TENNESSEE VALLEY AUTHORITY

WATTS BAR NUCLEAR PLANT

ENVIRONMENTAL CONTROL MANUAL

CHAPTER 4

EROSION/STORM WATER POLLUTION PREVENTION PLAN

Revision #2

Unit 0

NON-QUALITY RELATED

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(Type Name)

SPONSORING ORGANIZATION: Environmental Control

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REVISION LOG

REVISION OR CHANGE NUMBER	EFFECTIVE DATE	AFFECTED PAGE NUMBERS	DESCRIPTION OF REVISION/CHANGE
Rev. 2	11/1/93	All	Major revision to implement EPA requirements for erosion/storm water pollution control plans. Requires use of excavation permits per SSP-7.04 and review by Environmental Section for erosion control practices. Establishes a site Storm Water Polluton Prevention Team; provides for monthly inspections by Environmental control; describes site drainage areas; provides a list of significant material stored on-site; establishes reporting levels and requirements.

TABLE OF CONTENTS

Section	Title	Page
	Revision/Change Log	2
	Table of Contents	3
1.0	**INTRODUCTION**	4
1.1	Purpose	2
	Applicability	4
2.0	**DETAILS**	4
2.1	Requirements and Responsibilities	4
2.1.1	WBN Site Vice President	4
2.1.2	Plant Manager	4
2.1.3	Responsible Work Control Organizations	5
2.1.4	Site Environmental Manager (SEM)	5
2.1.5	Corporate Environmental Manager	6
2.1.6	Resource Group	7
2.1.7	Land resources	7
3.0	**RECORDS**	7
3.1	QA Records	7
3.2	Non-QA Records	8
4.0	**DEFINITIONS**	8
5.0	**REFERENCES**	9
5.1	Source Documents	9
5.2	Interface Documents	9
	Appendix A, Erosion/Storm Water Control Inspection Record	10
	Appendix B, Storm Water Drainage Areas	11
	Appendix C, Description of Exposed Significant Material	19
	Appendix D, Parameter Reporting Levels for Storm Water Discharge	24
	Figure 1, Storm Water Drainage Map	25
	SOURCE NOTES	26

1.0 INTRODUCTION

1.1 Purpose

Chapter 4 of the Watts Bar Nuclear Plant (WBN) Environmental Compliance Manual (ECM) describes how WBN will control soil erosion and storm water runoff from exposed areas. The primary purpose of this chapter is to provide guidance for industrial facilities that are subject to requirements under the Environmental Protection Agency (EPA) General Permits for storm water discharges associated with industrial activity. The goal of this plan is to improve the water quality by reducing the pollutants contained in storm water discharges.[1,2]

1.2 Applicability

This Chapter covers all activities at WBN which results in bare areas, loading and unloading areas, outdoor storage areas, outdoor activities, land disturbances, dust and particulate generation, improper piping, waste management and disposal areas and practices. This includes construction, modifications, and operational activities. Since onsite erosion is primarily due to rainfall runoff, methods to control water-generated erosion are the primary focus of this Chapter.

2.0 DETAILS

2.1 Requirements and Responsibilities

2.1.1 WBN Site Vice President

Responsible for the overall management of the power generation and maintenance programs such that the material storage and handling at WBN are fully consistent with the requirements of this ECM.

2.1.2 Plant Manager

A. Responsible for the day-to-day management of activities related to operating the plant such that all materials are stored and handled in such a manner as to prevent their release into the environment.

B. Responsible for the coordination and implementation of requirements identified in this plan for the TVA Watts Bar Nuclear Plant.

2.1.3 Responsible Organizations

A. Obtain excavation permits in accordance with SSP-7.04, Work Permits.

B. Notify the Site Environmental Manager (SEM) prior to excavation that will contribute to the discharge of high suspended solids water (muddy water) to a stream, ditch, drain, or catch basin.

C. If the excavation will disturb an area of one acre or more, a written Erosion/Storm Water Control Plan must be prepared at least a week in advance describing the proposed action and the proposed mitigative measures to be taken to control erosion.

NOTE No actions are to be taken until the SEM has approved the mitigation plan for erosion/storm water control.

D. Implement the appropriate erosion/storm water control measures as specified in the Erosion/Storm Water Control Plan and/or as directed by the SEM. Maintain the Erosion/Storm Water Control Plan as an attachment to the work initiating document.

E. Take appropriate actions to upgrade or maintain the erosion control devices as directed by the SEM.

F. Furnish personnel and material to perform any upgrading or maintenance of erosion control devices as requested by the SEM.

G. Apply Best Management Practices (BMPs) such as DIVERSION DIKES, COLLECTION DITCHES, regrading, SEDIMENT TRAPS, STRAW BALES, ROCK CHECK DAMS, RETENTION SWALES, GROUND COVERINGS, and revegetation to control erosion and SEDIMENT RUNOFF. These techniques may be used individually or in combination.

2.1.4 Site Environmental Manager (SEM)

A. Responsible for the overall management of the Watts Bar Erosion/Storm Water Control Plan to assure the adequacy of erosion and storm water runoff pollution prevention.

B. Review all proposed actions which will result in erodible areas.

C. Approve any written Erosion/Storm Water Control Plans, or revise the plan, and advise the initiating organization of what additional controls are to be used.

D. Direct responsible organizations in the implementation of appropriate onsite erosion and sediment control measures.

2.1.4 **Site Environmental Manager (SEM)** (Continued)

E. Ensure appropriate annual employee training in erosion/storm water pollution control is provided for all plant personnel who might reasonably be expected to perform the duties identified in this plan. Additionally, the Nuclear Power Environmental Awareness Handbook provides site employees a basic understanding and knowledge of spill control and storm water runoff contamination.

F. Inspect areas monthly where erosion/storm water control measures are being taken to ensure control measures are working properly.

G. Document all inspection and erosion/storm water control work in an Erosion/Storm Water Control Inspection Record (Appendix A).

H. Escort Regulatory Agencies and other personnel on site inspections.

I. Responsible for the annual review and updating of this plan including the WBN Description of Exposed Significant Material (Appendix D), and the Non-Storm Water Discharge Assessment.

J. Sign-off as responsible corporate official certifying the Non-Storm Water Discharge Assessment.

NOTE The Non-Storm Water Discharge Assessment and the Pollution Prevention Team rosters are updated annually and maintained on file in the Environmental Control Section.

K. Serve as Storm Water Pollution Prevention Team Leader.

L. Direct the annual storm water sampling effort.

M. Submit report level exceedance (See Parameter Reporting Levels for Storm Water Discharges, Appendix D) and the annual monitoring results in accordance with State requirements.

2.1.5 **Corporate Environmental Manager**

A. Prepare for submittal the environmental permit applications.

B. Provide input as team member of the Storm Water Pollution Prevention Team.

C. Evaluate the storm water monitoring data for compliance with the regulations.

2.1.6 Resource Group

Responsible to the SEM for the following:

A. Peforms the monthly erosion control inspections and coordinates corrective actions with the Land Resources personnel.

B. Completes the proper inspection forms and notifies the SEM of all problems related to the inspection.

C. Recommends to the SEM preventative maintenance activities and BMPs.

D. Works as part of the Environmental Emergency Response Team.

E. Provides input as team member of the Storm Water Pollution Prevention Team.

F. Performs the annual storm water sampling collection in accordance with the regulations and guidelines and gathers the storm water data such as air temperature, storm event duration, starting and ending of the storm event, the magnitude and the time between storm events.

G. Provides the WBN narrative description for Storm Water Drainage Areas (Appendix B).

2.1.7 Land Resources

Responsible to the SEM for the following:

A. Responsible for installing and maintaining the silt fences and straw bales on the WBN site.

B. Supervise the application of the herbicides at the plant.

C. Provide input as team member of the Storm Water Pollution Prevention Team.

3.0 RECORDS

3.1 QA Record

None

3.2 Non-QA Records

The following documents are non-QA records and are verified, packaged and transmitted for retention in accordance with the Document Control and Records Management System (DCRM).

- *Erosion/Storm Water Control Inspection Record* (Appendix A)

These records are maintained for at least three years.

4.0 DEFINITIONS

The following terms are controlled by this Chapter:

A. BEST MANAGEMENT PRACTICE (BMP) - Measures used to reduce the amount of pollution entering surface water, air, land, or ground water and may take the form of a process, activity, or physical structure.

B. COLLECTION DITCH - Ditch to collect storm water runoff and transport runoff in a controlled manner to a holding pond or other containment structure.

C. DIVERSION DIKE - Used to route storm water runoff away from exposed areas.

D. GROUND COVER - Cloth-like covering made from natural materials and used to hold grass and seeds in place until vegetation can take hold. Usually left in place, since it will decompose.

E. RETENTION SWALE - A hollow or depression where sediment is allowed to settle out.

F. ROCK CHECK DAM - Sediment control dams constructed of rocks used to reduce or prevent excessive erosion by reduction of flow velocities.

G. SEDIMENT TRAP - A small holding pond were sediment is allowed to settle out and water evaporates or percolates into the soil.

H. SILT FENCE - Man-made material erected across an exposed area to filter out sediment but allow water to pass through.

4.0 **DEFINITIONS** (Continued)

I. STRAW BALE SEDIMENT BARRIER - Temporary berms, diversions, or other barriers constructed of straw bales and used to retain sediment onsite by retarding and filtering storm water runoff.

J. STORM WATER - The flow of water or surface runoff resulting from rainfall.

5.0 **REFERENCES**

5.1 **Source Documents**

A. EPA, Guidelines for Erosion and Sediment Control Planning and Implementation, EPA-R2-72-015, August 1972

B. EPA, Processes, Procedures, and Methods to Control Pollution Resulting from All Construction Activities, EPA-430/9-73-007, October 1973

C. NPDES Permit TN0020168, Part 1.B.1.e and Part III.1

D. SSP-13.03, *Environmental Compliance*

E. *Storm Water in Tennessee, A Training Manual for Manufacturers*, The University of Tennessee Center for Industrial Services, 1992

F. EPA, Storm Water Management for Industrial Activities Developing Pollution Prevention Plant and Best Management Practices, EPA 832-R-92-006, September 1992

G. NPDES Permit TNR001343

H. Erosion and Sediment Control Plan, submitted to EPA Region 4, July 1984. Letter from M. R. Rivers, TVA to P. J. Traina, EPA Region 4, Atlanta, Georgia, July 1983.

5.2 **Interface Documents**

SSP-7.04, Work Permits

APPENDIX A
Page 1 of 1

EROSION/STORM WATER CONTROL INSPECTION RECORD

Date: _____ Inspected By: _____

Area(s) Inspected: Erosion/Storm Water Control Measures:

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

	Yes	No	N/A
Corroded drums or drums without plugs or covers	|_|	|_|	|_|
Corroded or damaged storage tanks	|_|	|_|	|_|
Torn bags or bags exposed to rain water	|_|	|_|	|_|
Leaking pumps and/or hose connections	|_|	|_|	|_|
Broken or cracked dikes or berms	|_|	|_|	|_|

Comments and Recommendations: _____

_____

_____

_____

_____

_____

Corrective Actions Taken: _____

_____

_____

_____

_____

_____

Erosion/Storm Water Pollution Prevention Plan Requires Revision |_| Yes |_| No

Inspector's Signature: _____ Reviewed By: _____

APPENDIX B
Page 1 of 8

STORM WATER DRAINAGE AREAS

Area 1:

The total surface area drained is 51 acres with approximately 43 acres of impervious surfaces. Area 1 includes a parking lot and roof drainage from portions of the Turbine, Auxiliary, and Control Room Bldgs; the number one Reactor Bldg; the Additional Diesel Generator Bldg; the Service Building; Power Stores Bldg; and the Makeup Water Treatment Plant. In addition, a portion of the switchyard is located in this area. Ground cover consists of crushed stone, gravel, pavement and some grass.

Area 1 also contains the Oil Storage Room located off the Service Bldg. loading dock, and the Chemical Storage area. Lubricating and insulating oils and various chemicals are stored under roofs of several buildings in this area. Diesel fuel offloading is performed in this area. The gasoline storage tank was removed from this area on December 12, 1991. If a spill occurs, then the flow is into the yard drainage system which drains into the yard holding pond (YHP). This area along with Area 2 has the largest potential for oil and chemical spills.

