9-3. Observations of Abnormalities by Species in Back River and Middle River, 7 March 1984.

Observation	Brown Bullhead	White Perch	Pumpkinseed	Gizzard Shad	Yellow Perch	Spotfin Shiner	Channel Catfish
Body							
Muscular atrophy	X						
Healed/healing scars	X						
Nodule/tumor	X						
Spinal curvature (lordosis)	X						
Unusual coloration	X						
Small whitish spots	X						
Small dark spots	X						
Lesions		X					
Fungus—smooth, opaque slime		X					
Fins							
Erosion/fin rot	X	X		X			
Hemorrhages (reddened membranes)	X	X					
Regenerated fins, rays	X	X	X				
Missing fin	X						
Gills							
Filament erosion	X	X					
Arch cysts	X						
Filament cysts							X
Gill raker erosion		X					
Gill filament spots					X		
Eyes							
Blind	X	X					
Parasites							
Ergasilus		X	X		X		
Leech		X	X				
Number examined grossly	234	118	43	6	6	2	1
Total observation types	14	10	3	1	2	0	1

Table 9-4. Observations of Abnormalities by Species in Back River and Middle River, 14 March 1984.

Observation	Brown Bullhead	White Perch	Pumpkinseed	Channel Catfish	Yellow Perch	Three-Spinned Stickleback	Blueback Herring
Body							
Muscular atrophy			X				
Healed/healing scars	X						
Nodule/tumor	X		X				
Fungus—smooth, opaque slime					X		
Deformed jaw			X				
Pughead			X				
Fins							
Erosion/fin rot	X		X	X			
Hemorrhages (reddened membranes)	X	X					
Regenerated fins, rays	X		X				
White cysts	X			X	X		
Black cysts	X						
Gills							
Filament erosion			X				
Gill raker erosion		X					
Pale gill filament			X				
Eyes							
Blind	X	X					
Parasites							
Ergasilus		X	X		X		
Leech		X	X	X	X		
Lernea				X			
Number examined grossly	153	45	53	9	8	1	1
Total observation types	8	5	10	4	4	0	0

The lower diversity of species in Back River and dominance by brown bullhead suggest that this species is more abundant in an environment that is not generally favorable to survival of the endemic fauna. Brown bullheads are described as pollution-tolerant and omnivorous by Scott and Crossman (1973), characteristics which allow survival under stressful water quality conditions and adaptation to varying types of food items. Because the basic water quality variables measured in this study were in the normal range, it is possible that another variable, or perhaps food quality, accounts for the finding that white perch were only collected at the mouth of Back River. Although the Back River fish community reflects a degraded environment, the average number of fish caught per trawl was similar to that of Middle River. This suggests that these rivers may have been equally productive during the study period though the quality of the catch obviously differed.

With regard to the condition of fish in Back River and Middle River, the most consistent trend was a higher incidence of fin abnormalities (erosion and regenerated rays) among brown bullheads in upper Back River and compared to specimens collected farther downstream. The lack of bullheads in the Middle River reference area, however, did not allow a determination of whether a similar upstream/downstream trend existed in an unpolluted area. Although not strictly comparable, it was noted that similar fin abnormalities occurred frequently among pumpkinseeds collected upstream in Middle River. There is reason, therefore, to question whether the incidence of fin erosion (possibly due to a bacterium; myxobacterium [Post 1977]) is related to the Back River sewage treatment plant outfall.

Robertson and May (undated report) reported that brown bullheads collected from Back River in June 1982 exhibited branchiitis, an inflammation of the gill epithelium. This condition increased in severity with the proximity of specimens to the sewage outfall. In another study, the authors found that branchiitis was induced in white perch by exposure to chlorinated or unchlorinated sewage effluent, again with the severity related to the effluent concentration. This trend in anomalies could not be substantiated in the present study, because of the methods employed, but the suggestion of a relationship between sewage effluent chemicals and fish condition may be related to our finding of an absence of macroparasites on bullheads. Brown bullheads might be unsuitable hosts for *Ergasilus* and leeches, but the finding of a reduced incidence of these parasites on Back River pumpkinseed and white perch compared to Middle River specimens suggests that the sewage constituents which induce branchiitis may be toxic to external parasites. Such a finding would complicate the use of parasite loading as an indicator of fish habitat quality in Back River.

10. Comparison of Laboratory Toxicity Data and Receiving Water Biological Impact

Biological field data were collected only in the Back River outfall area. Based on the fathead minnow data, impact would be predicted at Stations B1, B2, B3, and B4. From the *Ceriodaphnia* data, impact would be expected at all six stations. The data from Microtox® effluent tests predict impact at all stations.

The number of species collected was entirely too few to confidently compare test data and impact. Among the macrozooplankton, one species comprised more than 99 percent of all individuals, and other species were at such low numbers that comparisons are unduly influenced by 1 or 2 species. For the benthos, 4, 5, 3, and 2 species were collected at Stations B1 to B4, respectively, and 8 and 12 species were collected at Stations B5 and B6, respectively, but only 1 of those was collected at Stations B1 through B4 (probably a salinity-related event). For fish, a maximum of three species was collected at a station. The unseasonably cool weather, the salinity gradient and the uncertain water quality of all Back River stations makes the causes of so few species very uncertain. If one ignores the small numbers, the trend displayed by number of species and the toxicity are very similar, i.e., B6 and B5 are less impacted than the rest and B1 seems to be somewhat less affected than Stations B2, B3, and B4 (Tables 4-10, 7-3, 8-3, and 9-1).

Therefore, the comparison of toxicity data and field impact as has been done in other reports in this series will not be made. The daphnid, Microtox®, and fathead effluent toxicity over-estimated ambient toxicity at some of the stations.

11. Effluent Fractionation Testing

Complex effluents are usually mixtures of dissolved and suspended organic and inorganic components. It is not cost-effective to chemically identify and toxicologically evaluate each individual component of a complex effluent. Chemical fractionation procedures (Parkhurst et al. 1979; Walsh and Garnas 1983) are useful in dividing complex aqueous effluents into simpler subfractions, which can then be individually screened for biological activity (i.e., toxicity) to determine if chemical identification of a subfraction's constituents is warranted. The purpose of this fractionation study was to identify the primary toxic components of complex effluents through chemical fractionation, acute toxicity testing, and chemical analyses.

The approach was to

- determine the relative toxicity of each subfraction of the whole effluent and
- establish which subfraction exhibits the highest degree of toxicity and attempt to identify chemically the toxic constituents.

11.1 Fractionation Test Results

11.1.1 Ceriodaphnia 48-Hour Acute Tests

The acute *Ceriodaphnia dubia* tests on whole effluent from the Back River and Patapsco POTWs produced relatively similar results for the four samples tested. The LC50 values (Table 11-1, and Figure 11-1) for the 3-day composite and the 7-day composite were closer for the Patapsco POTW samples (2.05 versus 3.58 percent) than for Back River POTW (1.20 and 14.6, respectively).

For the Back River POTW samples, the organic fraction of both composites was found to exhibit toxic effects on *Ceriodaphnia*; the inorganic fractions were not toxic. Upon testing of the base/ neutral and acid/phenol subfractions with the 3-day composite organic fraction, it was found that both subfractions exhibited some toxicity, although there was an absence of a concentration/effect relationship over a range of concentrations (Table G-1). The highest mortalities were noted in the next-to-lowest effluent concentrations tested (3 percent effluent). Both the base/neutral and acid/phenol organic subfractions of the 7-day composite also exhibited toxic effects, but the acute tests failed to elicit a concentration/ effect response over the range of concentrations tested (Table G-1). Maximum mortalities observed (50 percent) occurred in the 100 percent effluent concentration for both 3- and 7-day composites, so the LC50 values were not calculated but were estimated to be approximately 100 percent.

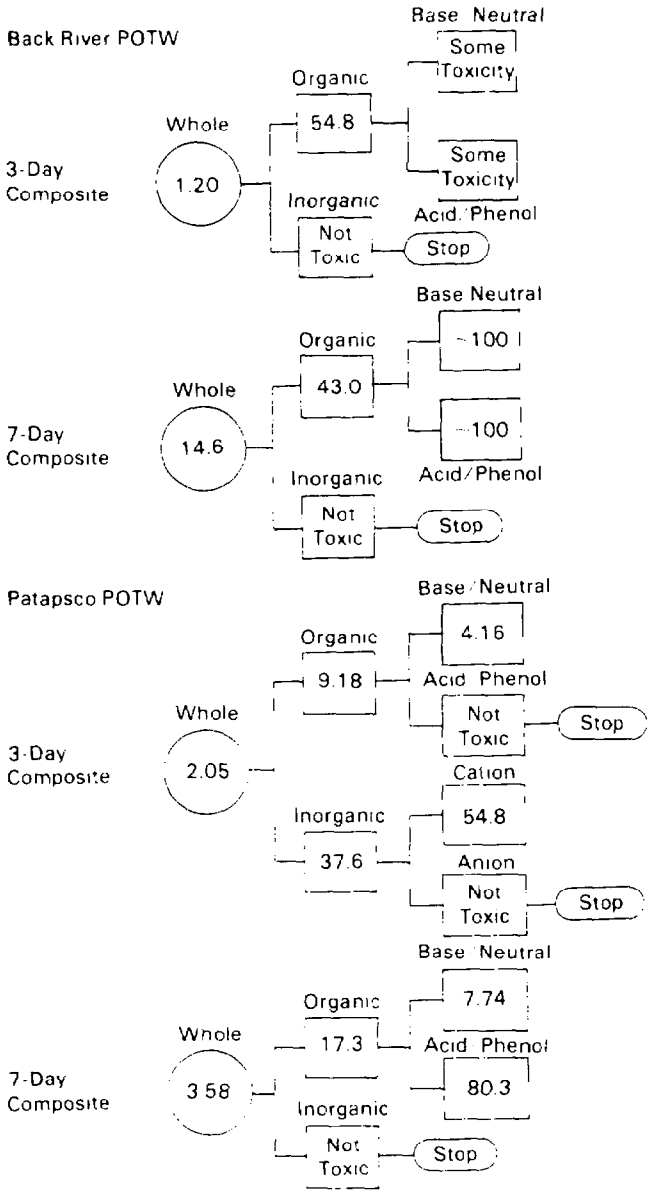
The Patapsco POTW results were slightly more complicated. The 3-day composite whole effluent sample had an LC50 value of 2.1 percent, the organic fraction had an LC50 of 9.3 percent and the inorganic fraction had an LC50 of 37.6 percent. The base/

Table 11-1. LC50 Values (in % Effluent) Calculated by Moving Average Method, Based on *Ceriodaphnia dubia* 48-Hour Acute Tests[a]

	Whole Effluent	Inorganic Fraction	Cation Fraction	Anion Fraction	Organic Fraction	Base/Neutral Fraction	Acid/Phenol Fraction
Back River POTW							
3-Day Composite Mean	1.20	Not Toxic	NA	NA	54.8[b]	Not calculated	Not calculated
95% Confidence Limits	<0.01-4.95						
7-Day Composite Mean	14.6	Not toxic	NA	NA	43.0	~100	~100
95% Confidence Limits	7.9-31.3				28.5-74.0		
Patapsco POTW							
3-Day Composite Mean	2.05	37.6	54.8[a]	Not toxic	9.18	4.16	Not toxic
95% Confidence Limits	0.5-4.13	24.7-61.8			5.96-16.2	0.97-11.2	
7-day Composite Mean	3.58	Not toxic	Not required	Not required	17.3[b]	7.74	80.3[b]
95% Confidence Limits	2.19-6.32					1.96-22.5	

[a]See Figure 11-1.
[b]Calculated by the binomial procedure.

Figure 11-1. **Schematic results (LC50 in percent effluent) of *Ceriodaphnia* acute tests on effluent fractions.**



neutral fraction of the 3-day composite sample exhibited acute toxicity to *Ceriodaphnia* (4.16 percent LC50), whereas the acid/phenol fraction did not. The inorganic fraction was further split into cation and anion fractions. The LC50 value for the cation fraction was 54.8 percent, whereas the anion fraction did not result in sufficient mortality to calculate an LC50 value (Table G-1). Thus, the majority of the toxicity noted in the 3-day Patapsco POTW composite was attributable to the base/neutral subfraction but there was some toxicity in the cation fraction. The toxicity response to the 7-day Patapsco POTW composite was similar to that noted for the Back River POTW samples in that the inorganic fraction was not toxic (Table 11-1). The organic fraction was less toxic (17.3 percent LC50) than the whole effluent (3.58 percent LC50). The base/neutral and acid/phenol subfractions both displayed some toxicity, although the LC50 values indicate that the base/neutral subfraction was considerably more toxic (7.7 percent LC50) than the acid/phenol subfraction (80.3 percent LC50).

In summary, the whole-effluent toxicities of the Back River and Patapsco POTWs were similar, but, after fractionation, the organic fraction (which contributed the most to the overall toxicity of the four samples tested) of the Back River POTW effluent had considerably less toxicity than the whole effluent. In contrast, the organic fraction of the Patapsco composites was nearly as toxic as the whole effluent, and most of the toxicity of this fraction was traceable to the base/neutral subfraction.

11.1.2 Microtox® Tests

The fractionation results of the Microtox® test were different from the *Ceriodaphnia* tests. The whole effluent, which exhibited the second greatest toxicity to *Ceriodaphnia* (Patapsco POTW 3-day composite), was the least toxic according to the Microtox® tests (Table 11-2 and Figure 11-2). Conversely, the Back River POTW 7-day composite whole effluent, which displayed the geatest toxicity according to the Microtox® tests, was the least toxic according to the *Ceriodaphnia* tests.

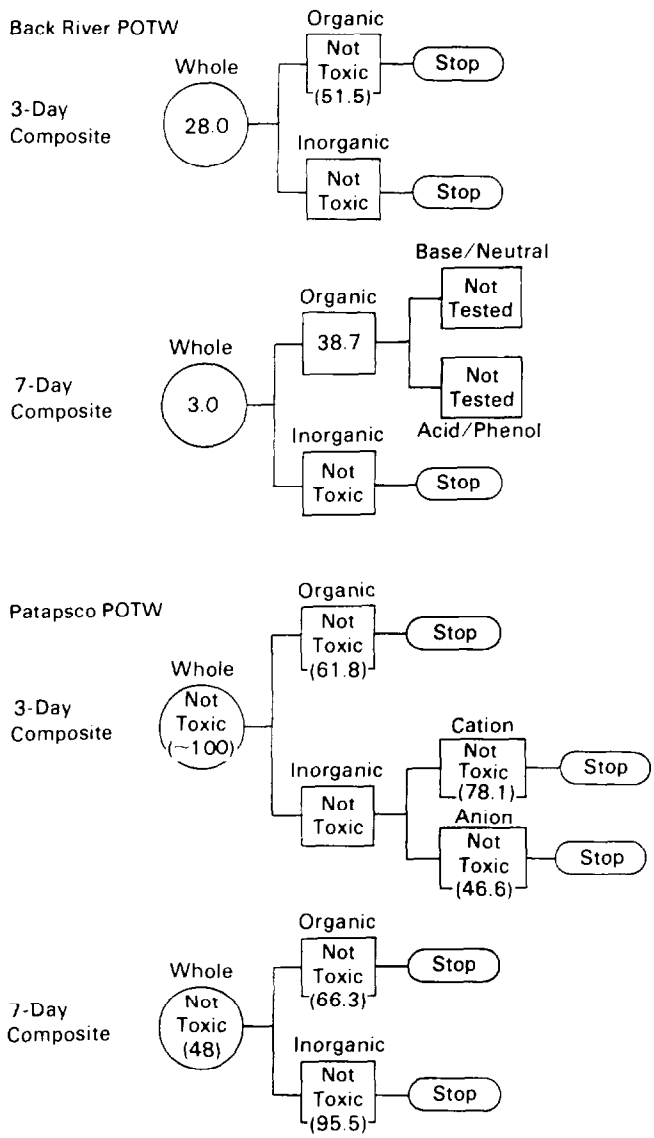
Only the Back River POTW whole effluent samples displayed toxicity in the Microtox® tests. The 7-day composite was the more toxic of the two effluent samples from Back River POTW (3.0 percent EC50 value compared to 28 percent for the 3-day composite). Neiter the organic nor inorganic fraction of the 3-day composite proved toxic according to Microtox® EC50s. The 7-day organic fraction was slightly toxic, with an EC50 value of 38.7 percent effluent. Samples with Microtox® EC50 values greater than 45.5 percent were classified as nontoxic because those values must be extrapolated. Extrapolated values (Table 11-2 and Figure 11-2) are provided only as a rough indication of toxicity. Because the organic fraction displayed limited toxicity, and since the Microtox® instrument was temporarily inaccessible when the organic samples were processed, the base/neutral and acid/phenol subfractions were not tested for Microtox® toxicity. The inorganic subfraction was not toxic according to Microtox® EC50 values. The Microtox® EC50 results agreed with the acute *Ceriodaphnia* tests in suggesting that the inorganic fractions of the Back River POTW effluent were not toxic.

Table 11-2. EC50 Values (in percent Effluent) Based on Beckman Microtox® Acute Tests[a]

	Whole Effluent	Inorganic Fraction	Cation Fraction	Anion Fraction	Organic Fraction	Base/Neutral Fraction	Acid/Phenol Fraction
Back River POTW							
3-Day Composite	28.0	Not toxic	NA	NA	Not toxic (51.5)[b]	NA	NA
7-Day Composite	3.0	Not toxic	NA	NA	38.7	Not tested	Not tested
Patapsco POTW							
3-Day Composite	Not toxic (~100)	Not toxic (>45.5)	Not toxic (78.1)	Not toxic (46.6)	Not toxic (61.8)	NA	NA
7-Day Composite	Not toxic (48)	Not toxic (95.5)	NA	NA	Not toxic (66.3)	NA	NA

[a]See Figure 11-2.
[b]Any Microtox® EC50 >45.5 percent is extrapolated and is considered not toxic.