Buildings in this area have internal sumps for collection and containment of spills. Spills occurring outside of buildings are minimized and controlled by a site specific Spill Prevention Control and Countermeasures (SPCC) Plan. There have been no spills experienced in this area which would expose residual materials to storm water.

Herbicides and fertilizers are applied twice per year during the growing season in this area to control vegetative growth in yard areas and fence rows and enhance the growth of grass in lawn areas.

The YHP can provide temporary retention for chemical spills and is equipped with an oil skimmer to prevent oil spills from leaving the pond.

Area 2:

The total surface area drained is 49 acres which includes approximately 47 acres of impervious surfaces. This area, which includes the other halves of the Turbine, Auxiliary, and Control Room Bldgs; the number two Reactor Bldg; the Diesel Generator Bldgs, the Lube Oil Tanks; Diesel Fuel Tanks; Cooling Towers (Nos. 1 & 2); Power Stores Yard; Corrosion Monitoring Laboratory; and the Condenser Cooling Water Bldg. is otherwise primarily hard surface area. Primary spills can occur from the chemical storage areas, and from the other sources of oil and hazardous chemicals. Drainage from this area is via drainage basin, and subsurface piping to the YHP.

APPENDIX B
Page 2 OF 8

STORM WATER DRAINAGE AREAS

Area 2: (Continued)

The switchyard is also located in this area, and if any insulating oil is spilled from one of the transformers, it will flow through the limestone underlayment into the drainage ducts which are part of the yard drainage system that drains to the YHP.

Buildings in this area have internal sumps for collection and containment of spills. Spills occurring outside of buildings are minimized and controlled by a site specific SPCC Plan. There have been no spills experienced in this area which would expose residual materials to storm water.

Herbicides and fertilizers are applied approximately twice per year during the growing season in this area to control vegetative growth in parking areas and enhance the growth of grass in lawn areas.

Area 3:

The total surface area drained is 134 acres which includes approximately 22 acres of impervious surfaces. This area consists mainly of wetlands, the desilting basins, and several office buildings. Ground cover consists primarily of natural vegetation. There are no fuel offloading facilities in this area or major equipment storage areas or other sources or oil or chemicals, hence no spills have been experienced in this area which would expose residual materials to storm water.

Area 3 also contains the large construction parking lot. It is gravel with drainage piping going to the trench A drainage ditch which drains directly into the river. In addition, several office buildings are located in this area.

Herbicide and fertilizers are applied twice per year during the growing season in this area to control vegetative growth in yard areas and enhance the growth of grass in lawn areas.

All storm water drainage from this area passes through a wetlands area and to the waters of the U. S.

Area 4:

The total surface area drained is 100 acres which includes approximately 3 acres of impervious surfaces. This area consists mainly of the cooling water intake and the diffuser discharge (DSN 101). The intake pumping station houses the sodium bromide and sodium hypochlorite (the Biocide injection area outside the intake pumping station, and microbiological induced corrosion chemical injection dike, respectively) both with secondary confinement. Spills that escape the secondary confinement dike will flow into the waters of the U.S.

APPENDIX B
Page 3 of 8

STORM WATER DRAINAGE AREAS

Area 4: (Continued)

On June 12, 1990, one hundred and twenty gallons of 12 percent sodium hypochlorite were released from a broken pipe. The spill was contained near the IPS, neutralized with sodium bisulfite, and cleaned up.

A broken supply line which fed a temporary pumping system located adjacent to the yard holding pond, spilled diesel fuel into the pond and less than one gallon of fuel reached the water of the U. S., on September 28, 1989.

Herbicides and fertilizers are spot-applied twice per year during the growing season in this area to control vegetative growth and enhance the growth of grass in the area.

Storm water drainage is primarily overland and through a major intermittent stream which collects runoff from other areas and drains into the waters of the U. S.

Area 5:

The total surface area drained is 35 acres which includes approximately 28 acreas of the YHP. The remaining 7 acres adjacent to the YHP is naturally vegetated and considered pervious.

There is no material storage in this area and subsequently no material loading or access areas. There have been no spills experienced in this area which expose residual materials to storm water.

Herbicides are applied twice per year during the growing season to control vegetative growth along the rip-rap.

Storm water drainage is directly into the YHP. The YHP is routed via a drainage system to the diffuser discharge into the river.

Area 6:

The total surface area drained is 27 acres which includes approximately 3 acres of impervious surface. This area, which includes the Evaporation/Percolation Pond TSP, is primarily naturally vegetated. Oil or chemical storage within this area is minimal. Ground cover is primarily grass. There have been no spills experienced in this area which would expose residual materials to storm water.

APPENDIX B
Page 4 of 8

STORM WATER DRAINAGE AREAS

Area 6: (Continued)

Herbicides are spot-applied twice per year during the growing season to control vegetative growth along fence rows.

General surface water drainage is directed from the high points of land to the drainage ditch which drains to the river.

Area 7:

The total surface area drained is 12 acres which includes approximately 7 acres of impervious surfaces. This area consists of the temporary waste storage area, the metal cleaning waste ponds (1-MG lined pong, 5-MG unlined pond), the Low Volume Waste Treatment Pond (LVWTP), and the alum sludge ponds which are each self-contained and receive only pumped influents. The LVWTP provides confinement for oils and chemicals. Any spillage or leaks from the temporary waste storage area is contained in the secondary confinement spill pan. Leaks from the secondary confinement spill pan or waste oil tanker will be contained by the berm surrounding the areas. The berm area has a valved drain to the LVWTP.

Herbicides are applied twice a year during the growing season to control vegetative growth around the treatment ponds.

Storm water drainage flows primarily overland into the Low Volume Waste Treatment Pond.

Area 8

The total surface area drained is 32 acres of which there are approximately 8 acres of impervious surfaces. This area consists of the special waste storage area, the asbestos waste storage bin, the old dairy farm and the red barn, and the heliport. The lower section is predominately a hilly, tree covered narrow strip of land with a long ravine. The upper section is a flat grasses area which was used for the heliport.

The general direction of surface water drainage is toward the Tennessee River via the ravine and the intermittent stream to the river. Oil and chemical storage in this area is minimal. There have been no spills experienced in this area which would expose residual materials to storm water.

Herbicide is spot-applied to fence rows twice a year during growing season to control vegetative growth.

APPENDIX B
Page 5 of 8

STORM WATER DRAINAGE AREAS

Area 9:

The total surface area drained is 45 acres which includes approximately 11 acres of impervious surfaces. This area consists of a portion of the construction warehouses yard 2, and a portion of the old demolition landfill. Ground cover consists of crushed stone, gravel around the warehouses, and natural vegetation on top of the landfill. Storm water drainage is overland and via several intermittent streams.

Oil and chemicals are stored within this area. There have been no spills experienced in this area which would expose residual materials to storm water.

Herbicide is spot-applied to fence rows twice a year during growing season to control vegetative growth. Fertilizer is applied to the old demolition landfill as needed to enhance the growth of the ground cover.

The plant SPCC plan provides for environmental protection from the waters of the U. S.

Area 10:

The total surface area drained is 40 acres with approximately 4 acres of impervious surfaces. This area consists of a portion of the construction warehouse yard 2 buildings, and a portion of the old demolition landfill. Ground cover consists of crushed stone, gravel on the upper end, and trees and grass on the lower end.

Oil and chemical materials are stored within this area. There have been no spills experienced in this area which would expose residual materials to storm water.

Herbicide is spot-applied to fence rows twice a year during growing season to control vegetative growth.

The plant SPCC plan provides for environmental protection of the waters of the U. S.

Area 11:

The total surface area drained is 83 acres which includes approximately 18 acres of impervious surfaces. This area includes the old warehouses, a part of the new demolition landfill, and the major part of the construction warehouse yard 2. The area around the warehouse building consists of crushed stone, gravel, and pavement. The rest is primarily naturally vegetated.

APPENDIX B
Page 6 of 8

STORM WATER DRAINAGE AREAS

Area 11:

Limited quantities of oil and chemicals are stored in this area.

Herbicides and fertilizers are applied twice per year during the growing season in this area to control the vegetative growth in yard areas and fence rows and enhance the growth of grass in lawn areas.

General surface water drainage is directed from the high points of land through and around the horseshoe pond to a perennial stream and then to the river.

Area 12:

The total surface area drained is 153 acres which includes approximately 1 acre of imperious surfaces. This area includes a portion of the new demolition landfill. It is almost entirely in natural vegetation. Oil and chemical storage in this area is nonexistent.

Herbicides and fertilizers are applied twice per year during the growing season in this area to control the vegetative growth around fence rows and enhance the growth of grass in specified areas.

General surface water drainage is directed to an unnamed tributary of Yellow Creek and then into the river.

Area 13:

The total surface area drained is 138 acres with approximately 20 acres of impervious surfaces. This area includes portions of the construction warehouse yards 1 and 2 buildings (Huts 7, 8, and 19), the borrow soil areas, the sewage treatment plant, the construction oil shed, and the paint and thinner storage. Ground cover consists of crushed stone, gravel around the warehouses; and trees and grass on the remaining north and south ends.

Material storage is consisting of paints, solvents, thinners and heavy equipment lubricating oils, all in 55-gallon containers or less. Drip pans and sorbent sand are in use in heavy equipment satellite service facility. There have been no spills experienced in this area which would expose residual materials to storm water.

APPENDIX B
Page 7 of 8

STORM WATER DRAINAGE AREAS

Area 13: (Continued)

Most of the warehouse drainage is routed to the construction holding pond (DSN 112). This pond provides temporary retention for chemical spills and is equipped with an oil skimmer to prevent oil spills from leaving the pond. Any spill that may occur in small quantities can be minimized and controlled by a site specific SPCC plan.

Herbicide is spot-applied to fence rows twice a year during growing season to control vegetative growth.

General surface water drainage is into the construction runoff pond which discharges into an unnamed tributary of Yellow Creek and then into the river.

Area 14:

The total surface area drained is 43 acres with approximately 33 acres of impervious surfaces. This area consists of a parking lot as well as several equipment buildings, and the westside collection depot. Gasoline and diesel fuel storage tanks are also in this area. Ground cover consists of crushed stone, gravel on the upper ends around the warehouses, and trees and grass on the lower end.

Limited quantities of oil and chemicals are stored within this area. There have been no spills experienced in this area which would expose residual materials to storm water.

Most of the warehouse drainage is routed to the construction holding pond (DSN 112). This pond provides temporary retention for chemical spills and is equipped with an oil skimmer to prevent oil spills from leaving the pond. Spills of waste oil, hazardous chemicals, or hazardous waste outside of the secondary confinement provided, can flow to the construction runoff holding pond where confinement for cleanup is provided for oils and other floating materials only and not for any water soluble chemicals.

Gasoline and diesel fuel offloading facilities are also in this area. Any spill that may occur in small quantities can be minimized and controlled by a site specific SPCC plan.

Herbicide is spot-applied to fence rows twice a year during growing season to control vegetative growth.

APPENDIX B
Page 8 of 8

STORM WATER DRAINAGE AREAS

Area 14: (Continued)

General surface water drainage is directed into the construction runoff pond which then discharges into an unnamed tributary of Yellow Creek and then into the river.

Area 15:

The total surface area drained is 19 acres with 18 acres of impervious surfaces. This area consists primarily of equipment buildings with limited quantities of oil and chemicals. Ground cover consists of crushed stone and gravel on the upper ends around these buildings.

There have been no spills experienced in this area which would expose residual materials to storm water.

All of the building drainage is routed to the construction holding pond (DSN 112). This pond provides temporary retention for chemical spills and is equipped with an oil skimmer to prevent oil spills from leaving the pond. Any spill that may occur in small quantities can be minimized and controlled by a site specific SPCC plan.

Herbicide is spot-applied to fence rows twice a year during growing season to control vegetative growth.

General surface water drainage is directed into the construction runoff pond which then discharges into an unnamed tributary of Yellow Creek and then into the river.

Area 16:

The total surface area drained is 5 acres with no impervious surfaces. This area is wholly wooded. No oils and chemicals are stored in this area.

General surface water drainage is directed from the high points of land down onto non-TVA property before joining the unnamed tributary of Yellow Creek and then into the river.

APPENDIX C
Page 1 of 5
DESCRIPTION OF EXPOSED SIGNIFICANT MATERIAL

MATERIAL	AREA SHOWN ON MAP	LOCATION ON SITE	QUANTITY (GALS)	STORAGE VESSEL OR METHOD	DESCRIPTION OF BMPs
DIESEL FUEL	1	5th D/G BLDG	69,100	TANKS (4)	YARD HOLDING POND
PCB OIL	1 & 2	AUXILIARY BLDG	6,252	TRANSFORMERS (26)	CONCRETE CURB AND FLOOR
PCB OIL	2	CCW BLDG	358	TRANSFORMERS (2)	CONCRETE CUB AND FLOOR
GASOLINE	14	CONSTR GAS TANK	15,000	TANK	CONSTR RUNOFF HOLDING POND
DIESEL FUEL	14	CONSTR DIESEL FUEL TANK	16,000	TANK	CONSTRUCTION RUNOFF HOLDING POND
FYRQUEL EHC	14	CONSTR WH HUT 6 YARD 1	880	DRUMS	CONSTRUCTION RUNOFF HOLDING POND
DIESEL FUEL	1 & 2	D/G BLDG	276,400	TANKS (21)	YARD HOLDING POND
LUBRICATION OIL	1 & 2	D/G BLDG	6,530	VARIOUS	YARD HOLDING POND
PCB OIL	5	INTAKE PUMPING STATION	646	TRANSFORMERS (2)	CONCRETE CURB AND FLOOR
LUBRICATION OIL	1 & 2	MECHANICAL MAINTENANC STORAGE ROOM	1,412	DRUMS/CANS	EMERGENCY OIL AND FIRE PROTECTION SUMP
KEROSENE	1 & 2	MECHANICAL MAINTENANC STORAGE ROOM	45	DRUMS/CANS	EMERGENCY OIL AND FIRE PROTECTION SUMP
KEROSENE	2	POWER STORE STORAGE YARD 1	110	DRUMS	YARD HOLDING POND
LUBRICATING OILS	2	POWER STORE STORAGE YARD 1	24,230	DRUMS/CANS	YARD HOLDING POND
LUBRICATION OILS	1	POWER STORE SERVICE BLDG OIL ROOM	100	CANS	YARD HOLDING POND
PCB OIL	1	SERVICE BLDG	500	TRANSFORMERS (2)	CONCRETE CURB AND FLOOR
WASTE OIL	7	TEMPORARY WASTE STORAGE AREA		DRUMS	EARTHEN BERM WITH CLAY
PCB OIL	1 & 2	TURBINE BLDG	2,348	TRANSFORMERS (12)	CONCRETE CURB AND FLOOR
LUBRICATING OIL	1 & 2	TURBINE BLDG	3,300	PUMPS (4)	EMERGENCY OIL AND FIRE PROTECTION SUMP

APPENDIX C
Page 2 of 5
DESCRIPTION OF EXPOSED SIGNIFICANT MATERIAL

MATERIAL	AREA SHOWN ON MAP	LOCATION ON SITE	QUANTITY (GALS)	STORAGE VESSEL OR METHOD	DESCRIPTION OF BMPs
LUBRICATING OIL	1 & 2	TURBINE BLDG	29,400	TANKS (2)	EMERGENCY OIL AND FIRE PROTECTION SUMP
LUBRICATING OIL	1 & 2	TURBINE BLDG	100	55 GAL DRUMS	EMERGENCY OIL AND FIRE PROTECTION SUMP
EHC FLUID	1 & 2	TURBINE BLDG	140	RESERVOIR (1)	EMERGENCY OIL AND FIRE PROTECTION SUMP
EHC FLUID	1 & 2	TURBINE BLDG	140	RESERVOIR (1)	TURBINE BLDG SUMP
LUBRICATING OIL	1 & 2	TURBINE BLDG		UNITS (2)	EMERGENCY OIL AND FIRE PROTECTION SUMP
INSULATING OIL	1	YARD	74,000	TANKS (2)	YARD HOLDING POND
DIESEL FUEL	2	YARD	142,000	TANKS (2)	YARD HOLDING POND
LUBRICATING OIL	2	YARD	58,000	TANKS (2)	YARD HOLDING POND
INSULATING OIL	2	YARD	200	CAPACITORS (10)	YARD HOLDING POND
INSULATING OIL	2	YARD	165,000	TRANSFORMERS	YARD HOLDING POND
BORIC ACID	1 & 2	AUX BLDG	34,800	TANKS (3)	AUX BLDG SUMP
SODIUM HYDROXIDE	1	CHEMICAL STORAGE BLDG	12,000	TANK	CONCRETE WALL AND FLOOR
SULFURIC ACID	1	CHEMICAL STORAGE BLDG	12,000	TANK	CONCRETE WALL AND FLOOR
BETZ SLIMICIDE	4	BIOCIDE INJECTION AREA	500 LBS	TANK	CONCRETE CURB AND FLOOR
BETZ 06	4	MIC CHEMICAL INJECTION AREA	4,000	TANK	CONCRETE CURB AND FLOOR
BETZ 07	4	MIC CHEMICAL INJECTION AREA	6,400	TANK	CONCRETE CURB AND FLOOR
BETZ 30K	4	MIC CHEMICAL INJECTION AREA	6,400	TANK	CONCRETE CURB AND FLOOR
BETZ CU-1	4	MIC CHEMICAL INJECTION AREA	55	TANK	CONCRETE CURB AND FLOOR
BETZ CT-1	4	MIC CHEMICAL INJECTION AREA	280	TANK	CONCRETE CURB AND FLOOR

APPENDIX C
Page 3 of 5
DESCRIPTION OF EXPOSED SIGNIFICANT MATERIAL

MATERIAL	AREA SHOWN ON MAP	LOCATION ON SITE	QUANTITY (GALS)	STORAGE VESSEL OR METHOD	DESCRIPTION OF BMPs
PAINTS AND THINNERS	4	PAINT HOUSE YARD 2	7,000	DRUMS/CANS	N/A
AMMONIUM HYDROXIDE (29%)	2	POWER STORE STORAGE YARD 1	2,750	DRUMS	NONE
AMMONIUM DEGREASER	2	POWER STORE STORAGE YARD 1	55	DRUMS	NONE
CORTEC CORROSION INHIBITOR	2	POWER STORE STORAGE YARD 1	220	DRUMS	NONE
ETHYLENE GLYCOL	2	POWER STORE STORAGE YARD 1	1,395	DRUMS/CANS	NONE
HYDRAZINE	2	POWER STORE STORAGE YARD 1	1,650	DRUMS	NONE
ISOPROPANOL	2	POWER STORE STORAGE YARD 1	165	DRUMS	NONE
NALCO 8256	2	POWER STORE STORAGE YARD 1	275	DRUMS	NONE
NOX RUST INHIBITOR	2	POWER STORE STORAGE YARD 1	220	DRUMS	NONE
RUSTOLENE 30	2	POWER STORE STORAGE YARD 1	220	DRUMS	NONE
SODIUM HYDROXIDE (10%)	2	POWER STORE STORAGE YARD 1	50	DRUMS	NONE
THINNER	2	POWER STORE STORAGE YARD 1	440	DRUMS	NONE
1,1,1 TRI-CHLORO-ETHANE	2	POWER STORE STORAGE YARD 1	165	DRUMS	NONE
VARSOL	2	POWER STORE STORAGE YARD 1	550	DRUM	NONE
SODIUM HYPO-CHLORITE	13	WAREHOUSE HUT 17 YARD 1	140	BOTTLES	N/A
AMMONIA	13	WAREHOUSE HUT 17 YARD 1	75	BOTTLES	N/A

APPENDIX C
Page 4 of 5
DESCRIPTION OF EXPOSED SIGNIFICANT MATERIAL

MATERIAL	AREA SHOWN ON MAP	LOCATION ON SITE	QUANTITY (GALS)	STORAGE VESSEL OR METHOD	DESCRIPTION OF BMPs
ALUMINUM SULFATE HYDRATED	13	WAREHOUSE HUT 19 YARD 1	16,000 LBS	BAGS	N/A
BORIC ACID	13	WAREHOUSE HUT 19 YARD 1	60,125 LBS	DRUMS	N/A
LITHIUM HYDROXIDE	13	WAREHOUSE HUT 19 YARD 1	865 LBS	DRUMS	N/A
SODIUM META-BISULFITE	13	WAREHOUSE HUT 19 YARD 1	500 LBS	BAGS	N/A
SODIUM CARBONATE	13	WAREHOUSE HUT 19 YARD 1	4,600 LBS	BAGS	N/A
SODIUM HYDROXIDE	13	WAREHOUSE HUT 19 YARD 1	100	CANS	NONE
FREON 11	13	WAREHOUSE HUT 19 YARD 1	3,750 LBS	30 LBS CYLINDERS	N/A
FREON 11	13	WAREHOUSE HUT 19 YARD 1	1,400 LBS	100 LBS CYLINDERS	N/A
REFRIGEANT R-12	13	WAREHOUSE HUT 19 YARD 1	1,800 LBS	30 LBS CYLINDERS	N/A
REFRIGERANT R-22	13	WAREHOUSE HUT 19 YARD 1	3,750 LBS	30 LBS CYLINDERS	N/A
REFRIGERANT 502	13	WAREHOUSE HUT 19 YARD 1	3,750 LBS	30 LBS CYLINDERS	N/A
REFRIGERANT 502	13	WAREHOUSE HUT 19 YARD 1	960 LBS	30 LBS CYLINDERS	N/A
FREON TF SOLVENT	13	WAREHOUSE HUT 31 YARD 1	4,400	DRUMS	N/A
PAINTS AND THINNERS	2	PAINT TRAILER	2,953	CANS	N/A
METHYL ALCOHOL	1	OIL ROOM ON LOADING DOCK	24	BOTTLES	NONE
ACETONE	1	OIL ROOM ON LOADING DOCK	67	BOTTLES	NONE

APPENDIX C
Page 5 of 5
DESCRIPTION OF EXPOSED SIGNIFICANT MATERIAL

MATERIAL	AREA SHOWN ON MAP	LOCATION ON SITE	QUANTITY (GALS)	STORAGE VESSEL OR METHOD	DESCRIPTION OF BMPs
ETHYL ALCOHOL	1	OIL ROOM ON LOADING DOCK	96	BOTTLES	NONE
PAINT	1	OIL ROOM ON LOADING DOCK	10	CANS	NONE
2-PROPANOL	1	OIL ROOM ON LOADING DOCK	134	BOTTLES	NONE
PAINT AND THINNER	1	PAINT SHOP TRAILER	1,219	CANS	N/A
SODIUM HYPO-CHLORITE	13	SEWAGE TREATMENT PLANT	120	DRUMS	PLASTIC BERM INSIDE COVERED BUILDING
AMMONIUM HYDROXIDE	1 & 2	TURBINE BLDG	500	TANK	TURBINE BLDG SUMP
HYDRAZINE	1 & 2	TURBINE BLDG	250	TANK	TURBINE BLDG SUMP
ALUMINUM SULFATE	1 & 2	TURBINE BLDG	600 LBS	BAGS	N/A
ALUMINUM SULFATE	1 & 2	TURBINE BLDG	360	TANK	TURBINE BLDG SUMP
LITHIUM HYDROXIDE	1 & 2	TURBINE BLDG	100 LBS	DRUM	N/A

APPENDIX D
Page 1 of 1

PARAMETER REPORTING LEVELS FOR STORM WATER DISCHARGES

EFFLUENT PARAMETER	*REPORT LEVELS (mg/l)*	*MINIMUM MEASURE-MENT FREQUENCY*	*SAMPLE TYPE*
BOD (5-DAY)	50	Annually	Composite
TSS	200	Annually	Composite
Ammonia as N	4	Annually	Composite
O&G	15	Annually	Grab
pH Range	4.0 to 9.0	Annually	Grab
Floating Matter, Color, Foam, and Oil Sheen	-	Annually	Visual Observation

Note

Pollutants levels exceeding a report level shall be reported to the Tennessee/Division of Water Pollution Control within 30 days after the discharger becomes aware of the results. The discharger shall provide the Division with an explanation of the pollutant's origin. Monitoring results shall be submitted on the storm water monitoring report form.

pH values outside the range of 4.0 to 9.0 standard units shall be considered to exceed the report level and shall be reported as such.