Figure 11-2. Schematic results (EC50 value in percent effluent) of Microtox® tests on effluent fractions.



The Patapsco POTW effluent, both 3-day and 7-day composites, were found not toxic in the Microtox® tests, in contrast to their toxicity to *Ceriodaphnia*. The inorganic and organic fractions were tested by Microtox® for both composites, and were found to be not toxic (EC50 values >45.5 percent). Because the cation and anion subfractions of the 3-day composite had been tested using the *Ceriodaphnia* 48-hour acute test, their toxicities were evaluated by Microtox® as well. Both subfractions proved not toxic (EC50 values >45.5 percent).

11.1.3 Chemical Analyses of Toxic Fractions

The base/neutral subfractions of the organic fraction of the 3-day and 7-day Patapsco POTW effluents were selected for chemical analyses due to the toxicity observed in the *Ceriodaphnia* acute tests. These subfractions were analyzed for pesticides, herbicides and PCBs by gas chromatography, and for base/neutral priority pollutants by gas chromatography/mass spectrometry (GC/MS) (Appendix G). Levels of pesticides, herbicides, and PCBs (Table 11-3) and base/neutral priority pollutants (Table 11-4) were below detection limits for both the 3-day and 7-day composite Patapsco POTW samples.

Results of the GC/MS analyses for base/neutral organic compounds, including reconstructed ion chromatograms and quantitation reports for samples, standards, spikes, and blanks, are included in Appendix G.

11.2 Summary

The organic fraction contributed the most to the overall toxicity of the four effluent samples tested. However, the toxicity of a particular waste was not always traceable to one particular subfraction (i.e., base/neutral or acid/phenol). For the Patapsco POTW, the base/neutral subfraction accounted for the majority of the observed toxicity. Chemical

analyses on the base/neutral subfractions did not identify the toxic components among the pesticides, herbicides, PCBs, and priority pollutants tested. Toxicity, as measured by the acute *Ceriodaphnia* tests, were different than the toxicity as measured by the Microtox® test.

Table 11-3. Levels of Pesticides, Herbicides, and PCBs in 3-Day and 7-Day Composite Patapsco POTW Effluents

Compounds		Concentration (μg/L) 3-Day	7-Day
Aldrin	Less than	0.001	0.001
alpha BHC	Less than	0.0006	0.0005
beta BHC	Less than	0.006	0.005
delta BHC	Less than	0.001	0.0009
Lindane	Less than	0.0007	0.0006
Chlordane	Less than	0.02	0.02
p,p'-DDE	Less than	0.002	0.002
p,p'-DDD	Less than	0.005	0.005
p,p'-DDT	Less than	0.007	0.007
Dieldrin	Less than	0.003	0.002
Endosulfan 1	Less than	0.003	0.002
Endosulfan 2	Less than	0.004	0.004
Endosulfan sulfate	Less than	0.008	0.007
Endrin	Less than	0.008	0.007
Endrin aldehyde	Less than	0.009	0.008
Heptachlor	Less than	0.003	0.003
Heptachlor epoxide	Less than	0.002	0.001
Methoxychlor	Less than	0.02	0.01
Mirex	Less than	0.009	0.008
Toxaphene	Less than	0.3	0.3
Aroclor 1016	Less than	0.03	0.03
Aroclor 1221	Less than	0.1	0.1
Aroclor 1232	Less than	0.04	0.04
Aroclor 1242	Less than	0.03	0.03
Aroclor 1248	Less than	0.03	0.03
Aroclor 1254	Less than	0.04	0.04
Aroclor 1260	Less than	0.05	0.05
2,4-D	Less than	0.02	0.01
2,4,5-TP	Less than	0.003	0.003

Table 11-4. Levels of Base/Neutral Compounds, Determined by GC/MS Analysis (EPA Method 625), for 3-Day and 7-Day Patapsco POTW Effluents

Base/Neutral Compounds		μg/L 3-day	7-Day
N-Nitrosodimethylamine	Less than	1.2	1.1
Bis(2-chloroethyl)ether	Less than	0.40	0.37
1,3-Dichlorobenzene	Less than	0.40	0.37
1,4-Dichlorobenzene	Less than	0.40	0.37
1,2-Dichlorobenzene	Less than	0.40	0.37
Bis(2-chloroisopropyl)ether	Less than	0.40	0.37
Hexachloroethane	Less than	0.40	0.37
N-Nitroso-di-n-propylamine	Less than	0.80	0.75
Nitrobenzene	Less than	1.2	1.1
Isophorone	Less than	0.40	0.37
Bis(2-chloroethoxy)methane	Less than	0.40	0.37
1,2,4-Trichlorobenzene	Less than	0.40	0.37
Naphthalene	Less than	0.40	0.37
Hexachlorobutadiene	Less than	0.40	0.37
Hexachlorocyclopentadiene	Less than	1.2	1.1
2-Chloronaphthalene	Less than	0.40	0.37
Acenaphthylene	Less than	0.40	0.37
Dimethyl phthalate	Less than	0.40	0.37
2,6-Dinitrotoluene	Less than	0.40	0.37
Acenaphthene	Less than	0.40	0.37
2,4-Dinitrotoluene	Less than	0.40	0.37
Fluorene	Less than	0.40	0.37
Diethyl phthalate	Less than	0.40	0.37
4-Chlorophenyl phenyl ether	Less than	0.40	0.37
N-Nitrosodiphenylamine	Less than	0.40	0.37
1,2-Diphenylhydrazine	Less than	0.40	0.37
4-Bromophenyl phenyl ether	Less than	0.40	0.37
Hexachlorobenzene	Less than	1.2	1.1
Phenanthrene	Less than	0.40	0.37
Anthracene	Less than	0.40	0.37
Di-n-butyl phthalate	Less than	0.40	0.37
Fluoranthene	Less than	0.40	0.37
Benzidine	Less than	8.0	7.5
Pyrene	Less than	0.40	0.37
Butyl benzyl phthalate	Less than	0.40	0.37
Benzo(a)anthracene	Less than	0.40	0.37
3,3'-Dichlorobenzidine	Less than	1.2	1.1
Chrysene	Less than	0.40	0.37
Bis(2-ethylhexyl) phthalate	Less than	4.0	3.7
Di-n-octyl phthalate	Less than	0.40	0.37
Benzo(a)pyrene	Less than	0.40	0.37
Indeno(1,2,3-cd)pyrene	Less than	0.80	0.75
Dibenzo(a,h)anthracene	Less than	0.80	0.75
Benzo(g,h,i)perylene	Less than	0.80	0.75
Unresolved Isomeric Pairs			
Benzo(b)fluoranthene+ benzo(k)fluoranthene	Less than	0.80	0.75

References

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard Methods for the Examination of Water and Wastewater, 15th edition. APHA, Washington, 1,134 pp.

Beckman Microtox® System Operating Manual. 1982. Beckman Instructions 015-555879. Beckman Instruments, Inc. Microbics Operations, Carlsbad, CA 92008.

Courtemanch, D. L. 1983. The use of a coefficient of community loss to assess environmental degradation. Presented at the 31st Annual Meeting, North American Benthological Society, 27-29 April, 1983, LaCrosse, WI.

Cronin, L. E., J. C. Daiber, and E. M. Hulbert. 1962. Quantitative seasonal aspects of zooplankton in the Delaware River Estuary. Chesapeake Sci., 3:63-93.

Dovel, W. L. 1971. Fish eggs and larvae of the upper Chesapeake Bay. NRI Spec. Rept. No. 4. Natural Resources Institute, Univ. Md. 71 pp.

Ecological Analysts, Inc. (EA). 1974. Perryman Site: Power Plant Site Evaluation, Aquatic Biology, Final Report. PPSE 2-2. Prepared for Maryland Power Plant Siting Program, Annapolis, MD. 193 pp.

Ecological Analysts, Inc. (EA). 1980. C. P. Crane Power Plant: An Environmental Assessment and Ecological Survey of the Aquatic Biota. Annual Report, January-December 1979. Prepared for Baltimore Gas and Electric Company.

Ecological Analysts, Inc. (EA). 1981. C. P. Crane Power Plant: An Environmental Assessment and Ecological Survey of the Aquatic Biota, Final Report, 1978-1980. Prepared for Baltimore Gas and Electric Company. EA Report BGE02K2.

Hamilton, M. A. 1984. Statistical Analysis of the Seven-Day *Ceriodaphnia reticulata* Reproductivity Toxicity Test. EPA Contract J3905NASX-1. 16 January. 48 pp.

Heinle, D. R. and D. A. Flemer. 1975. Carbon requirements of a population of the estuarine copepod *Eurytemora affinis*. Marine Biology, 31:235-247.

Hunter, J. R. 1975. A one-dimensional dynamic and kinematic numerical model suitable for canals and estuaries. Chesapeake Bay Institute, The Johns Hopkins University. Spec. Rept. 47, Ref. 75-10.

Marking, L. L. and V. K. Dawson. 1973. Toxicity of Quinaldine Sulfate to Fish. Invest. Fish. Contrib. No. 48. U.S. Fish and Wildlife Service, Washington, DC. 8 pp.

Mount, D. I. and T. J. Norberg. 1984. A seven-day life cycle cladoceran toxicity test. Environ. Toxicol. Chem., 3(3):425-434.

Mount, D. I. and T. J. Norberg-King (1986). The Validity of Effluent and Ambient Toxicity Test for Predicting Biological Impact, Kanawha River, Charleston, West Virginia. EPA/600/3-86/006.

Norberg, T. J. and D. I. Mount. 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. Environ. Toxicol. Chem., 4(5).

Parkhurst, B. R., C. W. Gehrs, and I. B. Rubin. 1979. Value of chemical fractionation for identifying the toxic components of complex aqueous effluents. *In*: Aquatic Toxicology (L. L. Marking and R. A. Kimerle, eds.), pp. 120-130. ASTM STP 667. American Society for Testing and Materials, Philadelphia, PA.

Post, G. 1977. Glossary of Fish Health Terms. Fish Health Sect., Amer. Fish. Soc., Washington, DC. 48 pp.

Robertson, P. G. and E. B. May. No date. Pathological stress of fish linked to POTW discharges. Tech. Anal. Div., Off. Environ. Programs, Md. Dept. Health and Mental Hygiene. 6 pp.

Scott, W. B. and E. G. Crossman. 1973. Freshwater Fishes of Canada. Bull. 184. Fish. Res. Bd. Can., Ottawa. 966 pp.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Company, New York, NY.

Stimpson, K. S., D. J. Klemm, and J. K. Hiltunen. 1982. A Guide to the Freshwater Tubificids (Annelida: Clitellata: Oligochaeta) of North America. EPA/600/3-82/033. April. 61 pp.

U.S. EPA. 1979. Methods 624 and 625: GC/MS methods for priority pollutants. Federal Register, 44(233):69532-69558.

Walsh, G. E. and R. L. Garnas. 1983. Determination of bioactivity of chemical fractions of liquid wastes using freshwater and saltwater algae and crustaceans. Environ. Sci. Technol., 17(3):180-182.

Appendix A
Toxicity Tests and Analytical Methods

A.1 Sampling and Sample Preparation

Sampling of Patapsco and Back River POTW was done using ISCO* samplers set to collect an aliquot every 15 minutes and to composite the sample into a five-gallon polyethylene container. About 15 L of sample was collected each 24-hour period and a new composite sample was taken each day. On the first two collection days, 9 and 10 March, unseasonably cold weather froze the ISCO samplers and a grab sample had to be used.

The Back River and Middle River ambient samples were taken at low slack tide as a grab sample, at 0.5 meters in depth. The three Patapsco River ambient samples were grab samples taken between 8:00 a.m. and 12:00 noon each day. About 16 L were collected in collapsible polyethylene containers.

Reconstituted water was made using the formula of Marking and Dawson (1973) (moderately hard option) at the Environmental Research Laboratory in Duluth, Minnesota, and stored in five gallon polyethylene jugs. Water was kept at room temperature until used. All effluents were diluted with reconstituted water. The salinity test was set up using seawater diluted with the same reconstituted water stock to make the appropriate salinity test concentrations. The seawater was provided by the EPA-Narragansett and was from their laboratory seawater supply.

Effluent dilutions were made using polypropylene or polyethylene laboratory ware. The values were measured using graduated cylinders of various sizes and 4 L beakers for mixing. Samples were warmed to 25°C and then aerated until supersaturation was removed as measured by dissolved oxygen levels of 8.5-9.0 mg/L. For the effluent dilution tests, 100 percent effluent and 100 percent dilution water were warmed separately and aerated before being mixed. All samples were used within six hours of collection. Two liters of each exposure water was made and 170 ml was used for the *Ceriodaphnia* tests and the remainder used for the fathead minnow test. Because of BOD in some samples, the daily renewal volume for the fathead minnow test was reduced to 1 L in the Back River ambient samples on day 4 of testing.

After the 2 L was prepared, DO, pH, conductivity and/or salinity was measured. When the daily renewal was made, DO was measured in one compartment of each chamber in the fathead minnow test and in one cup of the *Ceriodaphnia* test in each exposure. At least once, DO was measured in the fathead minnow tests soon after the lights were turned on to determine diurnal DO cycles, but none were found.

A.2 *Ceriodaphnia* Test Methods

The protocol followed in general that of Mount and Norberg (1984) with a few exceptions. A hard, transparent, plastic, one-ounce cup was used in place of 30-ml glass beakers, and the cups were discarded after use. Each day, a new and different sample of effluent or ambient water was used. The initial measurements, for pH, DO, salinity, and conductivity were made on the 2 L volume and are pertinent for both tests. For the final DO measurement, one cup from each exposure condition was used to measure final DO.

A new food formulation was used which consisted of three parts: (1) 5 g/L of dry yeast; (2) 5 g/L of Cerophyl®,* , stirred overnight and filtered through a plankton net; and (3) 5 g/L of trout chow, aerated vigorously for seven days, settled and decanted. The yeast suspension and the supernatant from the Cerophyl® and trout chow are mixed in equal parts, and new food was made every seven days. The mixture was kept refrigerated as are the Cerophyl® and yeast components, while the trout chow supernatant remained frozen until the mixture was made. In our experience, this food was suitable for a wide variety of water types, including reconstituted water. Because the suspended solids concentrations are ~1,800 mg/L, which is less than half the solids contained in the yeast suspension, this mixture is fed 0.1 ml per day per *Ceriodaphnia* rather than 0.05 ml as was recommended for yeast (Mount and Norberg 1984).

*ISCO, Inc., Lincoln, Nebraska

*Cerophyl® was obtained from Agri-Tech Kansas City, Missouri As of January 1985 Cerophyl® was no longer produced by that manufacturer

All test animals were less than 2-hours-old and were produced from adults that were 11-14 days of age. The cultures were at pH 7.1 and no acclimation to pH was necessary when the test animals were placed in the exposure chambers.

A.3 Fathead Minnow Test Method

The methods for the fathead minnow test followed closely those described by Norberg and Mount (1985). The test chambers were 30.5 x 5.2 x 10.2 cm, and divided into four compartments; this design allowed four replicates for each concentration. Less than 24-hour-old posthatch fathead minnow larvae were air shipped from the Duluth culture to the mobile laboratory, and were assigned to the exposure chambers immediately upon arrival. The fish were assigned to the test compartments by pipetting one or two fish at a time to each replicate test chamber until all replicates had 10 fish in each or 40 per concentration. Uneaten brine shrimp were removed daily by siphoning the tanks during test solution renewal. At the same time, the volume in the test chamber was drawn down to 1 cm, after which 2 L of new test solution was added. Because the Back River ambient samples had a significant BOD, the volume put in each chamber daily was reduced to 1 L on day 4 of the test to improve the surface-to-volume ratio. A 16-hour light photoperiod was used.

After 7 days of exposure, the fish were preserved in 4 percent formalin. Prior to weighing, they were rinsed in distilled water. Then each group was dried for 18 hours in preweighed aluminum pans and weighed on a five-place analytical balance.

A.4 *Ceriodaphnia* Statistical Analyses

The statistical analyses of the *Ceriodaphnia* data were performed using the procedure of Hamilton (1984) as modified by J. Rogers (1984). The essential features of the analysis are that a mean young production per live adult is calculated for each day young were observed, and these means are summed over the period of the test to give a 7-day estimated mean production per adult, ignoring mortality (all data method). In this way, the adults which die during the test do not reduce the estimate of young production. The variance and confidence intervals of the estimates were derived from a distribution generated by the bootstrap method, using a sample size of 999. The multiple comparisons for effluents were made using Dunnett's test. Multiple comparisons for ambient toxicity tests are made using Tukey's Honestly Significant Difference Test. The multiple comparison procedures were modified to compensate for different variances and degrees of freedom for different tests.

The survival, defined as the number of adults alive at the beginning of the last observation period was transformed using an arcisine transformation for binomial proportions. The variance and confidence intervals of the transformed survival and the correlation of the survival and reproduction estimates were derived from the bootstrap method as above. The multiple comparisons for the survival followed the same procedures as for the reproduction.

A.5 Fathead Minnow Statistical Analysis

The four mean group weights are statistically analyzed with the assumption that the four compartments behave as replicates. The method of analysis used assumes the variability in the mean treatment response as proportional to the number of fish per treatment. MINITAB (copyright Pennsylvania State University, 1982) was used to estimate a t-statistic for comparing the mean treatment and control responses using weighted regression with weights equal to the number of measurements in the treatments. The t-statistic is then compared to the critical t-statistic for the standrd Dunnett's test (Steel and Torrie 1960). Prior to the regression analysis, the survival data are arcsine transformed (which is a variance-stabilizing transformation).

A.6 Microtox® Testing Methods

The Microtox® System was utilized to conduct toxicity tests on both the effluent and ambient samples. Procedures for the tests followed those described in Beckman's "Microtox System Operating Manual." This toxicity test is based on increases or decreases in the natural light emissions of the luminescent marine bacteria *Photobacterium phosphoreum* (Beckman no date). All tests were performed on the Beckman Microtox® Model 2055 Toxicity Analyzer. Turbidity was determined not to be a problem with any sample. The color correction method was not used on any of the tests. The instrument was calibrated each day according to manufacturer's specifications. All data were recorded permanently on Beckman Microtox® chart paper.