There shall be no distinctly visible floating scum, oil or other matter contained in the storm water discharge.

The storm water discharge must result in no materials in concentrations sufficient to be hazardous or otherwise detrimental to humans, livestock, wildlife, plant life, or fish and aquatic life in the receiving stream.

The storm water discharge must not cause an objectionable color contrast in the receiving stream.

SOURCE NOTES
Page 1 of 1

Source Note	Source Document	Summary
1	NPDES Permit TN0020168, Part 1.B.1.e., Part III.1	State requirements for an Erosion Control and Storm Water Pollution Prevention Plan
2	NPDES Permit TNR001343	WBN General Permit for Storm Water Discharges

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 4

Principle Investigator: Cynthia L. Russell

Starting Date: October 15, 1992

Ending Date: October 22, 1992

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. This report presents findings of the first monthly toxicity bioassessment (Experiment 4) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluated acute, chronic, and subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows, Pimephales promelas, and the daphnid Ceriodaphnia dubia.

Tests showed no toxicity in the WBN diffuser discharge under operating conditions using samples collected daily from October 14-20.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Tennessee River water, collected from the Aquatic Research Laboratory (ARL) facility at Browns Ferry Nuclear Plant (TRM 293) near Athens, Alabama.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning October 14, 1992 (Isco composite initiated October 13), and continuing through October 20, 1992. Diffuser and intake collections were made between the hours of 1000 and 1210 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were intermittent during the study (Appendix A). Dilution factors ranged from 542-986.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

2.7 Sample Transport: Samples collected and shipped on 10/14, 10/15, 10/16, 10/19, and 10/20 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 10/17 and 10/18 were transported the same day via private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to ARL, Browns Ferry Nuclear Plant site.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 12.5 percent concentrations (diluted with Tennessee River from ARL TRM 293) in the fathead minnow and daphnid tests. Intake samples were run only at 100 percent in both tests. In addition, culture medium was run as a laboratory reference for fathead minnows and daphnids (medium = moderately hard reconstituted water).

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: October 10-11, 1992

3.1.6 Hatch Date/Time: October 14-15, 1992

3.1.7 Acclimation Water: Tennessee River Water, TRM 293

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.06 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing 10 percent filtered aquarium water from the fathead minnow culture unit and 1.0 ppb selenium. [1]

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 and ECKENFELDER INC. Laboratory Practices with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [2][3]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [2]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: October 15, 1992/1030 CDT

4.1.3 Date/Time Test Terminated: October 22, 1992/1000 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: Tennessee River Water (TRM 293)

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: Mean = 25.0°C (24.5-25.5)

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [2]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [2]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: October 15, 1992/1130 CDT

4.2.3 Date/Time Test Terminated: October 21, 1992/1115 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Tennessee River water (TRM 293)

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: Mean = 25.0°C (24.3-25.7)

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50×10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity

endpoints. Fishers Exact Test was used to evaluate *Ceriodaphnia* survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [2]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. Any known deviations were noted during the study and are reported herein. [2]

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Log Book and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and calibration information pertaining to ARL laboratory instruments is contained in a bound Laboratory Instrument Log Book and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [4]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [4]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [4]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [4]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [4]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [4]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)

Daphnids--7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water (enriched for daphnids) - fish and daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and *Ceriodaphnia* Reproduction, NOEC - Dunnett's Test or Steel's Many-one Rank Test

Ceriodaphnia Survival - Fisher's Exact Test

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results: No survival or growth effects were demonstrated for diffuser discharge or intake samples collected from October 14-20, 1992. Although one replicate in the Intake treatment had only 10 percent survival, the overall reduction was not statistically significant. This mortality does not appear to have been treatment related since survival was 100 percent in the other three replicates. The cause of the mortality is not known.

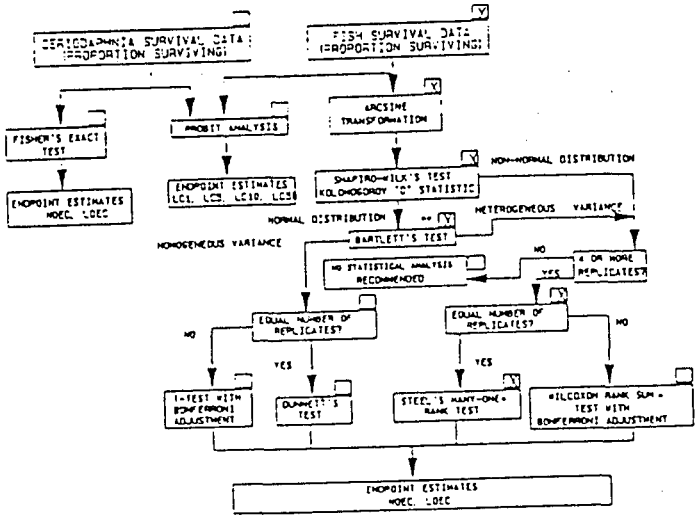
6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 4, October 15-22, 1992.

	Total Daily % Survival						
Treatment	Day 1	2	3	4	5	6	7
TR	100	100	100	100	100	100	100
Discharge-12.5%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	90	80	78	78
Medium	100	100	100	100	100	100	100

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 4, October 15-22, 1992.

SURVIVAL EFFECTS



* Test requires 4 replicates/treatment
** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 4, October 15-22, 1992.

Survival (%) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S *
TR	100	100	100	100							100	
Discharge-12.5%	100	100	100	100							100	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	100	100	100							100	
Discharge-100%	100	100	100	100							100	
Intake	10	100	100	100							78	
Medium	100	100	100	100							100	

*Indicates values significantly less than control (TR).

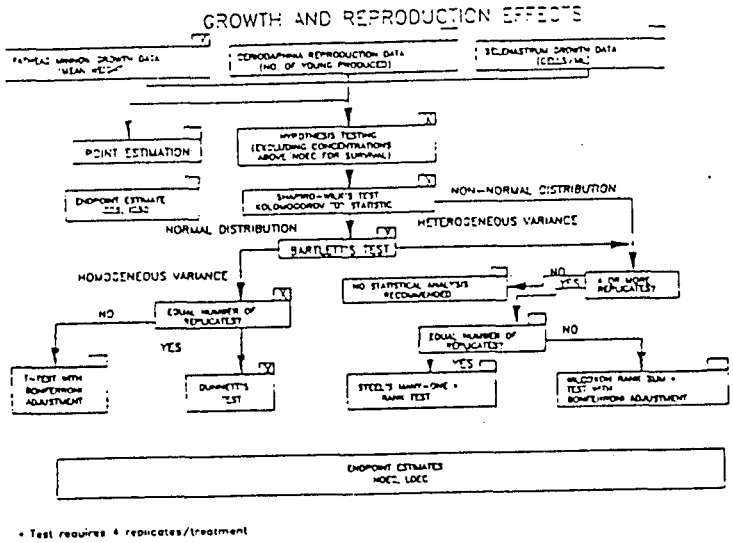
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-12.5%	4	10	18
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	18
Discharge-100%	4	10	18
Intake	4	10	16
Medium	4	10	18

*Values less than Critical Rank Sum are significantly less than the control (TR).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 4, October 15-22, 1992.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 4, Watts Bar Nuclear Plant, October 15-22, 1992.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.06.

This represents a 12.39% reduction in Dry Weight (mg).
T = 2.45 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
TR	0.41	0.45	0.45	0.47							0.45	
Discharge-12.5%	0.44	0.49	0.46	0.40							0.45	
Discharge-25.0%	0.44	0.45	0.44	0.46							0.45	
Discharge-50.0%	0.47	0.46	0.48	0.44							0.46	
Discharge-100%	0.47	0.46	0.44	0.48							0.46	
Intake	0.33	0.41	0.43	0.48							0.41	
Medium	0.45	0.48	0.49	0.50							0.48	

*Indicates values significantly less than control (TR). EMS = 0.001.

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from October 14-21, 1992.

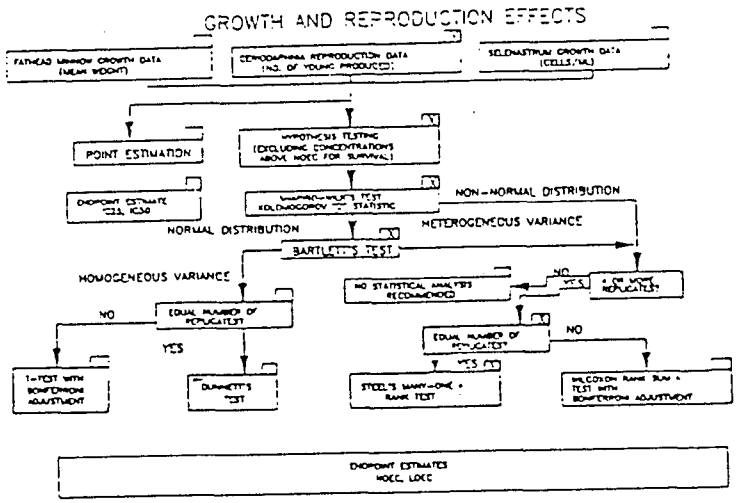
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 4, October 15-21, 1992.

Treatment	Total Daily % Survival					
	1	2	3	4	5	6
TR	100	100	100	100	100	100
Discharge-12.5%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	90
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	90	90
Intake	100	100	100	100	100	100
Medium	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 4, October 15-21, 1992.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 4, Watts Bar Nuclear Plant, October 15-21, 1992.

Reproduction (# young/female/6 days) Data

Treatment	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
TR	17	21	24	20	17	11	26	22	22	22	20.20	
Discharge-12.5%	27	22	24	17	24	22	21	25	22	25	22.90	
Discharge-25.0%	2	23	26	17	22	21	21	22	26	25	20.50	
Discharge-50.0%	30	23	27	17	25	23	24	22	26	26	24.30	
Discharge-100%	24	23	11	20	25	13	21	23	24	21	20.50	
Intake	18	23	25	10	13	19	22	11	24	22	18.70	
Medium	22	22	19	9	10	11	24	19	15	23	17.40	

*Indicates values significantly less than control (TR).

Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-12.5%	10	74	126.0
Discharge-25.0%	10	74	115.0
Discharge-50.0%	10	74	136.0
Discharge-100%	10	74	111.0
Intake	10	74	101.0
Medium	10	74	92.0

*Values less than Critical Rank Sum are significantly less than the control (TR).

6.3 Results: Watts Bar Nuclear Plant diffuser (DSN101) concentrations of total zinc and total phosphorus, October 13-20, 1992.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Concentrations of Total Zinc and Total Phosphorus, October 13-20, 1992.

6.4 Results: Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 4, October 15-22, 1992.

See: Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 4, October 15-22, 1992

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 4 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test: September 29-October 6, 1992/0945 CDT

6.5.2.1.2 LOEC--0.008 mg Cu/L
NOEC--0.003 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 36

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)

6.5.3 Ceriodaphnia

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of most recent acceptable test:
September 30-October 6, 1992/0800 CDT

6.5.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.02 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 34

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)

NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)

7.0 CONCLUSION

Tests conducted on WBN diffuser discharge and intake samples collected from October 14-20, 1992, did not show toxicity (survival, or growth or reproduction effects) to fathead minnows or Ceriodaphnia.

8.0 REFERENCES

1. Weber, C. I. (ed.), Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA/600/4-90/027 (September 1991).
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3. ECKENFELDER INC., Good Laboratory Practices for Aquatic Toxicology, June 1990, Nashville, TN.
4. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLABDSSW-2202

Appendix A

Calculated Allowable* Toxicity Based Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, October 13-20, 1992.