A.6.1 Microtox® Effluent Samples

All effluent test concentrations were prepared using serial dilutions of 2:1 or 3:1. The salinity of all samples was adjusted to 2 percent NaCl using Microtox® osmotic adjusting solution prior to the preparation of dilutions. The effluent samples were run in dupliate using four or five concentrations and a control. If 100 percent sample were to be tested, it was run separately from the serial dilutions with its own control. All 100 percent samples were treated identical to the ambient stations; this resulted in a final concentratoin being assayed of 90.1 percent. All

dilutions were made using Microtox® diluent. The lyophilized reagent bacteria was rehydrated using Microtox® reconstitution solution. Ten microliters of the reagent was then introduced into each of the 10 cuvettes to be charged with the test solutions. The reagent was allowed to acclimate for 15 minutes and at the end of this time period the light output from each cuvette was measured. Immediately after this initial reading (I_0), each cuvette was charged with test solution, and at the end of five minutes (I_5) and 15 minutes (I_{15}) the light output from each cuvette was recorded again. All data were recorded on Beckman Microtox® chart paper and normalized using the Sharp Model EL1500 calculator. Toxic effects were defined as the concentration causing 50 percent reduction in light output after 5 or 15 minutes exposure to the effluent ($5EC_{50}$, $15EC_{50}$). Effect concentrations for those effluents tested at 100 percent (90.1 percent actual concentration) were based on extrapolations.

A.6.2 Microtox® Ambient Samples

All ambient samples were salinity adjusted to 2 percent NaCl using Microtox® osmotic adjusting solution. This adjustment resulted in a final test concentration of 90.1 percent. Each sample and control was run in duplicate or triplicate depending on the time available. The tests were initiated by pipetting 10 μl of rehydrated bacteria reagent into each of the cuvettes containing sample. Five and fifteen minutes after the introduction of the reagent, light measurements were recorded. These data were reduced by calculating the mean percent differences in light output between the control and each sample tested. These differences were interpreted as either an increase in light output (stimulation) or a decrease in light output (inhibition).

Appendix B
Hydrological Sampling and Analytical Methods

B.1 Patapsco River Survey

B.1.1 Dye Injection

A 20 percent solution of rhodamine WT dye was injected into the Patapsco POTW flow at the downstream end of the chlorine contact chamber, just upstream of the pump. Injection began at 1345 hours on 21 March and was terminated at 1550 hours on 22 March. During that time 37.3 lbs of solution were pumped, which is equivalent to 3.6×10^{-2} g/sec of pure dye.

The average flow through the plant on 22 March was 37.9 mgd (million gallons per day), or 1.66×10^{6} g/sec. Therefore, the average dye concentration at the discharge was

$$\frac{3.6 \times 10^{-2}}{1.66 \times 10^{6}} = 21.7 \text{ ppb} \qquad \text{(Equation B-1)}$$

B.1.2 Dechlorination

Chlorine residuals in the Patapsco POTW effluent are high enough to oxidize the rhodamine molecule. To prevent this, a 38 percent solution of sodium thiosulfate was injected along with the dye. The sodium thiosulfate is acted on preferentially by the chlorine and the rhodamine remains intact provided thiosulfate concentrations remain about 5.6 times the chlorine concentrations (APHA et al. 1981, p. 786).

The injection rate of the thiosulfate was 690 ml/min, which for a plant flow of 37.9 mgd will protect the rhodamine against chlorine residuals up to 0.6 mg/L.

B.1.3 Dye Sampling Procedures

Dye was sampled on 22 March from two boats, one making horizontal measurements and the other making vertical measurements. Each boat was outfitted with a Turner Designs Model 10 fluorometer in the continuous-flow configuration, a temperature sensing device, and a sampling pump. The fluorometer is capable of measuring Rhodamine dye to concentrations of 0.01 μg/l. Decay processes of the Rhodamine dye were considered to be minimal, if any. Standard fluorometric practices were used.

The boat making horizontal measurements had a rigid airfoil-shaped probe attached to its side. Polyethylene tubing was inserted through this probe and fed to the fluorometer intake. From the fluorometer, the tubing led to the temperature sensor and from there to the sampling pump and back over the side. The end of the probe was 0.5 m below the surface. The boat traversed the dye plume in a "ladder" fashion following the dye upstream and downsteam until fluorescence levels fell to background values.

The boat making vertical measurements had a weight affixed to the end of the sampling tubing, but was otherwise configured the same. Measurements were made from the surface to the bottom in 1-m increments.

The "horizontal" boat navigated using a Motorola Mini-Ranger system. The "vertical" boat used an electronic distance meter (EDM) with a person on shore who would note the distance and measure the angle between the boat and a reference direction using a surveyor's transit.

B.2 Back River and Middle River

B.2.1 Dye Injection and Sampling Procedures

Dye was injected from an anchored dinghy approximately 50 yd downstream of the treatment plant outfall. The dye was a 20 percent solution of rhodamine WT and was pumped into the water at a rate of 12 ml/min using a precision metering pump driven by a 12 VDC automotive battery. The pump was started at 1445 hours on 7 March 1984.

On the morning of 17 March, it was discovered that the battery had been stolen and, since the injection equipment had been seen to be working shortly before 1600 hours on 16 March, it is estimated that injection stopped around 1700 hours on 16 March.

Two boats were used to map the distribution of the dye. Each was equipped with a Turner Designs Model 10 fluorometer, a temperature sensing device, and a sampling pump. Water was drawn in through a probe mounted to the side of the boat 0.5 m below the surface, and was then passed through polyethylene tubing to the fluorometer, the temperature sensor, the sampling pump, and then back over the side. This

procedure enabled a continuous record of dye-induced fluorescence to be obtained as a boat traversed a river transect. The temperature sensor is necessary because dye fluorescence is a function of temperature, and fluorometer readings must be related to instrument calibrations through a common temperature to which all values are corrected.

One boat sampled Transects 2A through 6 (Figure B-1), and the second boat sampled Transects 7 through 11. Transects 1 and 2 had to be abandoned because the water was too shallow. Mappings were done on 11, 13, 15, 17, and 20 March as summarized in Table B-1. Boat position was interpolated assuming a constant speed from bank to bank.

B.2.2 Tide Measurements

A Stevens Model F-68 recording tide gauge was placed at the mouth of the river on the south side at Cuckold Point. The record has several breaks due to icing conditions in the stilling well, as well as wave overtopping during unusually high seas. The breaks were filled in by correlating the usable record with the NOAA tide gauge at Fort McHenry and calculating the Back River tide by applying the derived amplitude and phase correction.

B.2.3 Description of One-Dimensional, Cross-Sectionally Averaged Model

The numerical model which was used to simulate the Back River hydrodynamics is an adaptation of Hunter's one-dimensional model (Hunter 1975) as it was applied to the Chesapeake and Delaware (C&D) Canal. The model computes tidal elevation, flow, salinity, and contaminant concentrations at interior points given assigned boundary values and interior sources and sinks. The model output was used as a correction to field measurements.

The computational algorithm is based on a finite difference representation of the momentum and continuity equations. Non-advective transport is con-

Figure B-1. Map showing the Back River segmentation scheme and water sampling locations.

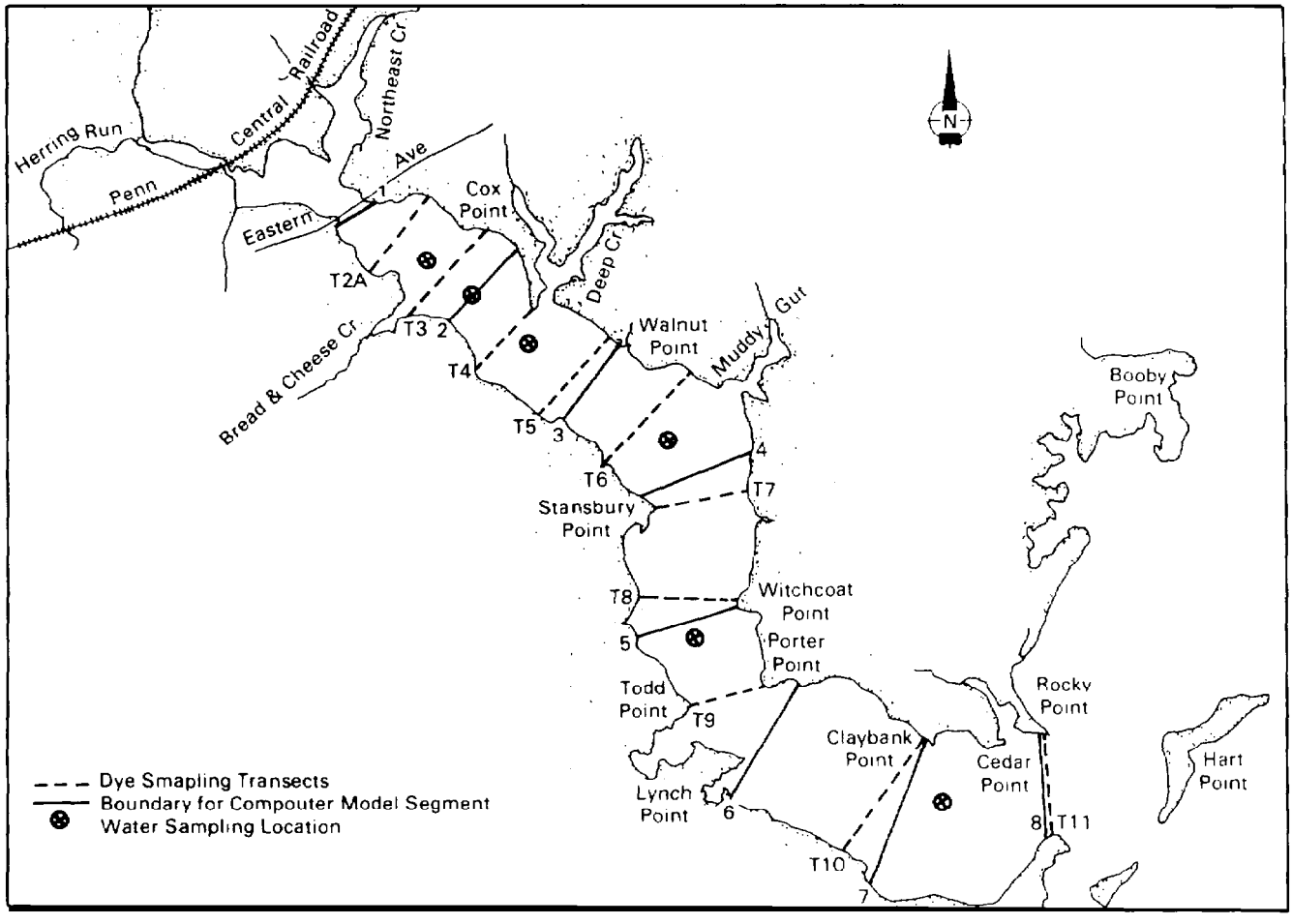


Table B-1. **Dye Plume Mappings (Transects and Times)**

Date	Sampling Station									
	2A	3	4	5	6	7	8	9	10	11
11 Mar 84	1416	1451	1504	1515	1541	1602				
13 Mar 84	1143	1154	1211	1222	1238	1251	1250	1240	1225	1214
	1322	1330	1341	1351	1404	1411	1402	1352	1338	1325
	1536	1544	1552	1602	1613	1631	1622	1613	1558	1544
15 Mar 84	0946	0955	1005	1019	1031	1036	1027	1018	1003	0950
	1143	1154	1210	1220	1234	1300	1250	1236	1223	1213
	1301	1310	1324	1334	1353	1414	1405	1355	1340	1326
	1417	1432	1447	1459	1511	1535	1525	1515	1500	1447
	1610	1619	1637	1646	1701	1759	1751	1742	1730	1718
17 Mar 84	1224	1239	1256	1312	1330	1346	1144	1127	1107	1045
20 Mar 84	1430	1422	1414	1354	1339	1325	1313	1255	1234	1220

trolled by an exchange coefficient which is itself a function of the hydraulic radius, Manning's "n," and a single-valued diffusion factor which is used to calibrate the model to observed data.

The model requires that the river be subdivided into sections, the sizes of which are constrained by the stability condition that the relation between the section lengths (ΔX) and the computational time step (Δt) consistent with the following

$$\Delta t < \frac{\Delta X}{gD} \qquad \text{(Equation B-2)}$$

where g is the acceleration due to gravity and D is river depth. The Back River was divided into seven sections 1,600 m long which allows a time step of 300 seconds.

Geometric data for the model schematization were taken from NOAA chart 12278. Required input includes "typical" values of total surface width, channel width, and depth for each section. The "typical" values of width were derived by averaging the widths from one-half a space step upstream to one-half a space step downstream. Total surface width includes side embayments; channel width does not. These side embayments act as storage areas only and do not directly participate in the transport of momentum. The dye concentration data were averaged over the cross section at each of the transects. To do this, each transect was divided into 20 segments, and the chart recording of dye fluorescence was also divided into 20 segments. The sum of the products of the segment areas and the dye concentrations divided by the total cross-sectional area yielded the cross-sectionally averaged dye concentration as required by the model. This procedure assumes that the dye is vertically mixed which is to be expected in shallow water with March weather conditions. Vertical measurements on 17 March confirmed the validity of this assumption.

Freshwater inflow to the Back River is dominated by the treatment plant flow. Surface run-off averages less than 0.2 m^3/sec, whereas typical plant flows are 3 or 4 m^3/sec. For this reason, river flow was neglected and hourly values of plant flow were input into Section 1 of the model.

B.2.5 Calibration of Model

Back River is only about 12 km in length which is much shorter than a tidal wavelength for the dominant M_2 constituent. This makes it very difficult to calibrate a model for hydrodynamic response, because tide gauges and/or current meters are not able to resolve the slight differences caused by changes in Manning's "n," which is the only parameter available for hydrodynamic calibration. In lieu of a calibration based on field data, Manning's "n" was set to 0.020, which is the value that was used when this model was applied to the C&D Canal and for a similar model of the Potomac River where field data were used for calibration.

The mixing and flushing characteristics of the model are adjusted by two parameters—the diffusion factor and the distance assigned to the "oceanic" source of the contaminant. The diffusion factor is used in calculating exchange coefficients as discussed above. The distance to the "oceanic" value of the contaminant is a length scale used in a model algorithm for predicting the influx of contaminant on the flood tide. The term "oceanic" refers to a reservoir of constant contaminant concentration.

Salinity was not included in the model because a sensitivity test indicated that salinity contributions

are not significant for salinity values at the mouth between 0 and 15 ppt.

The best fit to the observed dye data was obtained with the diffusion factor set at 150 and the distance to the "oceanic" source set at 10 km (approximately one tidal excursion).

Appendix C
Biological Survey Sampling and Analytical Methods

C.1 Plankton Survey

Oblique bottom and near surface tows were made at eight stations in Back River and Middle River (Figure 2-1) using a double sled fitted with two 505-μm mesh, 0.5-m nets. The sled was towed for 5 minutes at each depth for a total of 10 minutes. Tows were made only near surface at shallow stations. A General Oceanics Model 2030 digital flowmeter was mounted in the mouth of each net and a third one was mounted on the sled outside the net to facilitate detection of net clogging or meter malfunction. Tows were made against the current. Each sample was placed in a labeled 945-ml (1-qt) jar and preserved in 10 percent buffered formalin.

Water quality measurements consisting of temperature, dissolved oxygen, pH, and conductivity were taken concurrently with plankton sampling at each station.

Samples were examined in the laboratory under a dissecting microscope and all macrozooplankton, except the copepods, were enumerated, sorted into major taxonomic groups, and preserved in 75 percent ethanol for later identification. All organisms were identified to the lowest practical taxon and counted.

Copepod densities were so high that subsampling was required on all samples. *Eurytemora affinis* was the only species of copepod observed in the subsamples. Depending on sample density, the sample was either split with a Folsom plankton splitter, or 1.0- or 2.0-ml aliquots were taken with a Hensen-Stempel pipette. Each subsample was put into a Ward counting wheel and all copepods were counted. If necessary, additional subsamples were examined until at least 400 individuals were enumerated.

The number of copepods in the examined subsample, the volume of subsamples examined, and the adjusted volume of sample from which the subsamples were taken were recorded so that organism number could be converted to organism density during the initial phases of data tabulation. Density was determined from the equation

$$D = n(V_s/V_a) / K(R_f - R_i) \qquad \text{(Equation C-1)}$$

where

D = number of organisms/100 L (density)

n = number of organisms counted in aliquot

V_s = volume of diluted sample

V_a = volume of aliquot

R_f = final flowmeter reading

R_i = initial flowmeter reading, and

K = flowmeter calibration factor (100 L/count).

This calculated density was used in all later data analyses.

C.2 Benthic Macroinvertebrate Survey

A petite Ponar grab sampler (232 m^2) was used to collect three replicate samples at each station. Samples were washed in the field through a No. 30 mesh screen (595 μm) to remove fine silt and clay particles, placed in 945-ml labeled jars, and preserved in 10 percent buffered formalin.

Water quality measurements consisting of temperature, DO, pH, and conductivity were taken concurrently with benthos sampling at each station. Qualitative determinations of the sediment type were also made at each station.

Samples were sorted in the laboratory with the aid of a dissecting microscope. Organisms were enumerated, sorted into major taxonomic groups, and preserved in 75 percent ethanol for later identification. All organisms were identified to the lowest practical taxon using appropriate keys and references. Oligochaetes and chironomid larvae were mounted on microslides prior to identification.