Date	Zero Release (Hours)	WB Hydro Hourly Average Flow (cfs)	WBN DSN101 Daily Flow† (cfs)	Dilution (WBN:WB Hydro)	Allowable Toxicity TUc‡	Allowable Toxicity NOEC (% Effluent)
October 13	6	32189	37.7	1:853	≤ 855	≥ 0.12
October 14	6	33172	37.7	1:879	≤ 881	≥ 0.11
October 15	7	34029	37.7	1:902	≤ 904	≥ 0.11
October 16	6	31556	37.7	1:836	≤ 838	≥ 0.12
October 17	4	20935	37.7	1:555	≤ 556	≥ 0.18
October 18	5	20463	37.7	1:542	≤ 544	≥ 0.18
October 19	4	37210	37.7	1:986	≤ 988	≥ 0.10
October 20	0	29438	37.7	1:780	≤ 782	≥ 0.13

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream concentration of an effluent to which organisms can be exposed indefinitely without causing unacceptable effect.

†Estimated daily flows. Actual flows become considerably less as hours of zero release increase.

‡TUc = 100/NOEC.

PLABDSSW-2203(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, October 13-20, 1992

1992 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
October 14	Grab*	0.030	<0.1
October 15	Grab	<0.010	<0.1
October 16	Composite*	0.060	<0.1
October 16	Grab	0.090	--
October 18	Grab	0.010	--
October 19	Grab	0.020	<0.1
October 20	Composite	0.020	<0.1
October 20	Grab	0.110	<0.1

*Grab samples were at various times of the day and are not representative of exposure concentrations that fathead minnows and daphnids would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLABDSSW-2203(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 4, October 15-22, 1992

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
TR	25.0 (24.6-25.3)	25.0 (24.3-25.7)	8.2 (8.0-8.4)	5.5 (4.7-6.5)	7.7 (7.6-7.9)	7.9 (7.8-7.9)	7.6 (7.5-7.8)	8.1 (8.0-8.2)	178 (176-180)	183 (180-186)	61 (60-61)	69.6 (68.4-70.1)	-
Intake 100%	24.9 (24.5-25.3)	25.0 (24.3-25.7)	8.3 (8.3-8.3)	5.6 (4.9-6.5)	7.7 (7.5-7.9)	7.9 (7.5-8.2)	7.6 (7.5-7.9)	8.1 (8.0-8.2)	177 (173-182)	180 (177-184)	57 (57-58)	68.2 (66.7-68.4)	<0.10 (<0.10-<0.1
Discharge 12.5%	25.0 (24.7-25.4)	25.0 (24.3-25.7)	8.3 (8.1-8.4)	5.5 (4.3-6.4)	7.7 (7.6-7.9)	7.9 (7.8-8.0)	7.7 (7.5-7.8)	8.1 (8.0-8.2)	178 (176-180)	183 (179-188)	61 (60-61)	70.6 (70.1-71.8)	-
Discharge 25.0%	25.0 (24.7-25.5)	25.0 (24.3-25.7)	8.3 (8.3-8.4)	5.5 (4.8-6.5)	7.7 (7.6-7.9)	7.9 (7.8-8.0)	7.7 (7.5-7.8)	8.1 (8.0-8.2)	180 (178-181)	184 (181-188)	61 (60-61)	70.1 (70.1-70.1)	-
Discharge 50.0%	25.0 (24.6-25.4)	25.0 (24.3-25.7)	8.3 (8.3-8.3)	5.5 (4.6-6.6)	7.7 (7.5-7.8)	7.9 (7.6-8.1)	7.6 (7.5-7.7)	8.1 (8.0-8.2)	181 (180-182)	186 (185-188)	61 (61-62)	70.4 (70.1-71.8)	-
Discharge 100%	25.0 (24.7-25.4)	25.0 (24.3-25.7)	8.3 (8.3-8.3)	5.5 (4.6-6.5)	7.7 (7.5-7.8)	7.9 (7.5-8.1)	7.7 (7.6-7.8)	8.1 (8.0-8.2)	184 (182-187)	189 (187-195)	61 (60-64)	70.8 (70.1-73.5)	<0.10 (<0.10-<0.1
Medium	24.9 (24.6-25.3)	-	8.3 (8.3-8.4)	5.7 (5.0-6.7)	-	8.2 (8.2-8.3)	7.7 (7.5-7.9)	-	344 (338-348)	349 (347-354)	67 (66-68)	95.2 (94.1-97.5)	-
Enriched Medium	-	25.0 (24.3-25.7)	8.3 (8.3-8.4)	-	7.8 (7.6-7.9)	8.3 (8.2-8.3)	-	8.1 (7.9-8.2)	366 (357-382)	-	64 (62-66)	98.6 (97.5-100.9)	-

*mg/L as $CaCO_3$

PLABOSSW-2204

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 5

Principle Investigator: Cynthia L. Russell

Starting Date: November 18, 1992

Ending Date: November 25, 1992

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. This report presents findings of the second monthly toxicity bioassessment (Experiment 5) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute, chronic, and subchronic responses of three trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas), daphnids (Ceriodaphnia dubia), and a unicellular alga (Selenastrum capricornutum).

Tests conducted using diffuser discharge samples collected from November 17-23 showed no toxicity to fathead minnows or daphnids. Significant reduction in algal growth was demonstrated in 100, 50, and 25 percent DSN101 samples, however, the two percent sample showed no statistically significant reduction. Allowable toxicity, based on effluent dilution factors ranging from 404-951 during the study, was a NOEC $\geq$0.17 percent effluent (mean value, Appendix A). The algal NOEC of 1.54 percent effluent (point estimate linear interpolation) did not exceed the EPA recommended chronic toxicity Criterion of 1.0 TUc. Toxicity may have been related to zinc measured in the algal test composite sample near levels known to be toxic to Selenastrum growth. Exposure to intake samples resulted in significant reduction in daphnid reproduction but the toxicity did not persist to the discharge.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Tennessee River water, collected from the Aquatic Research Laboratory (ARL) facility at Browns Ferry Nuclear Plant (TRM 293) near Athens, Alabama.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning November 17, 1992 (Isco composite initiated November 16), and continuing through November 23, 1992. Diffuser and intake collections were made between the hours of 0930 and 1430 EST.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were continuous during the study (Appendix A). Dilution factors ranged from 404-951.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

2.7 Sample Transport: Samples collected and shipped on 11/17, 11/18, 11/19, 11/20, and 11/23 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 11/21 and 11/22 were transported the same day via private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to ARL, Browns Ferry Nuclear Plant site.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with Tennessee River from ARL, TRM 293) in the fathead minnow, daphnid and algal tests. Intake samples were run at 100 percent only in all tests. In addition, culture medium was run as a laboratory reference for fathead minnows, daphnids, and algae (medium = moderately hard reconstituted water for fathead minnows and daphnids, and algal medium for algae).

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: November 13, 1992

3.1.6 Hatch Date/Time: November 17, 1992/1100 CST - November 18, 1992/0610 CST

3.1.7 Acclimation Water: Tennessee River Water, TRM 293

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.04 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing 10 percent filtered aquarium water from the fathead minnow culture unit and 1.0 ppb selenium. [1]

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for_trout chow and alfalfa substituted for Cerophyll. [2]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only

every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

3.3 Species: Selenastrum capricornutum, alga

3.3.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory, obtained from EPA, Newtown, Ohio, August 25, 1992.

3.3.2 Culture water: Culture medium consists of reconstituted water following EPA methods for the Algal Growth Test. [2] Culture medium pH is adjusted to 7.5 (7.4-7.6, acceptable range) when necessary using 0.1 N NaOH or 0.1 N HCl.

3.3.3 Temperature of Culture: 25 ± 1°C

3.3.4 General Maintenance: Cultures are maintained in 250-500 mL flasks containing 100-250 mL algal medium in light and temperature controlled incubators. New cultures are started each week by inoculating flasks with a single colony from incubated agar plates containing macro- and micronutrients or by transferring 1 mL of inoculant from a culture flask into fresh autoclaved and filtered medium using aseptic techniques (see 3.3.2). Agar plates are prepared as needed. One half of the plates are stored at 4°C for contingency use, the remainder are incubated at 24 ± 2°C. Medium used in algae sterile culture is vacuum-filtered through 0.45 µm glass fiber filters.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [2]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

Temperatures measured in one test beaker on Day 2 and two test beakers on Day 3 were 26.1°C which is outside the prescribed range of 25±1°C. This deviation is not judged to have affected test results since the beakers were randomly distributed among treatments and all treatments had similar means and ranges.

4.1.2 Date/Time Test Initiated: November 18, 1992/0920 CST

4.1.3 Date/Time Test Terminated: November 25, 1992/0850 CST

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: Tennessee River Water (TRM 293)

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25(±)1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Dunnett's Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [2]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [2]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: November 18, 1992/0930 CST

4.2.3 Date/Time Test Terminated: November 24, 1992/0945 CST

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Tennessee River water (TRM 293)

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25(±)1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50×10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [2] Note: Culture medium was omitted from statistical analyses due to failure to meet acceptability criteria for controls.

4.3 Algal, Selenastrum capricornutum, Growth Test, EPA Test Method 1003.0. [4]

4.3.1 Modification/Deviations to Method 1003.0: Mean initial cell density of three samples checked was 15,054 cells/mL, determined by Sedgwick-Rafter cell counts. The EPA methods call for an initial cell density of 10,000 cells/mL.

4.3.2 Date/Time Test Initiated: November 20, 1992/0845 CST

4.3.3 Date/Time Test Terminated: November 24, 1992/0750 CST

4.3.4 Test Chamber: 250 mL borosilicate Erlenmeyer flasks
Volume per Chamber: 100 mL

4.3.5 Cell Enumeration Method: Coulter Counter Model ZM with Sedgwick-Rafter Cell counts used for QA verification.

4.3.6 Initial Cell Density: 15,054 cells/mL (Cell density based on Sedgwick-Rafter counts).

4.3.7 Number of Replicates per Treatment: 4 (In addition an uninoculated sample was prepared for each treatment to serve as a blank for final cell counts.)

4.3.8 Dilution Water: Tennessee River water (TRM 293.0; See section 2.2.)

4.3.9 Test Control: Tennessee River water (TRM 293.0; See section 2.2.)

4.3.10 Test Temperature: 25(±1)°C

4.3.11 Photoperiod: Continuous illumination

4.3.12 Renewal Period: None. This test method is a 96-h static bioassay. Flasks were randomly placed into the incubator at test initiation, removed and hand swirled daily, and randomly replaced into the incubator (New Brunswick Scientific Model G25). The incubator provided constant shaking at a rate of 100 cpm.

4.3.13 Physical and Chemical Parameters Measured: Parameters measured for all test samples and algal medium at test initiation were temperature, DO, pH, conductivity, alkalinity, hardness, and total residual chlorine. Results are reported in section 6.4. Temperatures were measured three times daily in extra flasks placed randomly in the incubator. Since some variation was seen at different locations in the incubator, test flasks were randomly rearranged in the incubator daily when flask contents were swirled.