C.3 Fish Survey

Fish were collected at six stations in Back River and at two reference stations in Middle River (Figures 3-1 and 3-2). At each station, a 4.9-m wide (16-ft) otter trawl was towed at 1 m/sec for 10 minutes (600 meters). Specimens were identified and counted. Up to 20 specimens of each species were also examined closely for morphological anomalies, evidence of diseases, and for parasites. This level of study included examination of the gills, arches, and the gill cavity surfaces. Additional specimens, if available, were only examined grossly, i.e., the gill cavity was not opened. Water quality parameters were also reported.

The number of specimens of each species was tallied by station. The variety of abnormalities was listed, and the incidence of conditions among the examined specimens was determined for several species.

Appendix D
Effluent Fractionation and Toxicity Testing Methods

D.1 Sampling

An effluent fractionation procedure was used to detect toxic constituents in the effluents of the Patapsco and Back River POTWs. Two composite effluent samples, one a 3-day composite, and one a 7-day composite, were analyzed from each plant, resulting in a total of four samples. The composites were 19 L (5 gal) each in volume. The 3-day and 7-day composites were initiated on the same day.

D.2 *Ceriodaphnia* Culture, Maintenance, and Testing

Ceriodaphnia dubia was cultured in EA's laboratory in moderately hard reconstituted water (Table D-1) spiked with 7 ml of 5 g/L yeast solution per liter of water four days prior to usage. Cultures were kept on a 16-hour light, 8-hour dark photoperiod at 25°C in an environmental chamber and are fed a solution of yeast and cerophyll daily, then thinned as necessary to maintain healthy, productive, cultures. Adults from these cultures were separated into lots of 300 at least one day prior to test initiation and put in 1-L culture bowls and fed heavily. The morning of the test, gravid adults were separated into lots of 100 and put into 4.5-in. culture dishes and fed. This ensured that neonates used were of a specified age, preferably less than 8 hours. During testing, organisms were fed 2 drops of yeast solution per cup.

Dilution water for test solutions was moderately hard reconstituted water spiked with yeast four days prior to testing. This water also served as control water.

Table D-1. Formulation for Moderately Hard Reconstituted Water and Final Water Quality Ranges

Reagent Added (mg/liter)			
$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl
96	60	60	4

Final Quality		
pH [a]	Hardness [b]	Alkalinity [b]
7.4-7.8	80-90	60-70

[a] Approximate pH after equilibrium.
[b] Expressed in mg/liter as $CaCO_3$.

Acute lethality tests lasting 48 hours were performed in 1-oz portion cups using the following test concentrations: 1.0, 3.0, 10.0, 30.0, and 100.0 percent plus a dilution water control. Each concentration had 10 replicates with one organism per replicate. Effluent and diluent were filtered through a 100-μm mesh to remove large particles or any organisms that may be present. Final volumes of 180 ml were mixed in 250 ml Class A graduated cylinders. Small volumes of effluent were first measured in Class A pipettes, then added to the graduate and brought to volume with dilution water. The entire 180 ml of test solution was poured into a dispenser calibrated to deliver 10 separate 15-ml portions. Neonates were then randomly added, one per cup.

Water quality determination was performed on the following schedule: pH, alkalinity, hardness, and conductivity at sample receipt; pH, DO, and temperature at each renewal on one replicate control, low, medium, and high test concentrations. Test vessels were kept at 25 ± 2°C on a 16-hour light, 8-hour dark photoperiod cycle at a light intensity of 50 f.c. Analytical methods were conducted according to APHA et al. (1980).

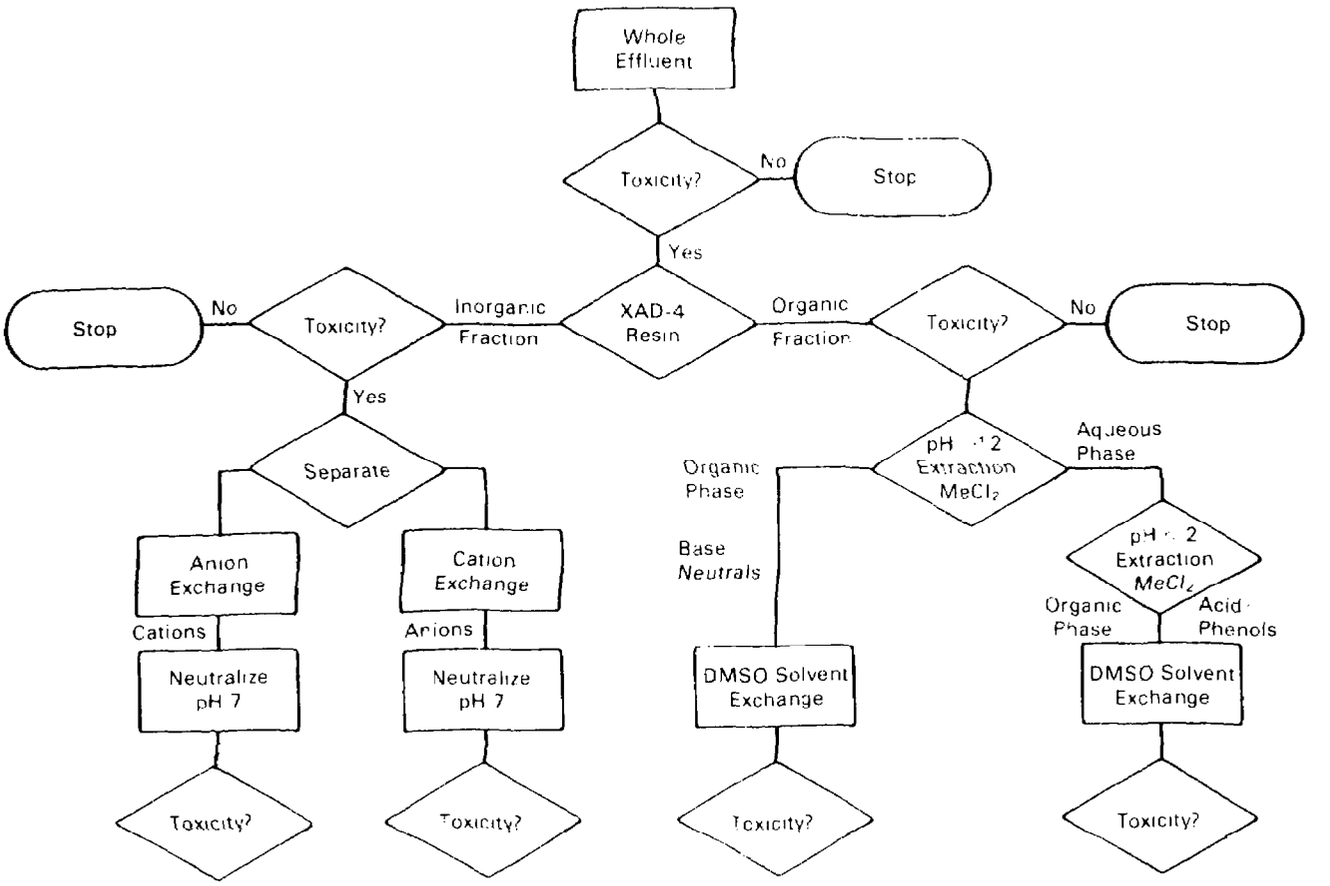
D.3 Microtox®

The Microtox® test is a luminescence inhibition test based on the proportionality between the light produced by a luminescent marine bacterium (*Photobacterium phosphoreum*) and its general respiratory metabolism. Toxic effects of chemicals which include reduction of metabolic rates are reflected in an attenuation of the bioluminescence of the bacteria. The bioluminescence response of the bacteria is quantified by a photometer in the Microtox® unit. The methods used for the Microtox® test followed those found in the Beckman Microtox® instruction manual.

D.4 Chemical Fractionation

To allow testing of the individual fractions of the effluents, the chemical fractionation procedure of Walsh and Garnas (1983) was followed (Figure D-1). The effluent was filtered through a prewashed Gelman Type A-E 1-μm pore size glass fiber filter to

Figure D-1. Fractionation and testing procedure.



remove solids, then eluted through a column of Rohm and Haas Amberlite XAD-4 resin.

The inorganic fraction included all chemicals not absorbed by the XAD-4 resin, which passed through with the aqueous effluent. Before use, the resin was prepared by repeated rinsing with deionized water, a 30-minute wash with 2 normal H_2SO_4, and a final de-ionized water rinse. Impurities were removed from the resin by rinsing with technical-grade acetone, followed by 12-hour sequential extractions with acetone and methanol in a Soxhelet extractor. XAD-4 column consisted of a 50-cc glass syringe, loosely plugged with glass wool, and filled with 50 ml (wet volume) of resin. At least 20 bed volumes of distilled water were used to displace the methanol from the column. A bored No. 6 teflon stopper coupled to a 3-cm piece of 8-mm outside diameter tubing was connected to the top of the column. Columns were prepared in advance and stored in a refrigerator until use.

During filtering, the 1-μm glass fiber filter mounted on a 142-mm filter holder, was fitted with a 20-μm nitex mesh prefilter to prevent clogging the glass fiber filter.

The aqueous inorganic fraction from the XAD-4 resin column was tested for toxicity following the procedures outlined in Sections D.2 and D.3. If toxicity was demonstrated, the inorganic fraction was further fractionated into anion and cation fractions. This was accomplished by a batch extraction procedure whereby a 4-L sample of water was adjusted to pH $>$ 10 and stirred for 24 hours with Dowex 1-X8 strong-base anion-exchange resin at a level of 10 gm dry resin/L water, to generate the cation fraction or adjusted to pH $<$ 4 and exposed to Dowex 50W-X8 strong-acid cation exchange resin to generate the anion fraction. Following treatment, the resin was removed from the sample by filtering through a glass fiber filter, and the pH was adjusted to neutrality.

The whole organic fraction was considered to be the fraction eluted from the XAD-4 resin column. This was accomplished by aspirating the column to remove excess water. The column was then eluted with 150 ml of nanograde acetone into a K-D concentrator flask. The resultant sample was concentrated to 25 ml under vacuum at room temperature and an aliquot was tested for toxicity using the methods described in Sections D.2 and D.3. If toxicity to the whole organic

fraction was found, further fractionation was performed by separating the base/neutral and acid/extractable subfractions following U.S. EPA Method 625 (U.S. EPA 1979) for priority pollutants. Prior to toxicity testing with these subfractions the methylene chloride was solvent exchanged with dimethyl sulfoxide (DMSO).

Appendix E
Toxicity Test Data

Table E-1. Routine Chemistry Data for the Ambient Tests, Baltimore Harbor, Maryland

Ambient Station	pH	Initial DO (mg/L) Mean	Initial DO (mg/L) Range	Final DO (mg/L) Mean	Final DO (mg/L) Range	Conductivity (μmhos) Mean	Conductivity (μmhos) Range	Mean Salinity (ppt ± SD)
Back River								
B1	6.9-7.5	8.3	7.5-8.9	5.5	2.3-8.4	1,429	1,250-1,650	0.5 ± 0.25-0.75
B2	6.9-7.5	8.5	7.8-8.8	5.1	1.9-8.4	1,451	1,300-1,700	0.57 ± 0.5-0.75
B3	6.8-7.5	8.6	8.3-9.0	5.1	2.9-7.7	1,464	1,300-1,600	0.64 ± 0.5-0.75
B4	6.9-7.4	8.5	7.8-8.9	5.5	4.3-8.0	1,568	1,350-2,300	0.68 ± 0.5-1.0
B5	7.0-7.7	8.7	8.3-9.0	6.4	4.9-9.1	2,043	1,650-2,800	1.0 ± 0.75-1.5
B6	7.0-8.0	8.6	8.2-8.8	7.1	5.8-10.4	2,779	2,200-3,500	1.5 ± 1.0-2.0
Patapsco								
P1	6.8-7.5	8.4	8.0-8.8	6.6	6.0-7.4	1,369	1,150-1,500	8 ± 6.5-8.8
P2	6.9-7.4	8.5	8.1-8.8	6.4	5.6-7.3	1,329	1,100-1,550	7.9 ± 6.3-8.6
P3	6.8-7.4	8.5	8.0-8.8	6.5	5.9-7.1	1,350	1,250-1,500	8.0 ± 7.0-9.0
Middle River								
M1	6.9-7.1	8.7	8.1-9.0	6.4	5.5-7.5	2,343	2,250-2,600	1.0 ± 1.0-1.1
M2	6.8-7.2	8.6	8.2-8.9	6.8	5.6-7.6	2,571	2,000-3,000	1.3 ± 1.0-1.5

Table E-2. Routine Chemistry Data for the Effluent Dilution and Salinity Tests

Test	Concentration	pH	Initial DO (mg/L) *Mean*	Initial DO (mg/L) Range	Final DO (mg/L) Mean	Final DO (mg/L) Range	Conductivity (μmhos) Mean	Conductivity (μmhos) Range	Mean Salinity (ppt ± SD)
Back River POTW	100[a]	6.8-7.1	*8.6*	8.6-8.7	5.1	--	925	900-950	0.28 ± 0.25-0.3
	30	7.1-7.4	*8.5*	8.2-8.9	5.3	3.6-7.6	666	610-700	--
	10	7.3-7.7	*8.5*	8.2-8.8	5.6	3.4-7.3	581	480-600	--
	3	7.3-7.7	*8.4*	8.2-8.7	6.1	4.2-7.5	508	470-575	--
	1	7.3-7.9	8.4	8.0-8.7	6.1	3.8-7.7	494	490-575	--
	Control	7.2-7.6	8.4	8.1-8.6	6.2	4.4-7.6	477	470-480	--
Patapsco POTW	100[a]	6.6	8.2	8.0-8.4	6.3	--	2,175	2,150-2,200	--
	30	6.6-6.9	8.2	7.4-8.7	5.7	4.8-6.4	1,056	950-1,150	0.31 ± 0.25-0.5
	10	7.0-7.3	8.4	8.0-8.8	6.2	4.8-7.5	723	700-725	--
	3	7.1-7.7	8.5	8.1-8.8	6.5	5.1-7.6	614	600-625	--
	1	7.2-7.8	8.5	8.1-8.8	6.5	5.2-7.5	546	475-600	--
	Control	7.2-8.0	8.4	8.1-8.8	6.2	5.0-6.9	493	470-550	--
Salinity	16[b]	6.9	8.9	--	8.2	--	26,000	--	--
	12	7.0-7.1	8.8	8.6-8.9	8.2	7.3-8.2	19,750	19,500-20,000	--
	8	7.1-7.7	8.2	6.6-8.6	7.3	6.5-8.3	13,750	11,500-13,500	--
	4	7.2-7.8	8.3	8.0-8.6	7.1	6.2-8.3	6,938	6,000-7,500	--
	2	7.3-7.7	8.2	8.0-8.4	7.2	6.4-8.3	3,831	3,700-4,300	--
	C	7.2-8.0	8.4	8.1-8.8	6.7	5.4-7.3	493	470-550	--

[a] *Concentrations* are in percent

[b] Concentrations are in parts per thousand (ppt).

Note: Reconstituted water was used for dilution in all tests.

Table E-3. **Final Dissolved Oxygen Levels for *Ceriodaphnia dubia* Effluent, Ambient, and Salinity Tests, Baltimore Harbor, Maryland**

Sample	Percent Effluent (v/v)	Mean DO (mg/L)	DO Range
Effluent			
Patapsco POTW	100	7.2	--
	30	7.3	--
	10	7.5	--
	1	7.8	--
	3	7.6	7.3-7.8
	Control	7.6	7.3-8.0
	3.0	7.4	--
	1.5	7.7	7.5-7.9
	0.75	7.4	7.3-8.0
	0.37	7.5	7.2-8.1
	Control		
Back River POTW	100	6.8	--
	30	6.8	5.3-7.2
	10	7.6	7.4-7.6
	3	6.1	7.3-7.6
	1	7.6	7.1-8.0
	Control	7.5	7.0-7.9
Ambient			
Back River			
B1		7.3	7.0-7.7
B2		7.3	7.0-7.6
B3		7.0	--
B4		7.3	--
B5		7.3	7.0-7.7
B6		7.5	7.0-7.8
Patapsco			
P1	7.7	--	
P2	--	--	
P3	7.8	--	
Control	7.8	7.4-8.4	
Middle River			
M1	7.8	7.1-8.2	
M2	7.6	7.0-8.0	
Salinity[a]	4	--	--
	2	7.8	7.2-8.4
	1	7.7	7.2-8.2
	0.50	7.6	7.1-8.1
	0.25	7.7	7.3-8.2

[a] Concentrations are in parts per thousand (ppt).
Note: Reconstituted water was used for dilution in all tests.

Appendix F
Biological Data

Table F-1. Results of a X^2 Test Performed on the Number of Macrozooplankton Taxa, Back River, March 1984

	Station							
	B1	B2	B3	B4	B5	B6	M1	M2
Number of taxa[a]	5	8	3	3	8	6	6	5
Expected number (based on average of M1 and M2)	5.5	5.5	5.5	5.5	5.5	5.5	-	-
X^2 contribution[c]	0	0.72	0.18	0.18	0.72	0		-

[a] Number of unique taxa, life stages by combining two replicate samples for each station for two collection dates.
[b] For individual station, the 1 degree of freedom X^2 with P = X^2 = 0.05 is 3.84.
Note: For all stations combined, the calculated X^2 = 3.18 (P = X^2 0.78 with 6 d.f.).

$$X^2 = \frac{(|E-O1|-0.5)^2}{E}$$ Correction factor incorporated for small (1 degree of freedom) dataset.