4.3.14 Statistics: Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [2] Decision processes followed for testing algal growth response are shown in section 6.3.2. Based on tests for normally distributed data and homogenous variances, the statistical test used for growth endpoint determination was the Dunnett's Test.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted

according to the protocol as described in this report and EPA/600/4-89/001. Any known deviations were noted during the study and are reported herein. [2]

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Log Book and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and calibration information pertaining to ARL laboratory instruments is contained in a bound Laboratory Instrument Log Book and specific instruments used were documented on an instrument record sheet, along with daily calibration record sheets, contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [3]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [3]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [3]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [3]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [3]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [3]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)
Daphnids--7-day chronic (NOEC)
Algal--4-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic, enriched medium (see Section 3.2.2) for daphnid chronic, and algal medium for algal chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth, Ceriodaphnia Reproduction and Algal Growth, NOEC - Dunnett's Test or Steel's Many-One Rank Test

Ceriodaphnia Survival - Fisher's Exact Test

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

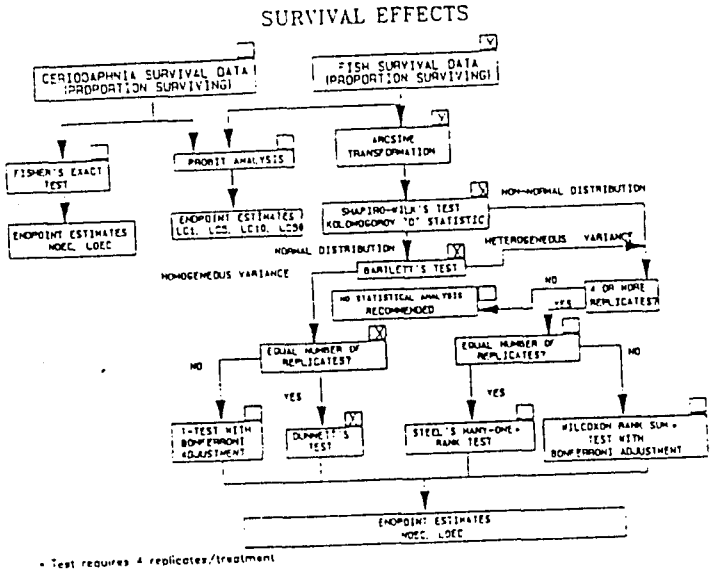
6.1.1 Summary of Results: No survival or growth effects were demonstrated for diffuser discharge or intake samples collected from November 17-23, 1992. Higher than expected mortality occurred in TR control and low concentrations of discharge which were diluted with TR. High variability among replicates, however, makes it difficult to attribute the mortality to toxicity in TR samples with certainty. The TR control met test acceptability criteria and no statistically significant reductions in survival occurred. Highest survival in the test (95 percent) occurred in the undiluted DSN101 and fish medium samples.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 5, November 18-25, 1992.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
TR	100	100	100	93	83	83	83
Discharge-2.0%	100	100	98	83	78	78	78
Discharge-25.0%	100	100	100	88	85	83	83
Discharge-50.0%	100	100	100	98	80	80	80
Discharge-100%	100	100	100	95	95	95	95
Intake	100	100	95	93	93	93	93
Medium	100	98	98	98	98	95	95

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 5, November 18-25, 1992.



6.1.2.3 Analysis of Survival Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 5, November 18-25, 1992.

Arcsine transformation was applied before data analysis. For this set of data, the minimum significant difference is 0.39.

This represents a 45.74% reduction in survival.
T = 2.45 Alpha 0/05 (one-tailed test)

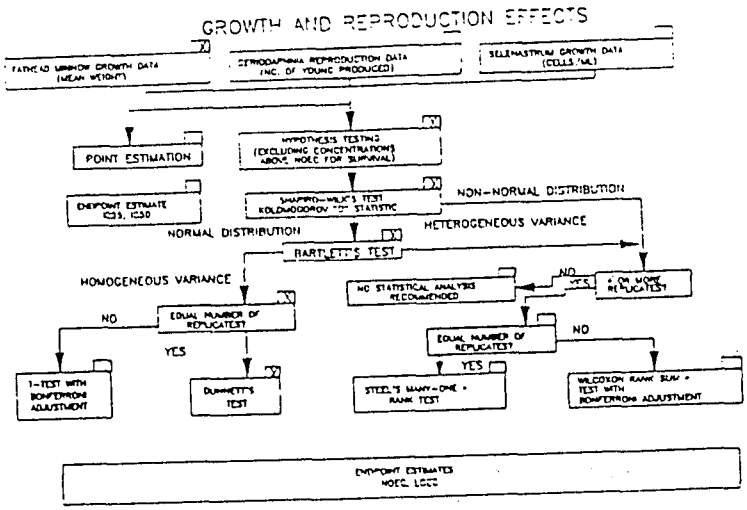
Survival (%) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S *
TR	70	100	100	60							83	
Discharge-2.0%	100	70	90	50							78	
Discharge-25.0%	40	90	100	100							83	
Discharge-50.0%	90	90	40	100							80	
Discharge-100%	100	100	100	80							95	
Intake	90	80	100	100							93	
Medium	90	100	100	90							95	

*Indicates values significantly less than control (TR); EMS = 0.061

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 5, November 18-25, 1992.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 5, Watts Bar Nuclear Plant, November 18-25, 1992.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.10.

This represents a 22.09% reduction in Dry Weight (mg).
T = 2.45 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

	Replicate										
Group	1	2	3	4	5	6	7	8	9	10	Mean S*
TR	0.44	0.41	0.49	0.56							0.48
Discharge-2.0%	0.44	0.53	0.51	0.63							0.53
Discharge-25.0%	0.50	0.45	0.46	0.43							0.46
Discharge-50.0%	0.48	0.48	0.50	0.37							0.46
Discharge-100%	0.48	0.39	0.40	0.56							0.46
Intake	0.46	0.48	0.38	0.44							0.44
Medium	0.51	0.42	0.51	0.54							0.50

*Indicates values significantly less than control (TR). EMS = 0.004.
IC_{25} = >100%

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No survival or reproduction effects were demonstrated for diffuser discharge samples collected from November 17-22, 1992. Exposure of daphnids to intake samples resulted in significantly reduced reproduction. This toxicity, however, did not persist to the discharge.

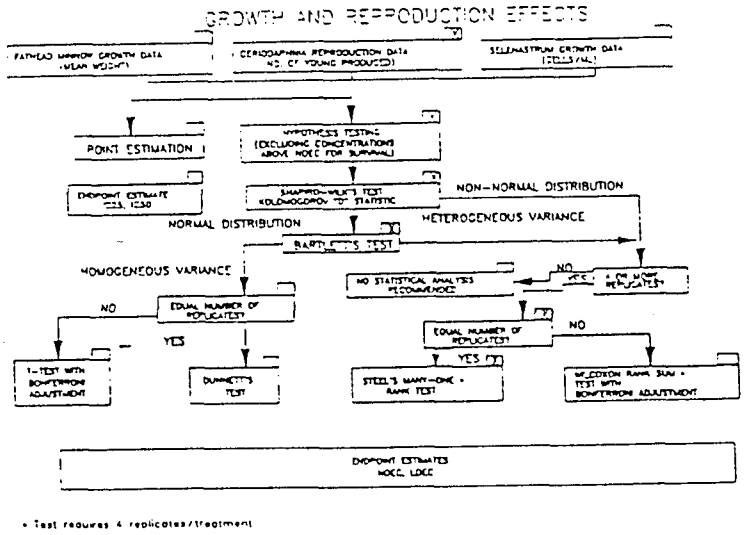
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 5, November 18-24, 1992.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
TR	100	100	100	100	100	100
Discharge 2.0%	100	100	100	100	100	90
Discharge- 25.0%	100	100	100	100	100	100
Discharge- 50.0%	100	90	90	90	90	90
Discharge- 100%	100	100	100	100	100	100
Intake	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 5, November 18-24, 1992.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 5, Watts Bar Nuclear Plant, November 18-24, 1992.

Reproduction (# young/female/6 days) Data

Treatment	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
TR	25	30	8	33	28	25	33	28	25	33	26.80	
Discharge-2.0%	23	33	29	18	15	19	29	32	28	23	24.90	
Discharge-25.0%	25	27	29	31	23	27	25	13	27	28	25.50	
Discharge-50.0%	29	29	29	32	11	27	19	0	24	3	20.30	
Discharge-100%	30	30	16	30	28	26	14	27	19	30	25.00	
Intake	15	14	23	20	7	19	24	30	23	22	19.70	*

*Indicates values significantly less than control (TR). IC_{25} >100%.

Steel's Many-one Rank Test

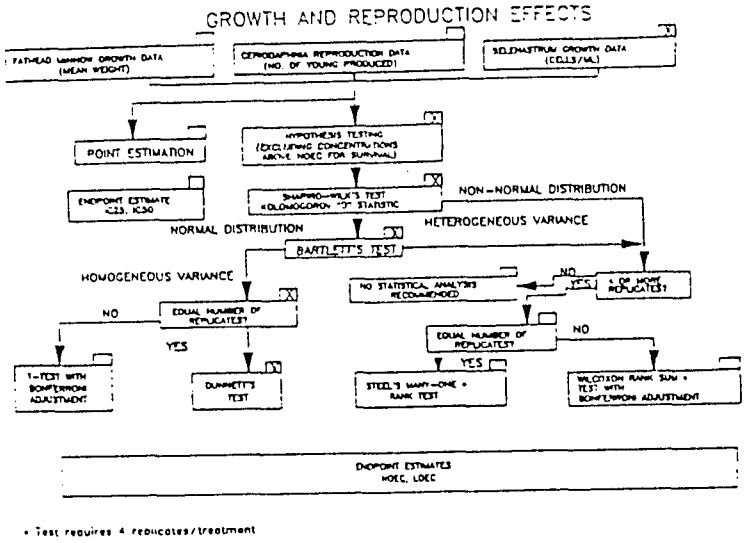
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-20.%	10	75	92.5
Discharge-25.0%	10	75	92.0
Discharge-50.0%	10	75	87.0
Discharge-100%	10	75	97.0
Intake	10	75	69.5 *

*Values less than Critical Rank Sum are significantly less than the control (TR).

6.3 Algal, Selenastrum capricornutum, Growth Test

6.3.1 Summary of Results: Diffuser discharge DSN101, collected November 19, 1992, demonstrated significant growth effects at 100, 50, and 25 percent concentrations. No reduction in growth occurred in the 2 percent concentration. The IC_{25} for DSN101 was 1.5364 percent. No growth effects were demonstrated in intake samples or medium.

6.3.2 Statistical Decision Process for Determining Toxicity Endpoints for 4-Day Exposure of the Alga, Selenastrum capricornutum, to Watts Bar Nuclear Plant DSN101 Discharge, Test 5, November 20-24, 1992.



* Test requires 4 replicates/treatment

6.3.3 Analysis of Growth Data Using Dunnett's Test for Algal 4-Day Growth Test, Watts Bar Nuclear Plant, Test 5, November 20-24, 1992.

Log transformation was applied before data analysis. For this set of data, the minimum significant difference is 441 x 10^3.

This represents a 33.46% reduction in No. cells/mL x 10^3
T = 2.45 Alpha = 0.05 (one-tailed test)

Algal Growth Data (No. cell/mL x 10^3)

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S
TR	1146	1273	1588	1306							1328	
Discharge-2.0%	724	798	962	1100							896	
Discharge-25.0%	518	444	492	667							530	*
Discharge-50.0%	248	266	258	276							262	*
Discharge-100%	102	244	122	132							150	*
Intake	1905	1511	1294	1134							1461	
Medium	1384	1887	1152	856							1319	

*Indicates values significantly less than the control (TR). EMS = 0.010
IC_{25} = 1.5364%

6.4 Physical/Chemical Parameters

6.4.1 Overall Test Temperature

6.4.1.1 Fathead Minnow: Mean == 25.4°C (24.8-26.1)

6.4.1.2 Ceriodaphnia: Mean = 25.1°C (24.5-25.6)

6.4.1.3 Algae: Mean = 25.1°C (24.7-25.5)

6.4.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 5, November 18-25, 1992.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, November 16-23, 1992.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 5, November 18-25, 1992

Appendix D Initial Water Chemistry Values for Algal Growth Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 5, November 20, 1992

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 5 showed chronic results consistent with ARL control chart ranges for fathead minnows, daphnids, and algae.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test: October 27-November 3, 1992/0935 CST

6.5.2.1.2 LOEC--0.008 mg Cu/L
NOEC--0.003 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 37

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)

6.5.3 Ceriodaphnia

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of most recent acceptable test: October 27-November 2, 1992/0830 CST

6.5.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.02 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 35

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)

NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)

6.5.4 *Selenastrum capricornutum*

6.5.4.1 Chronic Tests

6.5.4.1.1 Date/Time of most recent test: November 9-13, 1992/0845 CST

6.5.4.1.2 LOEC: <0.0004 mg Cu/L
NOEC: 0.0004 mg Cu/L

6.5.4.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 12

LOEC Range: <0.0004-0.003 mg Cu/L (four concentration increments)
NOEC Range: <0.0004-0.0015 mg Cu/L (three concentration increments)

7.0 CONCLUSION

Tests conducted using diffuser discharge samples collected from November 17-23 showed no toxicity to fathead minnows or daphnids. Significant reduction in algal growth was demonstrated in 100, 50, and 25 percent DSN101 samples, however, the two percent sample showed no statistically significant reduction. Allowable toxicity, based on effluent dilution factors ranging from 404-951 during the study, was a NOEC ≥0.17 percent effluent (mean value, Appendix A). The algal NOEC of 1.54 percent effluent (point estimate linear interpolation) did not exceed the EPA recommended chronic toxicity Criterion of 1.0 TUc. The composite sample collected November 18-19 and tested for algal toxicity contained 0.180 mg total Zn/L (Appendix B) and 80.4 mg/L hardness as $CaCO_3$ (Appendix D). This value exceeds the 4-day freshwater aquatic life criterion for zinc

of 0.088 mg/l (adjusted for hardness) and also exceeds the 0.030 mg Zn/L shown to inhibit Selenastrum growth (hardness not specified).[4] Exposure to intake samples resulted in significant reduction in daphnid reproduction but the toxicity did not persist to the discharge.