Table F-2. Abundance (No./m^3) of Macrozooplankton Collected from Back River and Middle River, 12 March 1984

	Station			Station			Station		
Taxa	B1L	B1R	Mean	B2L	B2R	Mean	B3L	B3R	Mean
E. affinis	63.693	45.769	54.731	105.649	103.423	104.536	201.492	215.362	208.427
M. edwardsi	-	-	-	0.021	-	0.010	-	0.031	0.015
Ostracoda	-	0.016	0.008	-	-	-	-	-	-
Chaoborus	-	0.016	0.008	-	0.008	0.008	-	0.016	0.008

	Station			Station			Station		
Taxa	B4L	B4R	Mean	B5L	B5R	Mean	B6L	B6R	Mean
Daphnia	-	0.016	0.008	0.014	-	0.007	-	0.029	0.014
E. affinis	492.192	504.108	498.150	290.704	274.229	282.466	1,118.296	952.334	1,035.315
M. edwardsi	0.052	0.037	0.044	0.240	0.120	0.180	0.233	0.299	0.266
Gammarus	-	-	-	0.014	0.031	0.022	-	-	-
Hemiptera N.	-	-	-	0.014	-	0.007	-	-	-
Nematoda	-	-	-	-	0.016	0.008	-	-	-
N. americana	-	-	-	0.014	-	0.007	-		-

	Station			Station		
Taxa	M1L	M1R	Mean	M2L	M2R	Mean
Daphnia	0.225	-	0.112	0.021	-	0.010
E. affinis	791.957	1,429.329	1,110.643	385.541	391.545	388.543
M. edwardsi		-	-	0.099	0.054	0.076

Table F-3. Abundance (No./m^3) of Macrozooplankton Collected from Back River and Middle River, 16 March 1984

	Station			Station			Station		
Taxa	B1L	B1R	Mean	B2L	B2R	Mean	B3L	B3R	Mean
E. affinis	15.243	22.459	18.851	117.916	142.137	130.026	50.565	65.941	58.253
M. edwardsi	0.022	0.016	0.019	0.056	--	0.028	0.048	0.063	0.056
Ceriodaphnia	0.063	--	0.032	--	0.039	0.020	--	--	--
Gammarus	--	--	--	0.023	--	0.012	--	--	--
Ostracoda	--	--	--	0.014	--	0.007	--	--	--
Chironomidae P.	--	--	--	0.014	--	0.007	--	--	--
Diptera P.	--	--	--	0.014	--	0.007	--	--	--
Chaoborus	--	--	--	--	0.016	0.008	--	--	--

	Station			Station			Station		
Taxa	B4L	B4R	Mean	B5L	B5R	Mean	B6L	B6R	Mean
Daphnia	--	--	--	--	0.076	0.038	0.014	0.023	0.018
E. affinis	368.905	350.774	359.840	936.747	1,040.629	988.688	272.122	235.941	254.032
M. edwardsi	--	0.016	0.008	0.061	0.054	0.058	0.044	0.073	0.058
Gammarus	--	--	--	0.014	--	0.007	--	0.030	0.015
Eubosmina	--	--	--	--	0.016	0.008	--	--	--
N. americana	--	--	--	--	--	--	0.014	--	0.007
L. plumulosus	--	--	--	--	--	--	--	0.023	0.012

	Station			Station		
Taxa	M1L	M1R	Mean	M2L	M2R	Mean
Daphnia	0.578	0.265	0.422	0.847	0.831	0.839
E. affinis	1,460.698	1,180.544	1,320.621	290.488	341.032	315.760
M. edwardsi	--	--	--	0.039	0.050	0.044
Collembola	0.014	0.017	0.016	--	--	--
Eubosmina	0.014	--	0.007	0.014	0.017	0.016
Diptera P.	0.014	--	0.007	--	--	--
A. proximoculi	--	0.017	0.008	--	--	--
Chaoborus	--	--	--	0.022	--	0.011

Table F-4. Analysis of Variance and Tukey's Studentized Range Test Results for *Eurytemora affinis*, Back River, March 1984

Dependent Variable: ln density (No. /m^3)

Source	Df	Squares	Square	F-Value	PR > F
Treatment	15	43.41	2.89	111.55	0.0001
Date	1	0.82	0.82	31.84	0.0001
Station	7	36.96	5.28	203.52	0.0001
Date x station	7	5.62	0.80	30.97	0.0001
Error	16	0.42	2.89		
Corrected Total	31	43.83	0.02		

Tukey's Studentized Range Test on Station Abundances

Station	M1	B5	B6	B4	M2	B2	B3	B1
Mean ln count	(7.1)	(6.3)	(6.2)	(6.1)	(5.9)	(4.8)	(4.7)	(3.5)

Table F-5. Water Quality Data from Back River and Middle River, 12 and 16 March 1984

Station	Time	Depth (m)	Salinity			pH		
			Surface	Middle	Bottom	Surface	Middle	Bottom
12 March 1984								
B1	1630	0.3	0.8	--	--	7.5	--	--
B2	1607	0.3	0.9	--	--	7.4	--	--
B3	1543	1.3	0.7	--	0.7	7.5	--	7.3
B4	1443	1.0	0.9	--	0.9	7.8	--	7.8
B5	1351	1.0	1.2	--	1.2	8.5	--	8.5
B6	1313	2.5	2.1	--	2.1	8.4	--	8.4
M1	1140	3.0	1.4	1.4	1.4	6.9	7.2	7.1
M2	1225	3.0	1.7	1.8	2.1	7.3	7.5	7.5
16 March 1984								
B1	1533	0.3	0.6	--	--	7.0	--	--
B2	1512	1.0	0.7	--	0.7	7.0	--	6.9
B3	1438	1.0	0.7	--	0.7	7.1	--	6.9
B4	1353	1.5	0.7	0.7	0.7	7.2	7.1	7.1
B5	1258	2.5	1.2	1.2	2.5	8.4	8.6	8.3
B6	1225	2.0	1.6	1.6	2.5	8.1	8.6	8.3
M1	1045	3.0	1.3	1.3	1.4	7.0	7.2	7.1
M2	1125	2.5	1.5	1.5	1.6	7.5	7.9	7.8

Station	Time	Tide	Temperature			DO		
			Surface	Middle	Bottom	Surface	Middle	Bottom
12 March 1984								
B1	1630	F	5.3	--	--	14.0	--	--
B2	1607	F	5.1	--	--	13.0	--	--
B3	1543	F	4.3	--	4.4	12.0	--	11.0
B4	1443	F	3.5	--	3.5	15.2	--	14.1
B5	1351	F	2.8	--	2.8	16.7	--	15.6
B6	1313	F	2.1	--	2.0	16.2	--	15.0
M1	1140	F[a]	2.8	2.6	2.5	13.2	12.6	12.8
M2	1225	F	1.9	1.9	1.8	14.8	13.3	13.6
16 March 1984								
B1	1533	F	9.9	--	--	8.9	--	--
B2	1512	F	9.1	--	8.2	6.3	--	6.2
B3	1438	F	9.4	--	8.7	6.5	--	6.0
B4	1353	F	9.7	7.2	7.1	7.1	9.9	9.8
B5	1258	LS	6.1	5.9	5.1	17.4	16.6	14.6
B6	1225	LS	6.0	5.0	4.0	17.4	15.6	13.6
M1	1045	E	4.4	3.8	3.4	14.2	13.7	13.4

[a]F = Flood, E= Ebb, LS = Low slack.

Table F-6. Results of a X^2 Test Performed on the Number of Benthic Macroinvertebrate Taxa, Back River, March 1984

	Station							
	B1	B2	B3	B4	B5	B6	M1	M2
Number of taxa[a]	4	5	3	2	8	12	10	13
Expected number (based on average of M1 and M2)	11.5	11.5	11.5	11.5	11.5	11.5		
X^2 contribution	4.26	3.13	5.56	7.04	0.78	0		

[a]Number of unique taxa life stages by combining three replicate samples for each station for two collection dates
[b]For individual station, the 1 degree of freedom X^2 with $P = X^2 = 0.05$ is 3.84
Note: For all stations combined, the calculated $X^2 = 23.77$ ($P = X^2 = 0.005$ with 6 d.f.)

$$X^2 = \frac{(|E-O|-0.5)^2}{E}$$

Correction factor incorporated for small (1 degree of freedom) dataset

O = Observed
E = Expected

Table F-7. Water Quality Data from Back River and Middle River, 19 March 1984

Station	Time	Depth (m)	Salinity			pH		
			Surface	Middle	Bottom	Surface	Middle	Bottom
B1	1435	1.5	0.6	--	0.7	7.6	--	7.5
B2	1405	1.0	0.7	--	0.8	7.6	--	7.5
B3	1330	2.0	0.7	0.8	0.8	7.8	7.4	7.4
B4	1300	2.0	0.9	0.9	1.2	8.2	7.9	7.9
B5	1130	3.0	1.4	2.1	2.1	8.0	8.4	8.2
B6	1050	3.0	2.6	3.4	3.5	8.2	8.1	8.1
M1	0905	3.0	1.3	1.3	1.3	7.1	7.2	7.1
M2	1000	4.0	0.7	2.3	2.7	7.8	7.9	7.9

Station	Time	Tide	Temperature			DO		
			Surface	Middle	Bottom	Surface	Middle	Bottom
B1	1435	1.5	11.2	--	9.7	8.9	--	9.0
B2	1405	1.0	11.0	--	8.5	9.4	--	9.6
B3	1330	2.0	10.1	8.7	8.7	11.3	10.6	11.0
B4	1300	2.0	9.4	9.0	7.4	14.9	13.8	12.4
B5	1130	3.0	8.2	6.3	6.2	13.9	13.9	13.8
B6	1050	3.0	6.4	5.0	4.9	13.9	13.2	13.2
M1	0905	3.0	6.4	6.3	6.5	11.7	11.4	11.0
M2	1000	4.0	5.5	4.9	4.8	13.2	12.8	12.8

Table F-8. Results of a X^2 Test Performed on the Number of Fish Taxa, Back River, March 1984

	Station							
	B1	B2	B3	B4	B5	B6	M1	M2
Number of taxa[a]	3	2	1	4	3	4	7	5
Expected number (based on average of M1 and M2)	6	6	6	6	6	6	--	--
X^2 contribution	1.04	2.04	3.38	0.38	1.04	0.38	--	--

[a]Number of unique taxa life stages by combining samples from two collection dates for each station.
[b]For individual station, the 1 degree of freedom X^2 with $P = X^2 = 0.05$ is 3.84
Note: For all stations combined, the calculated $X^2 = 8.01$ ($P = X^2 = 0.240$ with 6 d.f.).

$$X^2 = \frac{(|E-O|-0.5)^2}{E}$$

Correction factor incorporated for small (1 degree of freedom) dataset

O = Observed
E = Expected

Table F-9. Trends in Abnormalities Observed Among Brown Bullheads Collected in Back River and Middle River, 7 March 1984

Observation	Station B1	B2	B3	B4	B5	B6	B1-B3	B4-B6
Muscular atrophy	16.7% (1/6)						2.9% (1/35)	
Healed/healing scars		8.3% (1/12)		0.8% (1/126)	2.9% (2/69)	N O	2.9% (1/35)	1.5% (3/196)
Nodule/tumor				0.8% (1/126)		A		0.5% (1/196)
Spinal curvature (lordosis)				0.8% (1/126)		B		0.5% (1/196)
Unusual coloration				0.8% (1/126)		N O		0.5% (1/196)
Small whitish spots				0.8% (1/126)		R M		0.5% (1/196)
Small dark spots				0.8% (1/126)		A L		0.5% (1/196)
Fin erosion/rot	16.7% (1/6)	8.3% (1/12)		1.6% (2/126)	2.9% (2/69)	I T	5.7% (2/35)	2.0% (4/196)
Regenerated fin/rays		8.3% (1/12)				I E S	2.9% (1/35)	
Missing fin				1.6% (2/126)				1.0% (2/196)
Gill filament erosion		8.3% (1/12)					2.9% (1/35)	
Gill arch cyst			5.9% (1/17)				2.9% (1/35)	
Blind eye				0.8% (1/126)	1.4% (1/69)			1.0% (2/196)
Number examined closely	6	12	17	20	14	1	35	35
Number examined grossly	0	0	0	106	55	0	0	161
Total	6	12	17	126	69	1	35	196

Table F-10. Trends in Abnormalities Observed Among Brown Bullheads Collected in Back River and Middle River, 14 March 1984

Observation	Station B1	B2	B3	B4	B5	B6	B1-B3	B4-B6
Healed/healing scars		N O			2.6% (1/39)			1.1% (1/87)
Nodule/tumor		A B	4.0% (1/25)				1.5% (1/66)	
Fin erosion/rot	5.1% (2/39)	N O	12.0% (3/25)	2.1% (1/48)	5.1% (2/39)	N O	7.6% (5/66)	3.4% (3/87)
Regenerated fins/rays	10.3% (4/39)	R M	4.0% (1/25)			C	7.6% (5/66)	
White cysts on fins	2.6% (1/39)	A L				A T	1.5% (1/66)	
Black cysts on fins	2.6% (1/39)	I T				C H	1.5% (1/66)	
Blind eye		I E S			2.6% (1/39)			1.1% (1/87)
Number examined closely	20	2	20	18	20	--	42	38
Number examined grossly	19	0	5	30	19	--	24	49
Total	39	2	25	48	39	0	66	87

Table F-11. Trends in Abnormalities Observed Among White Perch Collected in Back River and Middle River, 7 March 1984

Observation	Station				River	
	B5	B6	M1	M2	Back	Middle
Body lesions		2.7% (2/74)		14.3% (1/7)	2.4% (2/84)	2.9% (1/34)
Body fungus—smooth, opaque slime		1.4% (1/74)	3.7% (1/27)		1.2% (1/84)	2.9% (1/34)
Fin erosion/rot			3.7% (1/27)			2.9% (1/34)
Regenerated fin/rays		2.7% (2/74)			2.4% (2/84)	
Gill filament erosion		5.0% (1/20)			3.3% (1/30)	
Gill raker erosion	20.0% (2/10)				6.7% (2/30)	
Blind eye		1.4% (1/74)			1.2% (1/84)	
Ergasilus	30.0% (3/10)	55.0% (11/20)	65.0% (13/20)	28.6% (2/7)	46.7% (14/30)	55.6% (5/27)
Leech		1.4% (1/74)	11.1% (3/27)		1.2% (1/84)	8.8% (3/34)
Number examined closely	10	20	20	7	30	27
Number examined grossly	0	54	7	0	54	7
Total	10	74	27	7	84	34

Table F-12. Trends in Abnormalities Observed Among Pumpkinseed and White Perch Collected in Back River and Middle River, 14 March 1984

Observation	Pumpkinseed					White Perch	
	B4-B6	M1	M2	Back River	Middle River	B6	M1
Muscular atrophy		2% (1/50)			1.9% (1/54)		
Nodule/tumor	N	2%		N	1.9%		
	O	(1/50)		O	(1/54)		
Deformed jaw		2%			1.9%		
	A	(1/50)		A	(1/54)		
Pughead	B	2%		B	1.9%		
	N	(1/50)		N	(1/54)		
Fin erosion/rot	O	6%		O	5.6%		
	R	(3/50)		R	(3/54)		
Regenerated fins/rays	M	14%	25%	M	14.8%		
	A	(7/50)	(1/4)	A	(8/54)		
Gill filament erosion	L		25%	L	4.2%		
	I		(1/4)	I	(1/24)		
Pale gill filaments	T	5%		T	4.2%		
	I	(1/20)		I	(1/24)		
Ergasilus	E	20%	50%	E	25.0%	15.0%	40.0
	S	(4/20)	(2/4)	S	(6/24)	(3/20)	(4/10)
Leech		30%	25%		29.6%	5.7%	10.0%
		(15/50)	(1/4)		(16/54)	(2/35)	(1/10)
Gill raker erosion							10.0% (1/10)
Blind eye							20.0% (2/10)
Number examined closely	4	20	4	4	24	20	10
Number examined grossly	0	30	0	0	30	15	0
Total	4	50	4	4	54	35	10

Table F-13. List of Fish Species and Families Collected, Back River and Middle River, March 1984

Family	Scientific Name	Common Name
Cyprinidae (minnows)	*Notropis spilopterus*	Spotfin shiner
Centrarchidae (sunfish)	*Lepomis gibbosus*	Pumpkinseed sunfish
Percichthyidae (temperate basses)	*Morone americana*	White perch
Percidae (perches)	*Perca flavescens*	Yellow perch
Ictaluridae (catfish)	*Ictalurus nebulosus* *Ictalurur punctatus*	Brown bullhead Channel catfish
Clupeidae (herring)	*Alosa aestivalis* *Drosoma cepedianum*	Blueback herring Gizzard shad
Gasterasteidae (sticklebacks)	*Gasterosteus wheatlandi*	Blackspotted stickleback

Appendix G
Support Chemical Fractionation Data

The results of the acute *Ceriodaphnia dubia* 48-hour LC50 tests for the Back River and Patapsco POTW effluents were discussed in Chapter 11, as part of the effluent fractionation procedure tests. The mortality data for these tests, in which 10 *Ceriodaphnia* were exposed to various concentrations of whole effluent and fractions derived from the effluent fractionation procedure described in Appendix D, are presented in Table G-1. As was discussed in Chapter 11, LC50s could not be calculated for certain of the tests, because of the absence of partial kills, or because of the absence of a valid dose-response relationship in the data.

The results of the chemical tests on the base/neutral subfraction of the organic fraction of the 3-day and 7-day composites of the Patapsco POTW effluents, which were the subfractions which displayed much of the toxicity observed in the samples tested, were discussed in Chapter 12. The documentation for the GC/MS analyses for the base/neutral priority pollutants is presented in this Appendix (Tables G-2 through G-8 and Figures G-1 through G-8). Reconstructed ion chromatograms and quantitation reports are presented for the standard (Figure G-1, Table G-2), the surrogate spike standard (Figure G-2, Table G-3), and blank (Figure G-3, Table G-4). A quantitation report is provided for the spike of the sample blank (Table G-5). Reconstructed ion chromatograms and quantitation reports are also provided for the 3-day composite (Figure G-4 and Table G-6), and the 7-day composite (Figure G-6 and Table G-7), while Figure G-5 presents the results of a library search to obtain a possible match for a compound noted in the 3-day composite. Documentation of the DFTPP tuning of the GC/MS is presented in Figures G-7 and G-8 and Table G-8.

Table G-1. *Ceriodaphnia dubia* Mortality in 48-Hour LC50 Tests on Back River and Patapsco POTW

Percent Effluent (v/v)	3-Day and 7-Day Composite Samples				
	Whole Effluent	Inorganic Fraction	Organic Fraction	Base/Neutral Fraction	Acid/Phenol Fraction
Back River POTW 3-Day Composite					
100	10	3	10	3	5
30	6	0	0	6	5
10	8	0	1	6	5
3	6	2	2	7	8
1	5	2	3	4	4
0 (control)	3	1	1	3	2
7-Day Composite					
100	10	0	10	5	5
30	2	0	1	3	1
10	5	1	1	3	0
3	3	0	3	4	3
1	1	1	2	2	1
0 (control)	2	0	0	0	0

Percent Effluent (v/v)	Whole Effluent	Inorganic Fraction	Cation Fraction	Anion Fraction	Organic Fraction	Base/ Neutral Fraction	Acid/ Phenol Fraction
Patapsco POTW 3-Day Composite							
100	10	10	10	2	10	10	2
30	10	2	0	2	10	10	4
10	10	1	0	0	2	4	4
3	4	1	0	0	2	4	2
1	5	1	0	0	0	5	2
0 (control)	3	0	0	0	1	0	2
7-Day Composite							
100	10	3	--	--	10	8	6
30	10	0	--	--	10	6	1
10	10	1	--	--	0	8	1
3	2	0	--	--	1	3	1
1	2	1	--	--	2	1	0
0 (control)	1	0	--	--	2	2	1

Note: Reconstituted water was used as dilution water.

Table G-2. Base/Neutral Standard Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis

Name	m/z	Scan	Time	Ref.	RRT	Meth.	Area (Hght)	Amount	% Tot.
D-8 Naphthalene (I.S. #1)	136	1482	10:50	1	1.000	A BB	1640290.	20.000 ppm	0 85
D10-Phenanthrene (I.S. #2)	188	2615	18:59	2	1.000	A BB	422573.	20.000 ppm	0.85
D12-Chrysene (I.S. #3)	240	3567	25:53	3	1.000	A BB	161155.	20.000 ppm	0.85
N-Nitrosodimethylamine	74	496	3:36	1	0.332	A BB	2326150.	50.000 ppm	2.12
Bis(2-Chloroethyl)ether	93	1040	7:33	1	0.697	A BB	2472650	50.000 ppm	2.12
1,3-Dichlorobenzene	146	1081	7:51	1	0.725	A BV	2740570.	50.000 ppm	2.12
1,4-Dichlorobenzene	146	1098	7:58	1	0.736	a BV	2659490.	50.000 ppm	2.12
1,2-Dichlorobenzene	146	1152	8:22	1	0.772	A BB	2406020.	50.000 ppm	2.12
Bis(2-Chloroisopropyl)ether	45	1198	8:42	1	0.803	A BB	3176600.	50.000 ppm	2.12
N-Nitroso-di-n-propylamine	70	1241	9:00	1	0.832	A BB	1242710.	50.000 ppm	2.12
Hexachloroethane	117	1240	9:00	1	0.831	A BB	1133950.	50.000 ppm	2.12
Nitrobenzene	123	1279	9:17	1	0.857	A BB	668276	50 000 ppm	2.12
Isophorone	82	1255	9:50	1	0.908	A BB	3226170.	50.000 ppm	2.12
Bis(2-Chloroethoxy)methane	93	1441	10:27	1	0.966	A BB	1611600.	50.000 ppm	2.12
1,2,4-Trichlorobenzene	180	1481	10:45	1	0.993	A BV	1456950.	50.000 ppm	2.12
Naphthalene	128	1500	10:53	1	1.005	A BB	5133090.	50.000 ppm	2.12
Hexachlorobutadiene	225	1563	11:21	1	1.048	A BB	982232.	50.000 ppm	2 12
Hexachlorocyclopentadiene	237	1809	13:08	1	1.212	A BB	286588.	50.000 ppm	2.12
2-Chloronaphthalene	162	1899	13:47	1	1.273	A BB	1994830.	50.000 ppm	2.12
Dimethyl phthalate	163	2045	14 50	1	1.371	A BB	2396960.	50.000 ppm	2.12
Acenaphthylene	152	2045	14 50	1	1.371	A BB	3688860.	50.000 ppm	2.12
2,6-Dinitrotoluene	165	2067	15:00	1	1.385	A BB	247250.	50.000 ppm	2.12
Acenaphthene	154	2112	15:20	2	0.908	A BB	1677720.	50.000 ppm	2.12
2,4-Dinitrotoluene	89	2201	15:58	2	0.842	A BB	121629.	50 000 ppm	2.12
Diethyl phthalate	149	2295	16:39	2	0.878	A BB	2701970.	50.000 ppm	2 12
Fluorene	166	2290	16:37	2	0.876	A VB	1734180.	50.000 ppm	2.12
4-Chlorophenylphenyl ether	204	2299	16:41	2	0.879	A BB	939726.	50.000 ppm	2.12
N-Nitrosodiphenylamine	169	2349	17:03	2	0.898	A BB	668102	50.000 ppm	2.12
1,2-Diphenylhydrazine	77	2353	17:05	2	0.900	A BB	2106620	50 000 ppm	2.12
4-Bromophenylphenyl ether	248	2468	17:55	2	0.944	A BB	428470.	50.000 ppm	2.12
Hexachlorobenzene	284	2509	18:13	2	0.959	A BB	547807	50.000 ppm	2.12
Phenanthrene	178	2623	19:02	2	1.003	A BV	1767770.	50.000 ppm	2.12
Anthracene	178	2640	19:10	2	1.010	A VB	2071970.	50.000 ppm	2 12
Di-n-butylphthalate	149	2874	20:51	2	1.099	A BB	1676360	50 000 ppm	2 12
Fluoranthene	202	3049	22:08	2	1.166	A BB	1578040.	50.000 ppm	2.12
Benzidine	184	3155	22:54	2	1.207	A BB	588.	50.000 ppm	2.12
Pyrene	202	3126	22:41	2	1.195	A BB	1133110.	50.000 ppm	2.12
Butylbenzyl phthalate	149	3405	24:43	3	0.955	A BV	267404.	50.000 ppm	2.12
3,3'-Dichlorobenzidine	252	3578	25:58	3	1.003	A BB	87263.	50 000 ppm	2.12
Benzo(a)anthracene	228	3562	25:51	3	0.999	A BV	663416.	50.000 ppm	2 12
Chrysene	228	3578	25:58	3	1.003	A VB	834147.	50.000 ppm	2.12
Bis(2-Ethylhexyl)phthalate	149	3629	26:20	3	1.017	A BV	395568.	50.000 ppm	2.12
Di-n-octyl phthalate	149	3855	27:59	3	1.081	A BB	377100.	50.000 ppm	2.12
Benzo(b)fluoranthene	252	3961	28:45	3	1.110	A BV	303478.	50.000 ppm	2.12
Benzo(k)fluoranthene	252	3973	28:50	3	1.114	A VB	320916.	50.000 ppm	2 12
Benzo(a)pyrene	252	4097	29:44	3	1.149	A BB	201020	50.000 ppm	2 12
Indeno(1,2,3-cd)pyrene	276	4738	34:23	3	1.328	A BB	89024.	50.000 ppm	2 12
Dibenzo(a,h)anthracene	278	4765	34:35	3	1.336	A BB	101606.	50.000 ppm	2.12
Benzo(g,h,i)perylene	276	4918	35:42	3	1.379	A BB	83677.	50.000 ppm	2 12

Table G-3. Surrogate Spike Standard Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis

Name	m/z	Scan	Time	Ref.	RRT	Meth.	Area (Hght)	Amount	% Tot
D-8 Naphthalene (I.S. #1)	136	1490	10:49	1	1.000	A BB	1284310	20.000 ppm	8.33
D10-Phenanthrene (I.S. #2)	188	2614	18:58	2	1.000	A BB	350927.	20.000 ppm	8.33
2-Fluorophenol (A/P Surr.)	112	779	5:39	1	0.523	A BB	3086270.	50.000 ppm	20.83
D-5 Phenol (A/P Surr.)	99	1034	7:30	1	0.694	A BB	1552090.	50 000 ppm	20 83
D5-Nitrobenzene (B/N Surr.)	128	1272	9:14	1	0.854	A BB	767043.	50 000 ppm	20.83
2-Fluorobiphenyl (B/N Surr.)	172	1874	13:36	1	1.258	A BB	2634180	50.000 ppm	20 83

Table G-4. Blank Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis

Name	m/z	Scan	Time	Ref.	RRT	Meth.	Area (Hght)	Amount	% Tot.
D-8 Naphthalene (I.S. #1)	106	1490	10:49	1	1.000	A BB	595682.	20.000 ppm	33.33
D10-Phenanthrene (I.S. #2)	188	2612	18:57	2	1.000	A BB	176072.	20.000 ppm	33.33
D12-Chrysene (I.S. #3 —	240	3564	25:52	3	1.000	A BB	35150.	20.000 ppm	33.33
N-Nitrosodimethylamine	Not Found								
Bis(2-Chloroethyl)ether	Not Found								
1,3-Dichlorobenzene	Not Found								
1,4-Dichlorobenzene	Not Found								
1,2-Dichlorobenzene	Not Found								
Bis(2-Chloroisopropyl)ether	Not Found								
N-Nitroso-di-n-propylamine	Not Found								
Hexachloroethane	Not Found								
Nitrobenzene	Not Found								
Isophorone	Not Found								
Bis(2-Chloroethoxy)methane	Not Found								
1,2,4-Trichlorobenzene	Not Found								
Naphthalene	128	1490	10:49	1	1.000	A BB	2068.	0.055 ppm	0.09
Hexachlorobutadiene	Not Found								
Hexachlorocyclopentadiene	Not Found								
2-Chloronaphthalene	Not Found								
Dimethyl phthalate	Not Found								
Acenaphthylene	Not Found								
2,6-Dinitrotoluene	Not Found								
Acenaphthene	Not Found								
2,4-Dinitrotoluene	Not Found								
Diethyl phthalate	Not Found								
Fluorene	Not Found								
4-Chlorophenylphenyl ether	Not Found								
N-Nitrosodiphenylamine	Not Found								
1,2-Diphenylhydrazine	Not Found								
4-Bromophenylphenyl ether	Not Found								
Hexachlorobenzene	Not Found								
Phenanthrene	Not Found								
Anthracene	Not Found								
Di-n-butylphthalate	149	2870	20:50	2	1.099	A BV	2492.	0.178 ppm	0.30
Fluoranthene	Not Found								
Benzidine	Not Found								
Pyrene	Not Found								
Butylbenzyl phthalate	Not Found								
3,3'-Dichlorobenzidine	Not Found								
Benzo(a)anthracene	Not Found								
Chrysene	Not Found								
Bis(2-Ethylhexyl)phthalate	Not Found								
Di-n-octyl phthalate	Not Found								
Benzo(b)fluoranthene	Not Found								
Benzo(k)fluoranthene	Not Found								
Benzo(a)pyrene	Not Found								
Indeno(1,2,3-cd)pyrene	Not Found								
Dibenzo(a,h)anthracene	Not Found								
Benzo(g,h,i)perylene	Not Found								

Table G-5. Spike Blank Quantitation Report for 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis

Name	m/z	Scan	Time	Ref.	RRT	Meth.	Area (Hght)	Amount	% Tot
D-8 Naphthalene (I.S. #1)	136	1490	10:49	1	1.000	A BB	595682.	20.000 ppm	7.87
D10-Phenanthrene (I.S. #2)	188	2612	18:57	2	1.000	A BB	176072.	20.000 ppm	7.87
2-Fluorophenol (A P Surr)	112	783	5:41	1	0.526	A BB	46531.	1.625 ppm	0.64
D-5 Phenol (A P Surr)	99	1037	7:32	1	0.696	A BB	192315.	13.357 ppm	5.26
D5-Nitrobenzene (B N Surr)	128	1273	9:14	1	0.854	A BB	704377.	98.995 ppm	38.95
2-Fluorobiphenyl (B N Surr.)	172	1872	13:35	1	1.256	A BB	2448540.	100.205 ppm	39.42

Table G-6. Quantitation Report for 3-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis

Name	m/z	Scan	Time	Ref.	RRT	Meth	Area (Hght)	Amount	% Tot
D-8 Naphthalene (I.S. #1)	136	1488	10:48	1	1.000	A BB	271288	20.000 ppm	33.13
D10-Phenanthrene (I.S. #2)	188	2611	18:57	2	1.000	A BV	123922.	20.000 ppm	33.13
D12-Chrysene (I.S. #3)	240	3566	25:53	3	1.000	A BB	25990.	20.000 ppm	33.13
N-Nitrosodimethylamine	Not Found								
Bis(2-Chloroethyl)ether	93	1037	7:32	1	0.697	A BB	2916.	0.357 ppm	0.59
1,3-Dichlorobenzene	Not Found								
1,4-Dichlorobenzene	Not Found								
1,2-Dichlorobenzene	Not Found								
Bis(2-Chloroisopropyl)ether	Not Found								
N-Nitroso-di-n-propylamine	Not Found								
Hexachloroethane	Not Found								
Nitrobenzene	Not Found								
Isophorone	Not Found								
Bis(2-Chloroethoxy)methane	Not Found								
1,2,4-Trichlorobenzene	Not Found								
Naphthalene	Not Found								
Hexachlorobutadiene	Not Found								
Hexachlorocyclopentadiene	Not Found								
2-Chloronaphthalene	Not Found								
Dimethyl phthalate	Not Found								
Acenaphthylene	Not Found								
2,6-Dinitrotoluene	Not Found								
Acenaphthene	Not Found								
2,4-Dinitrotoluene	Not Found								
Diethyl phthalate	Not Found								
Fluorene	Not Found								
4-Chlorophenylphenyl ether	Not Found								
N-Nitrosodiphenylamine	Not Found								
1,2-Diphenylhydrazine	77	2344	17:01	2	0.898	A BB	4116.	0.333 ppm	0.55
4-Bromophenylphenyl ether	Not Found								
Hexachlorobenzene	Not Found								
Phenanthrene	Not Found								
Anthracene	Not Found								
Di-n-butylphthalate	Not Found								
Fluoranthene	Not Found								
Benzidine	Not Found								
Pyrene	Not Found								
Butylbenzyl phthalate	Not Found								
3,3'-Dichlorobenzidine	Not Found								
Benzo(a)anthracene	Not Found								
Chrysene	Not Found								
Bis(20Ethylhexyl)phthalate	Not Found								
Di-n-octyl phthalate	Not Found								
Benzo(b)fluoranthene	Not Found								
Benzo(k)fluoranthene	Not Found								
Benzo(a)pyrene	Not Found								
Indeno(1,2,3-cd)pyrene	Not Found								
Dibenzo(a,h)anthracene	Not Found								
Benzo(g,h,i)perylene	Not Found								

Table G-7. Quantitation Report for 7-Day Patapsco POTW Base/Neutral Fraction Effluent Analysis

Name	m z	Scan	Time	Ref.	RRT	Meth.	Area (Hght)	Amount	% Tot.
D-8 Naphthalene (I.S. #1)	136	1488	10:48	1	1.000	A BB	244822.	20.000 ppm	30.16
D10-Phenanthrene (I.S. #2)	188	2609	18:56	2	1 000	A BB	130420	20.000 ppm	30.16
D12-Chrysene (I.S. #3)	240	3563	25.52	3	1 000	A BV	17752.	20.000 ppm	30.16
N-Nitrosodimethylamine	Not Found								
Bis(2-Chloroethyl)ether	93	1038	7:32	1	0.698	A BB	4416.	0.598 ppm	0.90
1,3-Dichlorobenzene	Not Found								
1,4-Dichlorobenzene	Not Found								
1,2-Dichlorobenzene	Not Found								
Bis(2-Chloroisopropyl) ether	Not Found								
N-Nitroso-di-n-propylamine	Not Found								
Hexachloroethane	Not Found								
Nitrobenzene	Not Found								
Isophorone	Not Found								
Bis(2-Chloroethoxy)methane	Not Found								
1,2,4-Trichlorobenzene	Not Found								
Naphthalene	Not Found								
Hexachlorobutadiene	Not Found								
Hexachlorocyclopentadiene	Not Found								
2-Chloronaphthalene	Not Found								
Dimethyl phthalate	Not Found								
Acenaphthylene	Not Found								
2,6-Dinitrotoluene	Not Found								
Acenaphthene	Not Found								
2,4-Dinitrotoluene	Not Found								
Diethyl phthalate	Not Found								
Fluorene	Not Found								
4-Chlorophenylphenyl ether	Not Found								
N-Nitrosodiphenylamine	Not Found								
1,2-Diphenylhydrazine	7	2344	17:01	2	0.898	A BB	1456.	0.112 ppm	0.17
4-Bromophenylphenyl ether	Not Found								
Hexachlorobenzene	Not Found								
Phenanthrene	Not Found								
Anthracene	Not Found								
Di-n-butylphthalate	149	2870	20:50	2	1 100	A BB	520.	0 050 ppm	0.08
Fluoranthene	Not Found								
Benzidine	Not Found								
Pyrene	Not Found								
Butylbenzyl phthalate	Not Found								
3,3'-Dichlorobenzidine	Not Found								
Benzo(a)anthracene	Not Found								
Chrysene	Not Found								
Bis(2-Ethylhexyl)phthalate	149	3622	26:17	3	1.017	A BB	5116.	5.871 ppm	8.85
Di-n-octyl phthalate	Not Found								
Benzo(b)fluoranthene	Not Found								
Benzo(k)fluoranthene	Not Found								
Benzo(a)pyrene	Not Found								
Indeno(1,2,3-cd)pyrene	Not Found								
Dibenzo(a,h)anthracene	Not Found								
Benzo(g,h,i)perylene	Not Found								

Table G-8. Mass List for DFTPP Analysis on 3-Day and 7-Day Patapsco POTW Base/Neutral Fraction Effluent