8.0 REFERENCES

1. Weber, C. I. (ed.), Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, EPA/600/4-90/027 (September 1991).
2. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkedick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1991).
3. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.
4. U.S. EPA. Quality Criteria for Water, 1986. EPA/440/5-86-001, May 1, 1986.

PLABDSSW-2299

Appendix A

Calculated Allowable* Toxicity Based Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, November 16-23, 1992.

Date	Zero Release (Hours)	WB Hydro Hourly Average Flow (cfs)	WBN DSN101 Daily Flow† (cfs)	Dilution (WBN:WB Hydro)	Allowable Toxicity TUc‡	Allowable Toxicity NOEC (% Effluent)
November 16	0	34292	37.7	1:909	≤911	≥0.11
November 17	0	34446	37.7	1:913	≤915	≥0.11
November 18	0	35888	37.7	1:951	≤953	≥0.10
November 19	0	36200	58.0	1:624	≤825	≥0.16
November 20	0	35575	64.0	1:556	≤557	≥0.18
November 21	0	29867	73.9	1:404	≤405	≥0.25
November 22	0	28042	64.0	1:438	≤439	≥0.23
November 23	0	34779	64.0	1:543	≤544	≥0.18
Average				1:667	≤694	≥0.17

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream concentration of an effluent to which organisms can be exposed indefinitely without causing unacceptable effect.

†Estimated daily flows.

‡TUc = 100/NOEC.

PLABDSSW-2300(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, November 16-23, 1992

1992 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
November 16	Grab*	0.072	<0.1
November 17	Grab	0.100	--
November 16-17	Composite*	1.100	<0.1
November 18	Grab	0.260	--
November 18-19	Composite	0.180	<0.1
November 23	Grab/Duplicate	0.052	<0.1
November 23	Grab/Duplicate	0.040	--

*Grab samples were at various times of the day and are not representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLABDSSW-2300(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 5, November 18-25, 1992

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
TR	25.4 (24.8-26.1)	25.1 (24.5-25.6)	8.3 (8.3-8.3)	5.6 (4.8-6.6)	7.7 (7.6-7.7)	7.9 (7.8-8.0)	7.5 (7.4-7.6)	8.0 (7.9-8.1)	158 (155-160)	162 (157-167)	55 (53-57)	61.8 (59.9-65.0)	-
Intake 100%	25.4 (25.0-26.0)	25.1 (24.5-25.6)	8.3 (8.3-8.4)	5.7 (5.0-6.5)	7.6 (7.6-7.7)	7.8 (7.4-8.0)	7.6 (7.4-7.6)	8.0 (7.9-8.1)	171 (168-184)	174 (169-186)	59 (56-65)	66.4 (61.6-77.0)	<0.10 (<0.10-<0.10)
Discharge 2.0%	25.5 (25.0-26.1)	25.1 (24.5-25.6)	8.3 (8.2-8.4)	5.4 (4.7-6.4)	7.6 (7.6-7.7)	7.9 (7.8-8.0)	7.5 (7.5-7.6)	8.0 (7.9-8.1)	158 (156-160)	162 (159-164)	55 (53-57)	60.6 (59.9-63.3)	-
Discharge 25.0%	25.4 (24.9-26.0)	25.1 (24.5-25.6)	8.3 (8.2-8.4)	5.4 (4.3-6.2)	7.6 (7.6-7.7)	7.9 (7.7-8.0)	7.6 (7.5-7.7)	8.0 (7.9-8.1)	162 (159-164)	165 (163-169)	55 (55-56)	61.8 (58.1-65.0)	-
Discharge 50.0%	25.5 (24.9-26.1)	25.1 (24.5-25.6)	8.3 (8.3-8.3)	5.5 (4.3-6.2)	7.6 (7.6-7.7)	7.9 (7.5-8.0)	7.6 (7.5-7.7)	8.0 (7.9-8.1)	167 (164-170)	170 (168-173)	58 (57-58)	64.7 (63.3-66.7)	-
Discharge 100%	25.5 (24.9-26.0)	25.1 (24.5-25.6)	8.4 (8.3-8.4)	5.7 (4.7-6.8)	7.6 (7.6-7.7)	7.8 (7.4-8.0)	7.6 (7.5-7.7)	8.0 (7.9-8.1)	174 (169-179)	178 (174-186)	60 (60-61)	67.9 (65.0-70.1)	<0.10 (<0.10-<0.10)
Medium	25.4 (24.8-26.0)	-	8.3 (8.2-8.4)	6.1 (5.1-6.6)	-	8.2 (8.1-8.2)	7.6 (7.5-7.7)	-	342 (336-350)	345 (338-353)	68 (66-70)	91.8 (88.9-94.1)	-
Enriched Medium	-	25.1 (24.5-25.6)	8.3 (8.2-8.4)	-	7.6 (7.5-7.7)	8.2 (8.1-8.2)	-	8.0 (7.9-8.0)	359 (348-372)	-	65 (63-67)	96.2 (94.1-97.5)	-

*mg/L as $CaCO_3$

PLABDSSW-2301

Appendix D

Initial Water Chemistry Values for Algal Growth Test,
Watts Bar Nuclear Plant Biomonitoring, Test 5, November 20-24, 1992

Treatment	Temp. (°C)	DO (mg/L)	pH (S.U.)	Cond. (umhos)	Hard. *	Alk. *	Total Residual Chlorine (mg/L)
TR	25.0	8.6	8.0	242	73.5	64	-
Discharge-2.0%	25.1	8.6	8.0	243	71.8	64	-
Discharge-25.0%	25.1	8.8	8.0	247	77.0	65	-
Discharge-50.0%	25.1	9.1	7.9	250	80.4	66	-
Discharge-100%	25.1	9.7	7.9	258	80.4	69	<0.1
Intake	25.2	9.8	7.7	250	77.0	64	<0.1
Medium	25.0	7.0	7.6	90	13.7	9	-

*mg/L as $CaCO_3$

PLABDSSW-2300(2)

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning December 15, 1992 (Isco composite initiated December 14), and continuing through December 21, 1992. Diffuser and intake collections were made between the hours of 0715 and 1115 EST.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were continuous during the study (Appendix A). Dilution factors ranged from 575-1015.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

2.7 Sample Transport: Samples collected and shipped on 12/15, 12/16, 12/17, 12/18, and 12/21 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 12/19 and 12/20 were transported the same day via private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to ARL, Browns Ferry Nuclear Plant site.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test and enriched medium for the daphnid test). Intake samples were run at 100 percent in both tests. In addition, Tennessee River water, collected from the Aquatic Research Laboratory (ARL) facility at Browns Ferry Nuclear Plant (TRM 293) near Athens, Alabama, was run at 100 percent only for the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: December 11, 1992

3.1.6 Hatch Date/Time: December 15, 1992/1000 CST - December 16, 1992/0615 CST

3.1.7 Acclimation Water: moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.05 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: December 16, 1992/0850 CST

4.1.3 Date/Time Test Terminated: December 23, 1992/0830 CST

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25 ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old 3 times daily *ad libitum*.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: December 16, 1992/1000 CST

4.2.3 Date/Time Test Terminated: December 22, 1992/1020 CST

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: enriched culture medium (see Section 2.2)

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25 ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. Any known deviations were noted during the study and are reported herein. [1]

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)
Daphnids--7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched medium (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and Ceriodaphnia Reproduction - Dunnett's Test or Steel's Many-One Rank Test

Ceriodaphnia Survival - Fisher's Exact Test

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

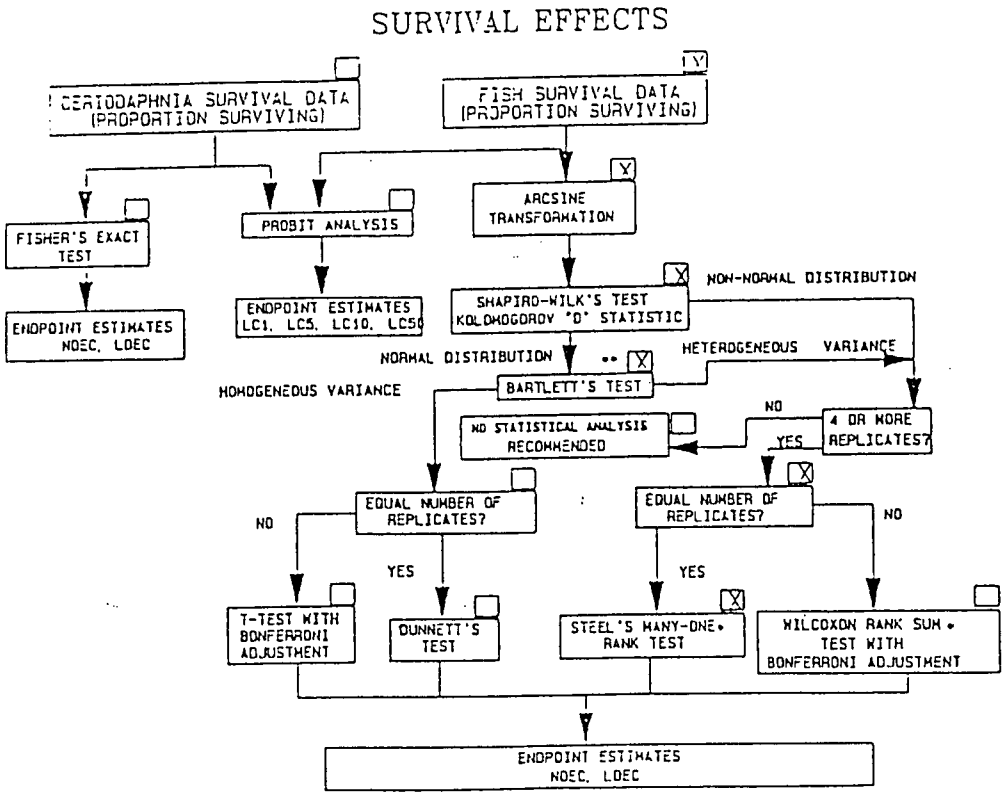
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from December 15-21, 1992.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 6, December 16-23, 1992.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	95	93	93	93

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 6, December 16-23, 1992.



* Test requires 4 replicates/treatment

** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 6, December 16-23, 1992.

Survival (%) Data

	Replicate											
Group	1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	100	100	100							100	
Discharge-100%	100	100	100	100							100	
Intake	70	100	100	100							93	

*Indicates values significantly less than control (Medium) IC_{25} >100%.