50 445 Mass	0.00 % RA	0.00 % RIC	2. Minima 0. Maxima Inten.	Mass	Min. Inten. 203 % RA	%RIC	Inten.
50.05 F	15.20	1.87	3336.	166.94 F	4.99	0.62	1096.
51.09 F	35.93	4.43	7888.	168.86 F	2.61	0.32	572.
52.21 F	2.30	0.28	504.	174.09 F	0.98	0.12	216.
54.85 F	1.68	0.21	368.	175.16 F	1.66	0.20	364.
56.13 F	1.80	0.22	396.	178.98 F	3.24	0.40	712.
57.06 F	3.94	0.47	844.	180.12 F	2.33	0.29	512.
63.07 F	1.62	0.20	356.	181.09 F	1.33	0.16	292.
65.07 F	1.02	0.13	224.	185.14 F	1.75	0.22	384.
68.98 F	41.98	5.18	9216.	186.11 F	11.48	1.42	2520.
73.91 F	5.43	0.67	1192.	187.11 F	3.26	0.40	716.
75.09 F	6.98	0.86	1532.	192.16 F	1.13	0.14	248.
77.02 F	46.36	5.72	10176.	193.09 F	1.04	0.13	228.
79.11 F	2.53	0.31	556.	198.03 F	100.00	12.34	21952.
80.06 F	2.13	0.26	468.	199.06 F	7.00	0.86	1536.
81.06 F	2.97	0.37	652.	204.08 F	2.82	0.35	620.
82.21 F	0.97	0.12	212.	205.08 F	5.12	0.63	1124.
83.23 F	1.06	0.13	232.	206.08 F	19.86	2.45	4360.
91.10 F	0.98	0.12	216.	207.12 F	2.95	0.36	648.
92.09 F	1.18	0.15	260.	211.05	2.35	0.29	516.
93.07 F	4.05	0.50	888.	217.00 F	5.74	0.71	1260.
98.05 F	2.92	0.36	640.	217.97 F	1.02	0.13	224.
99.09 F	2.73	0.34	600.	220.98 F	7.34	0.91	1612.
100.85 F	2.37	0.29	520.	221.97 F	1.51	0.19	332.
103.13 F	0.98	0.12	216.	223.06 F	1.60	0.20	352.
104.16 F	0.98	0.12	216.	224.06 F	11.42	1.41	2508.
105.15 F	0.97	0.12	212.	225.08 F	2.97	0.34	612.
107.02 F	11.83	1.46	2596.	227.08 F	5.39	0.67	1184.
108.08 F	1.99	0.25	436.	229.02 F	0.98	0.12	216.
110.03 F	25.58	3.16	5616.	244.00 F	9.27	1.14	2036.
111.09 F	3.12	0.38	684.	245.12 F	1.08	0.13	236.
116.95 F	9.69	1.20	2128.	246.05 F	1.64	0.20	360.
121.85 F	1.22	0.15	268.	255.03 F	43.22	5.33	9488.
123.10 F	1.37	0.17	300.	256.06 F	6.67	0.82	1464.
124.12 F	1.00	0.12	220.	258.16 F	2.68	0.33	588.
127.05 F	41.91	5.17	9200.	265.09	1.24	0.15	272.
128.14 F	3.41	0.42	748.	272.94 F	1.90	0.23	416.
129.08 F	15.03	1.85	3300.	274.03 F	3.64	0.45	800.
130.02 F	1.62	0.20	356.	275.03 F	18.26	2.25	4008.
135.09 F	1.48	0.18	324.	276.06 F	2.55	0.31	560.
137.20 F	1.26	0.16	276.	277.06 F	1.64	0.20	360.
141.16 F	2.24	0.28	492.	296.00 F	4.92	0.61	1080.
147.11 F	1.33	0.16	292.	323.06 F	2.13	0.26	468.
148.03 F	2.51	0.31	552.	334.03 F	1.26	0.16	276.
149.12 F	1.09	0.13	240.	364.94 F	2.44	0.30	536.
151.53	1.20	0.15	264.	372.03	0.97	0.12	212.
155.08 F	1.49	0.18	328.	422.97 F	4.12	0.51	904.
156.14 F	1.69	0.21	372.	440.97 F	6.87	0.85	1508.
157.64 F	1.38	0.17	304.	441.97 F	49.78	6.14	10928.
159.17 F	1.77	0.22	388.	442.97 F	9.89	1.22	2172.
161.20 F	1.44	0.18	316.	444.03 F	0.97	0.12	212.

Figure G-1. **Base/neutrals standard reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis.**

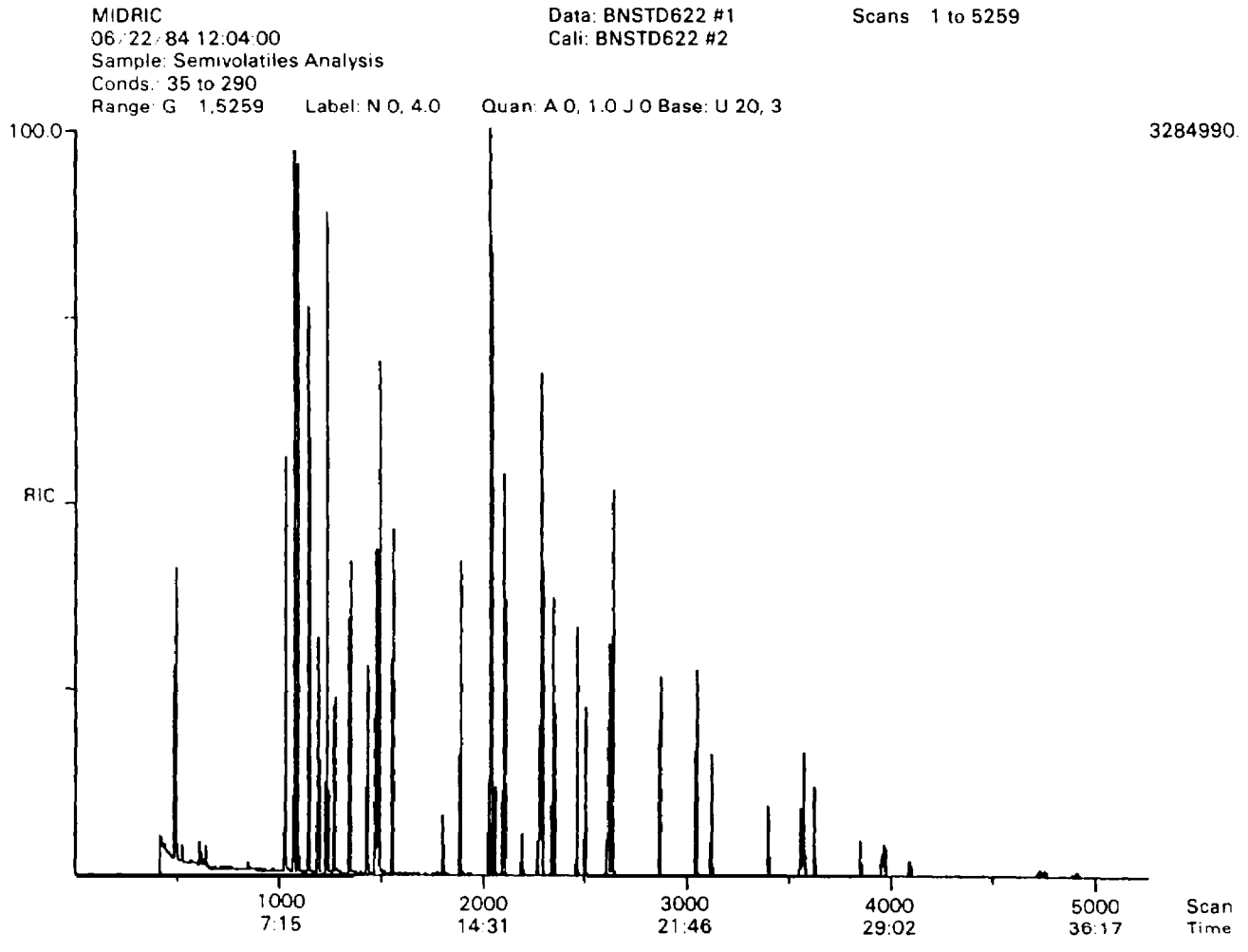


Figure G-2. **Surrogate spike standard reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis.**

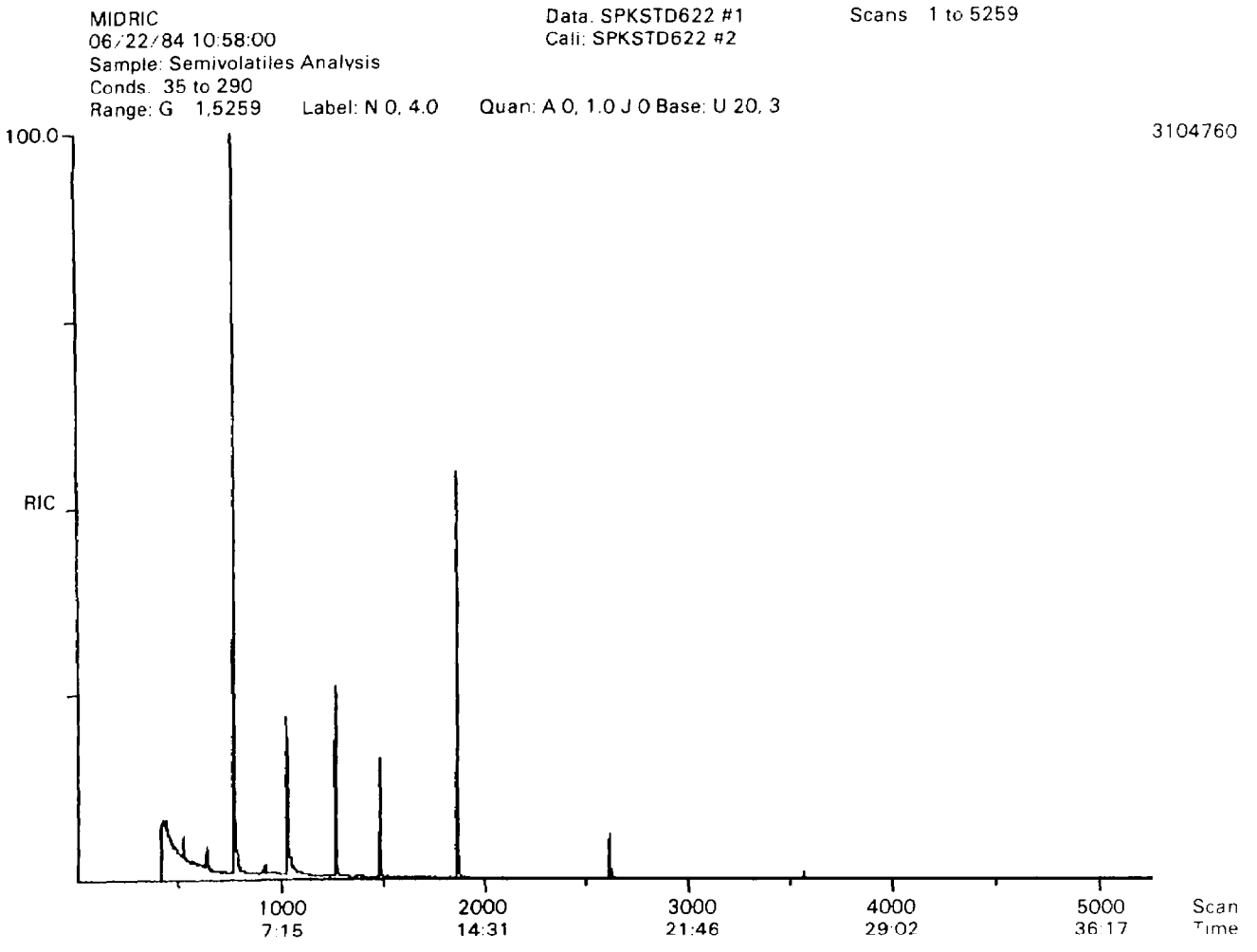


Figure G-3. **Blank reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis.**

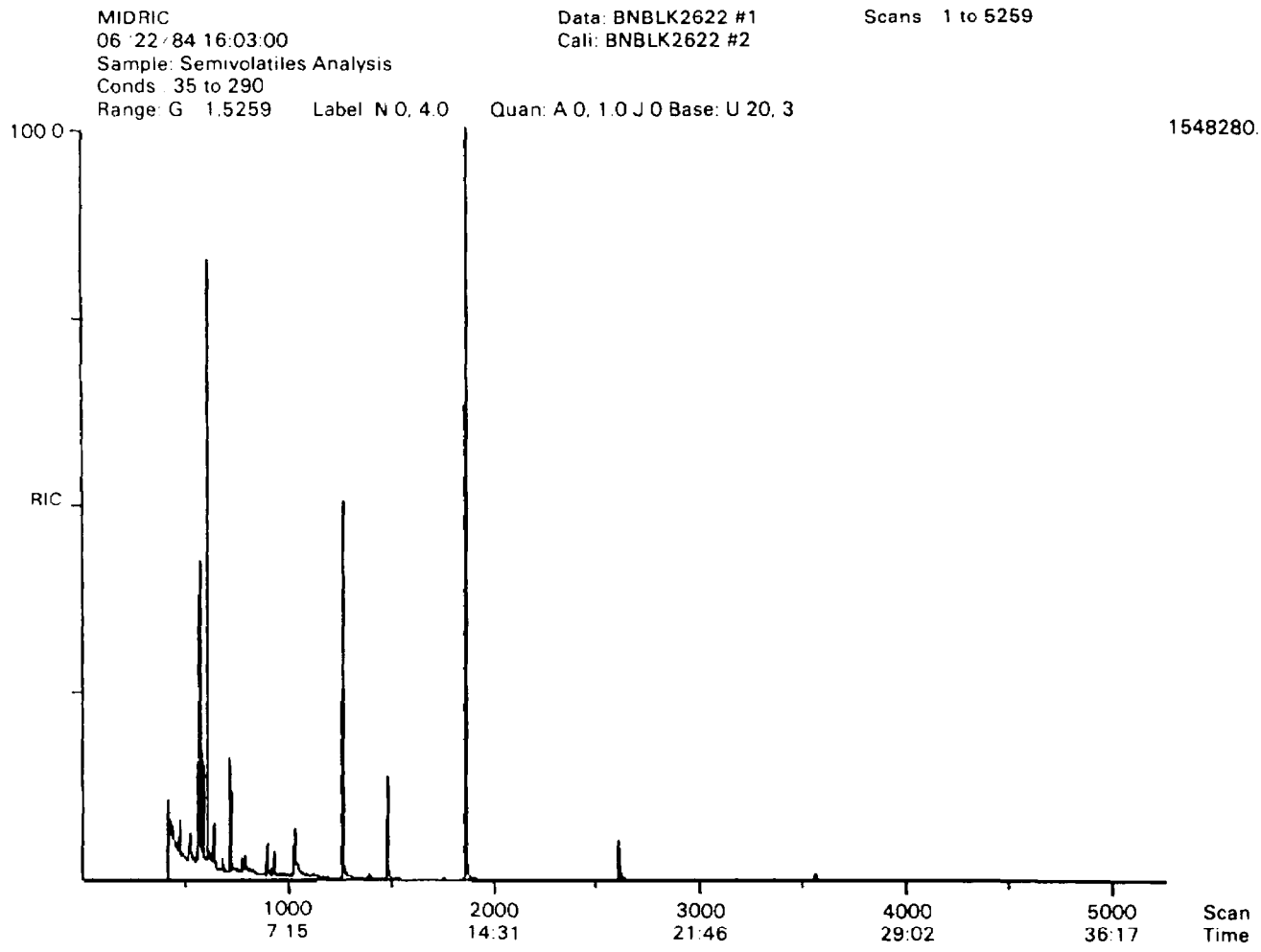


Figure G-4. Reconstructed ion chromatogram for 3-day Patapsco POTW base/neutral fraction effluent analysis.

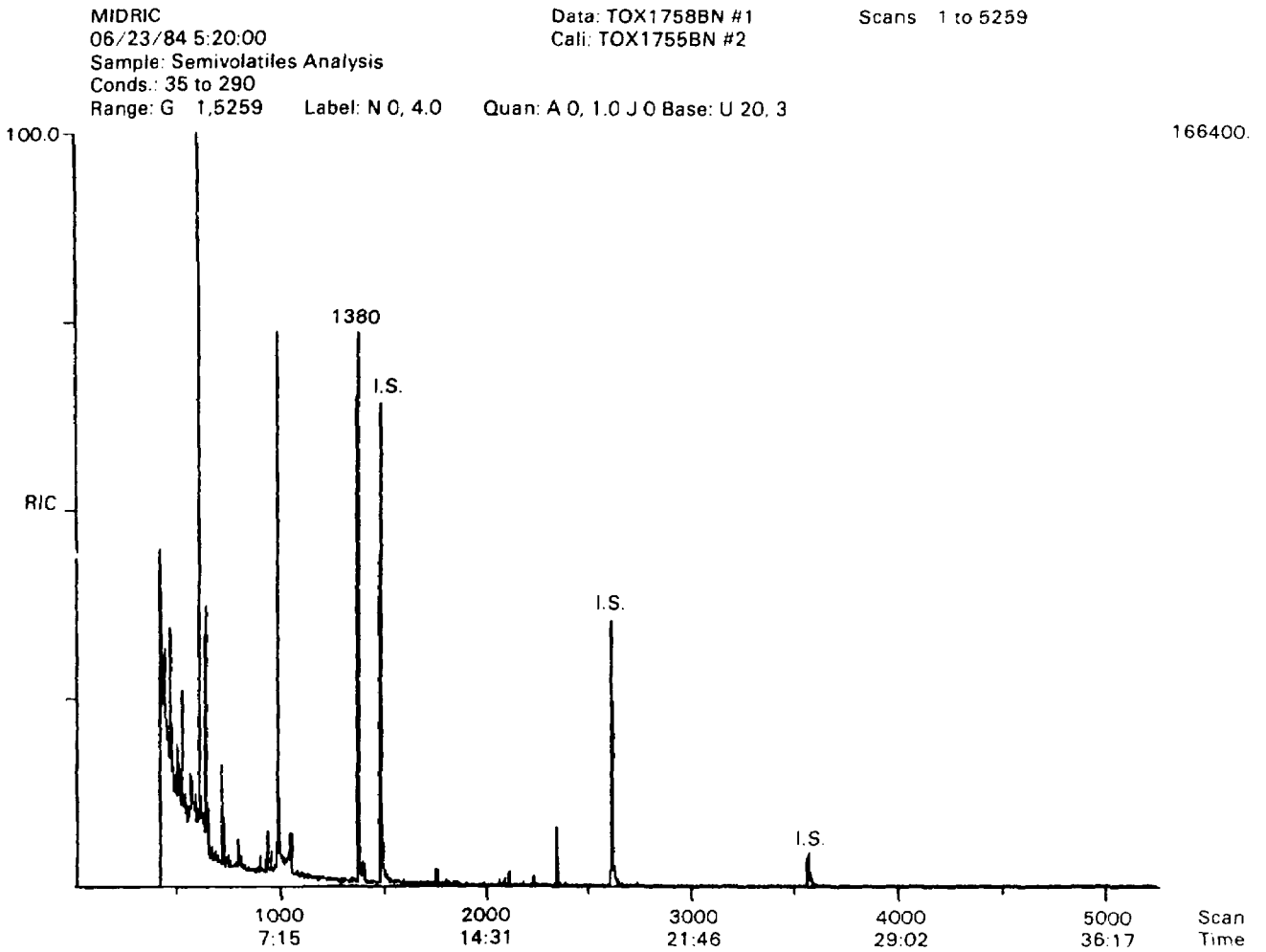


Figure G-5. **Library search for possible compound from 3-day Patapsco POTW base/neutral fraction effluent analysis.**

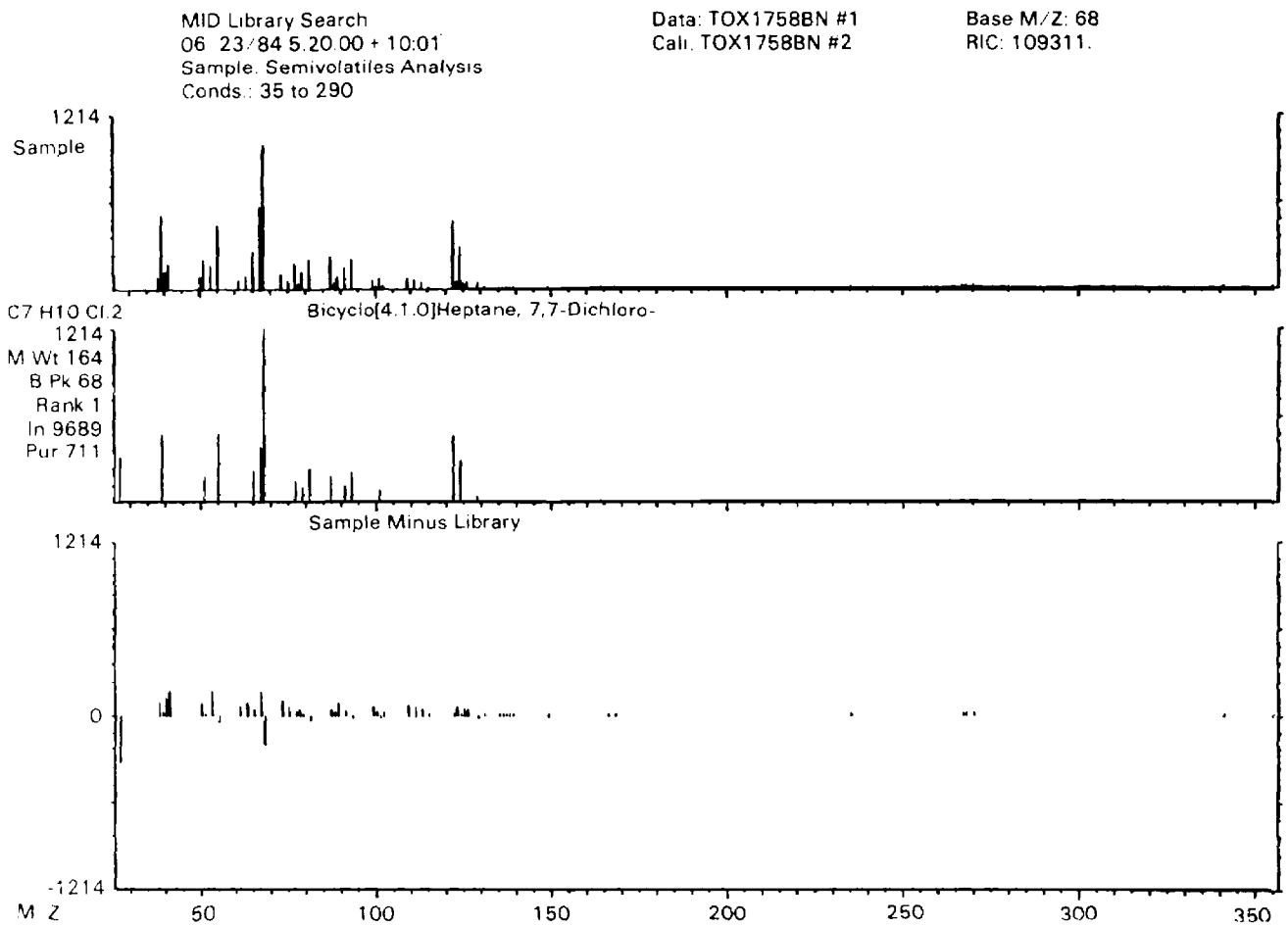


Figure G-6. Reconstruction ion chromatogram for 7-day Patapsco base/neutral fraction effluent analysis.

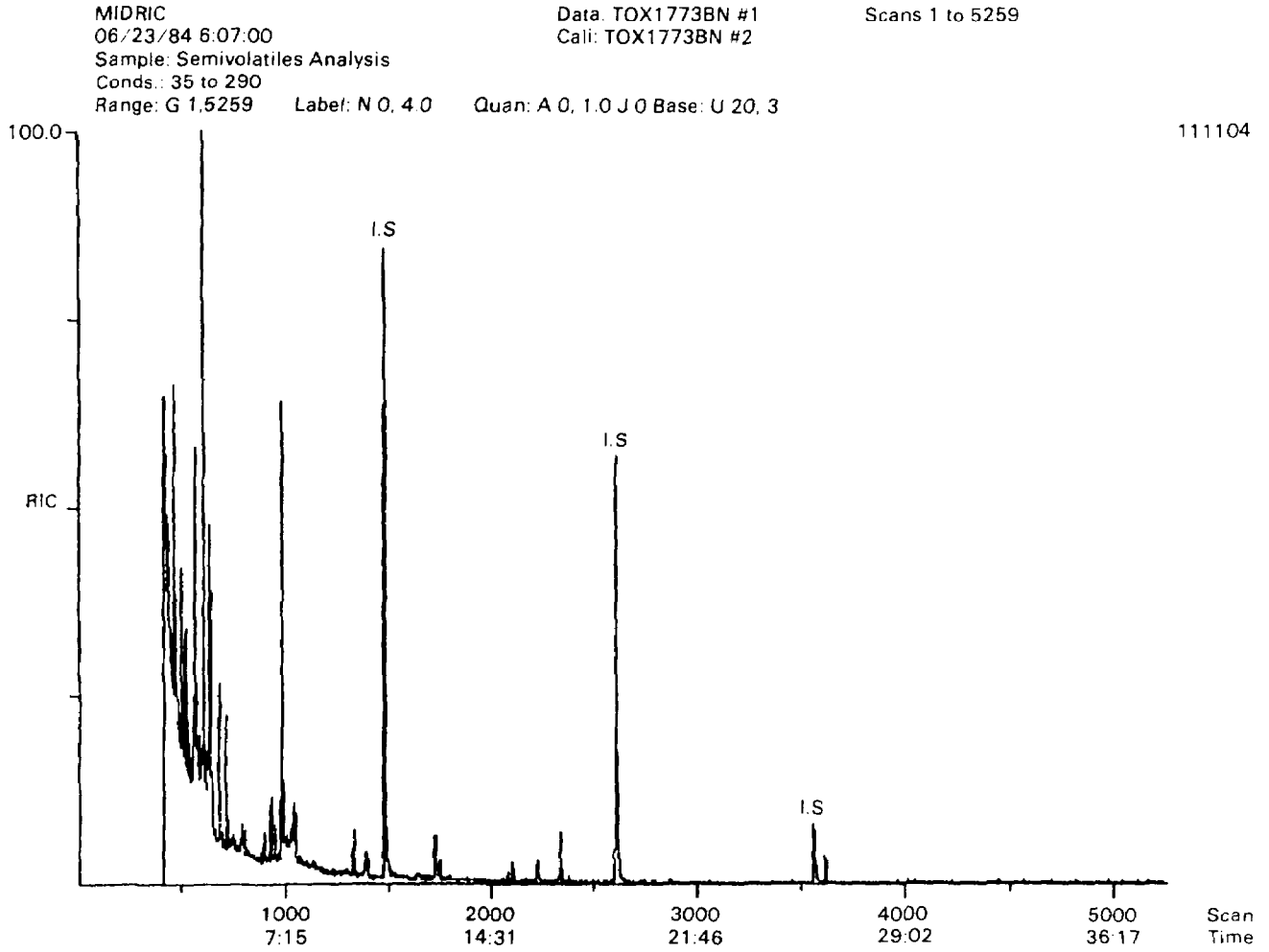


Figure G-7. **DFTPP reconstructed ion chromatogram for 3-day and 7-day Patapsco POTW base/neutral fraction effluent analysis.**

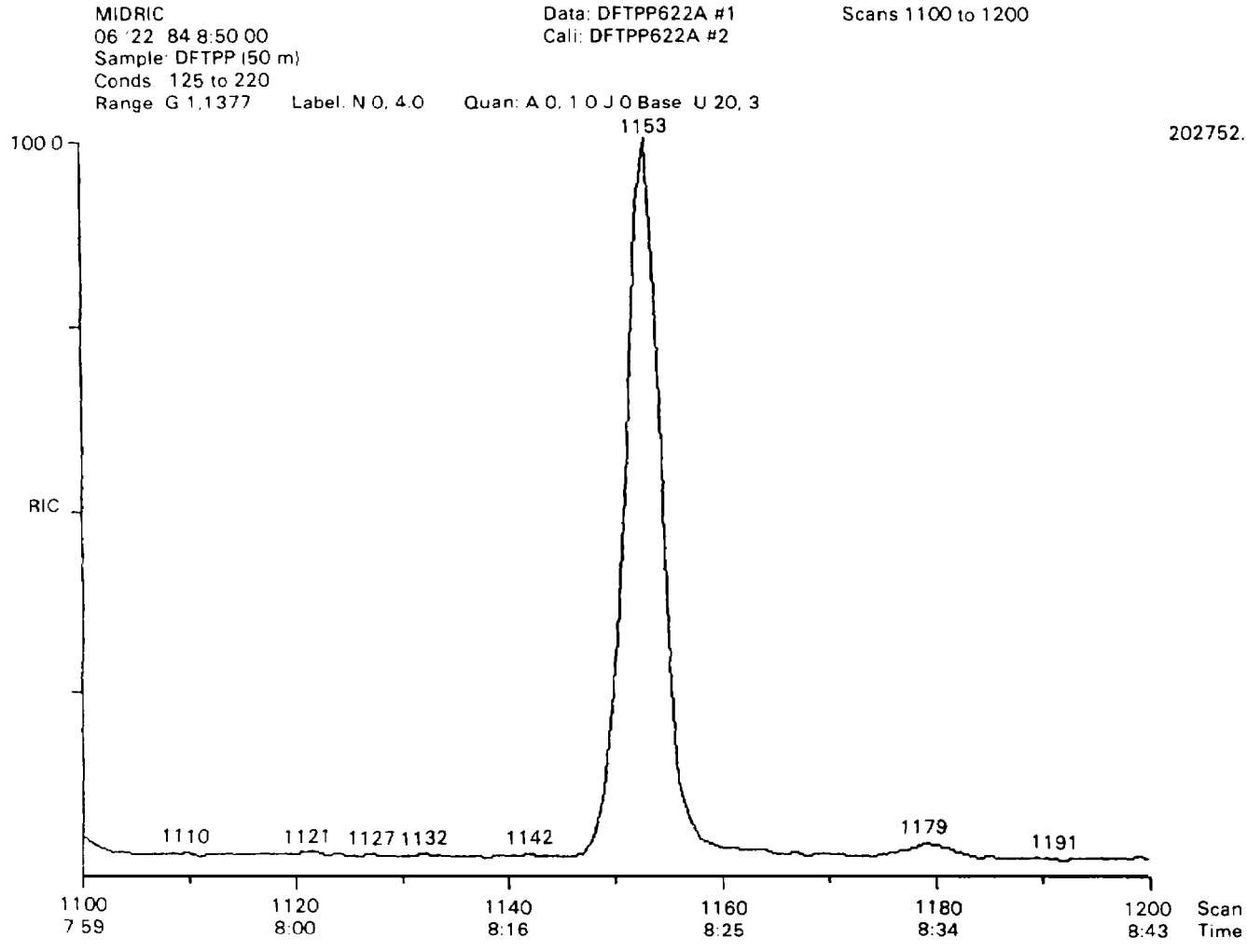
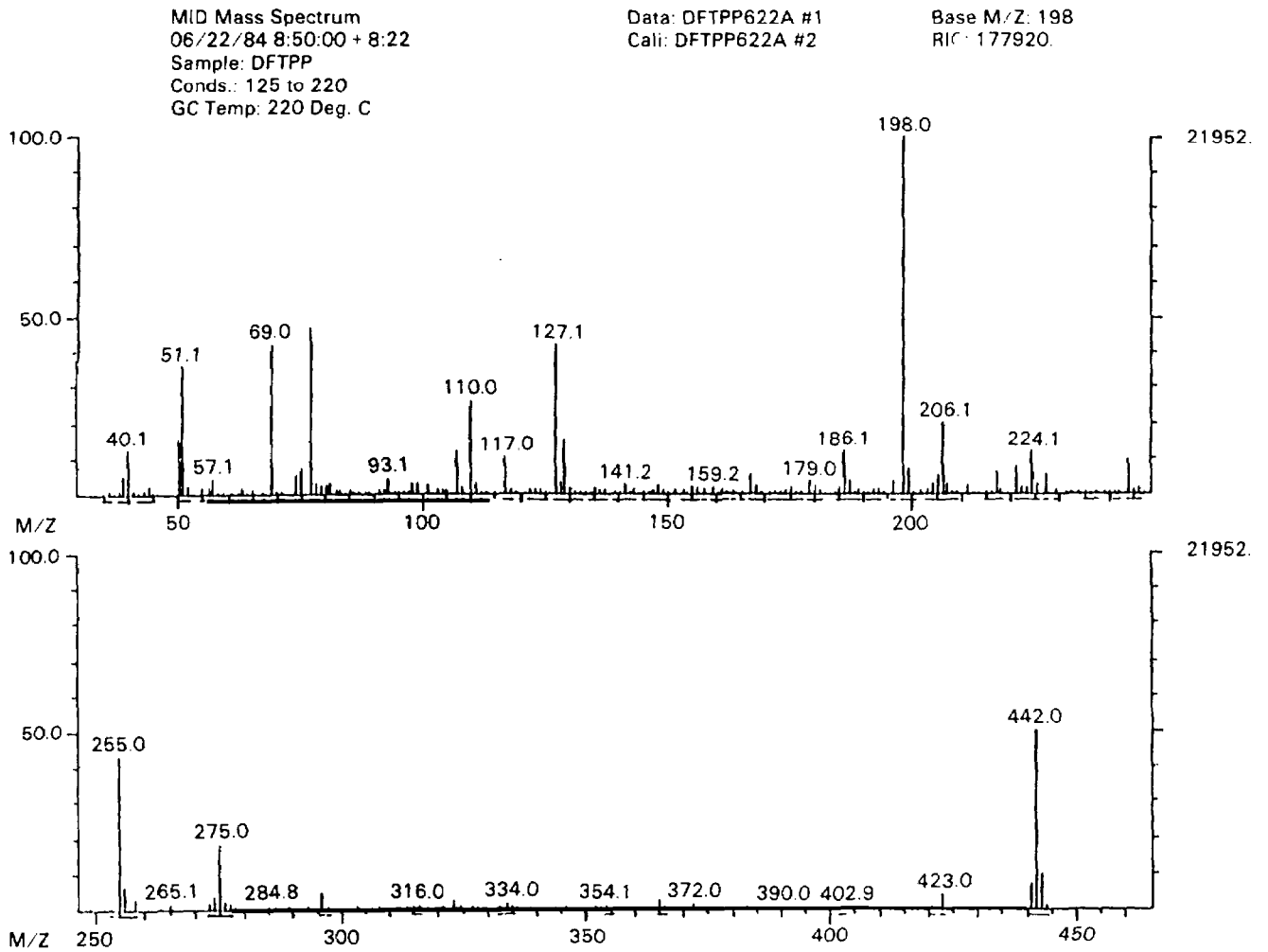


Figure G-8. DFTPP mass spectrum for 3-day and 7-day Patapsco base/neutral POTW fraction effluent analysis.



☆U.S. GOVERNMENT PRINTING OFFICE : 1986—646-116/40609

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

BULK RATE
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

Official Business
Penalty for Private Use, $300

Please make all necessary changes on the above label, **detach** or copy and return to the address in the upper **left-hand** corner.

If you do not wish to receive these reports CHECK HERE ☐, **detach**, or copy this cover, and return to the address in the **upper left-hand** corner.