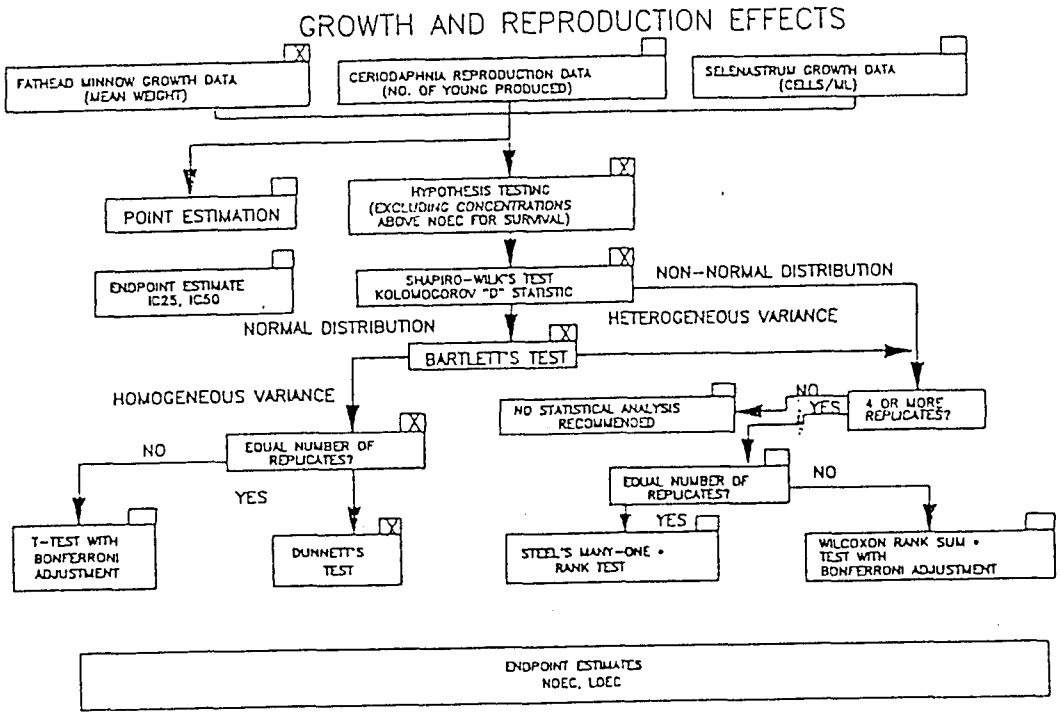
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	18
Discharge-100%	4	10	18
Intake	4	10	16

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 6, December 16-23, 1992.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 6, Watts Bar Nuclear Plant, December 16-23, 1992.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.11.

This represents a 19.25% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	0.57	0.63	0.49	0.57							0.57	
Discharge-2.0%	0.62	0.59	0.55	0.60							0.59	
Discharge-25.0%	0.64	0.58	0.59	0.63							0.61	
Discharge-50.0%	0.68	0.67	0.61	0.70							0.67	
Discharge-100%	0.67	0.56	0.61	0.53							0.59	
Intake	0.74	0.47	0.51	0.57							0.57	

*Indicates values significantly less than control (Medium).
EMS = 0.004. IC_{25} = >100%

6.2 <u>Ceriodaphnia</u> Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from December 15-20, 1992.

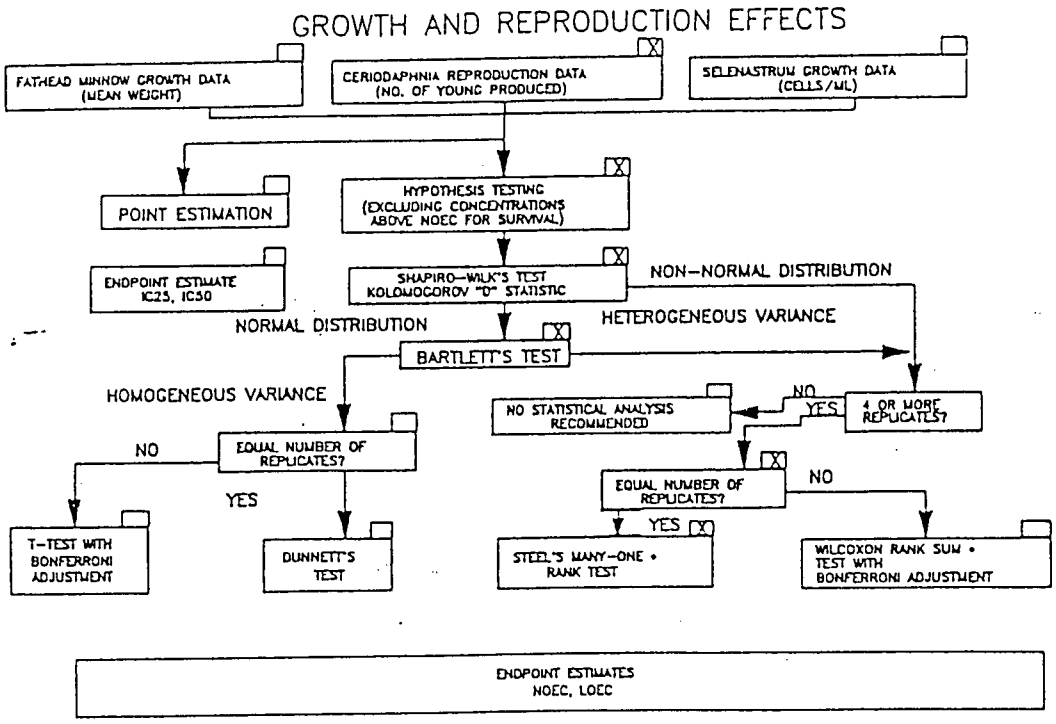
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for <u>Ceriodaphnia</u> Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 6, December 16-22, 1992.

Treatment	Total Daily % Survival 1	2	3	4	5	6
Medium	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	90	90
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100
Intake	90	90	90	90	70	70
TR	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 6, December 16-22, 1992.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 6, Watts Bar Nuclear Plant, December 16-22, 1992.

Reproduction (# young/female/6 days) Data

	Replicate											
Treatment	1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	15	19	27	29	27	34	19	34	23	27	25.40	
Discharge-2.0%	14	17	23	13	23	22	27	30	29	22	22.00	
Discharge-25.0%	25	17	27	31	29	36	0	35	19	27	24.60	
Discharge-50.0%	34	20	34	30	27	28	30	32	5	24	26.40	
Discharge-100%	27	18	8	23	13	27	29	31	29	27	23.20	
Intake	13	32	28	27	0	31	30	17	30	23	23.10	
TR	32	21	29	24	25	21	31	28	28	31	27.00	

*Indicates values significantly less than control (Medium). IC_{25} >100%.

Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	10	74	90.0
Discharge-25.0%	10	74	108.5
Discharge-50.0%	10	74	116.5
Discharge-100%	10	74	99.0
Intake	10	74	104.0
TR	10	74	114.5

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: Mean == 25.2°C (24.6-25.8)

6.3.1.2 Ceriodaphnia: Mean = 25.1°C (24.5-25.5)

6.3.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 6, December 16-23, 1992.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, December 16-23, 1992.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 6, December 16-23, 1992

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 6 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.4.2 Fathead Minnows

6.4.2.1 Chronic Tests

6.4.2.1.1 Date/Time of Most Recent Test: December 1-8, 1992/ 0945-1000 CST

6.4.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.0068 mg Cu/L

6.4.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 38

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)

NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)

IC_{25} Range: 0.0059-0.0109 mg Cu/L

6.4.3 Ceriodaphnia

6.4.3.1 Chronic Tests

6.4.3.1.1 Date/Time of most recent acceptable test: December 1-9, 1992/ 0930-1000 CST

6.4.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.02 mg Cu/L
IC_{25}: 0.153 mg Cu/L

6.4.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 36

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)

NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)

IC_{25} Range: 0.0111-0.1530 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from December 16-23 showed no toxicity (survival or growth or reproduction effects) to fathead minnows or daphnids.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1991).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.
3. U.S. EPA. Quality Criteria for Water, 1986. EPA/440/5-86-001, May 1, 1986.

PLARC501-1180

Appendix A

Calculated Allowable* Toxicity Based Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, December 15-22, 1992.

Date	Zero Release (Hours)	WB Hydro Hourly Average Flow (cfs)	WBN DSN101 Daily Flow† (cfs)	Dilution (WBN:WB Hydro)	Allowable Toxicity TUc#	Allowable Toxicity NOEC (% Effluent)
December 15	0	38250	37.7	1:1015	≤1015	≥0.09
December 16	0	38163	45.4	1:841	≤841	≥0.11
December 17	0	37629	65.4	1:575	≤576	≥0.17
December 18	0	43729	53.2	1:822	≤822	≥0.12
December 19	0	44188	39.9	1:1108	≤1108	≥0.09
December 20	0	44200	48.9	1:904	≤904	≥0.11
December 21	0	44071	49.2	1:896	≤896	≥0.11
December 22	0	44038	49.2	1:895	≤896	≥0.11
Average				1:882	≤822	≥0.11

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream concentration of an effluent to which organisms can be exposed indefinitely without causing unacceptable effect. [3]

†Estimated daily flows.

#TUc = 100/NOEC.

PLARC501-1181(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, December 15-22, 1992

1992 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
December 15	Grab*	--	<0.1
December 15-16	Composite*	0.040†	<0.1
December 16	Grab	--	<0.1
December 16-17	Composite	0.120†	<0.1
December 18	Grab	0.110	--
December 19	Grab	0.078	--
December 20	Grab	0.095	<0.1
December 21	Grab	0.098	<0.1
December 22	Grab	0.102	--

*Grab samples were at various times of the day and are not representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

†Zinc sulfate injection into WBN cooling water systems was not in service from 12/13/92 until 12/18/92 at 1330.

PLARC501-1181(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 6, December 16-23, 1992

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	25.2 (24.7-25.7)	-	8.3 (8.2-8.4)	6.5 (5.7-7.0)	-	8.2 (8.1-8.2)	7.7 (7.6-7.8)	-	338 (333-343)	343 (337-345)	65 (64-66)	90.0 (88.9-90.6)	-
Dschg 2.0% w/F.Med.	25.2 (24.7-25.7)	-	8.3 (8.2-8.4)	6.3 (5.2-7.2)	-	8.1 (8.0-8.2)	7.8 (7.6-7.8)	-	335 (330-339)	339 (333-344)	-	-	-
Dschg 25.0% w/F.Med.	25.3 (24.9-25.8)	-	8.3 (8.2-8.3)	6.2 (5.1-6.9)	-	8.0 (8.0-8.1)	7.8 (7.6-7.8)	-	299 (295-302)	305 (299-319)	-	-	-
Dschg 50.0% w/F.Med.	25.2 (24.8-25.7)	-	8.3 (8.2-8.3)	6.1 (5.1-6.8)	-	8.0 (7.9-8.0)	7.7 (7.6-7.8)	-	258 (255-261)	262 (260-266)	-	-	-
Enriched Medium	-	25.1 (24.5-25.5)	8.3 (8.2-8.4)	-	7.7 (7.6-7.9)	8.2 (8.1-8.2)	-	8.0 (7.8-8.1)	314 (306-332)	-	65 (63-66)	89.8 (87.2-92.3)	-
Dschg 2.0% w/E.Med.	-	25.1 (24.5-25.5)	8.3 (8.2-8.4)	-	7.8 (7.7-7.9)	8.2 (8.1-8.2)	-	8.0 (7.9-8.1)	310 (301-325)	-	-	-	-
Dschg 25.0% w/E.Med.	-	25.1 (24.5-25.5)	8.2 (8.0-8.3)	-	7.7 (7.5-7.9)	8.1 (8.0-8.1)	-	8.0 (7.9-8.1)	281 (273-292)	-	-	-	-
Dschg 50.0% w/E.Med.	-	25.1 (24.5-25.5)	8.2 (8.2-8.3)	-	7.7 (7.5-7.9)	8.0 (8.0-8.1)	-	8.0 (7.9-8.1)	245 (239-255)	-	-	-	-
Discharge 100%	25.2 (24.8-25.7)	25.1 (24.5-25.5)	8.3 (8.2-8.4)	6.1 (4.7-6.8)	7.7 (7.6-7.9)	7.9 (7.7-8.0)	7.7 (7.6-7.8)	8.0 (7.9-8.1)	176 (174-177)	180 (177-187)	59 (57-60)	67.7 (65.0-68.4)	<0.10 (<0.10-<0.10)
Intake 100%	25.2 (24.6-25.8)	25.1 (24.5-25.5)	8.3 (8.1-8.4)	6.3 (5.3-6.9)	7.7 (7.5-7.9)	7.9 (7.9-7.9)	7.7 (7.6-7.8)	7.9 (7.8-8.0)	169 (144-174)	173 (146-187)	56 (48-59)	64.7 (56.4-68.4)	<0.10 (<0.10-<0.10)
TR	-	25.1 (24.5-25.5)	8.3 (8.2-8.3)	-	7.7 (7.5-7.9)	8.0 (7.8-8.1)	-	7.9 (7.8-8.0)	145 (113-162)	-	49 (36-55)	57.6 (44.5-65.0)	-

*mg/L as $CaCO_3$

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 7

Principle Investigator: Cynthia L. Russell

Starting Date: January 15, 1993

Ending Date: January 22, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. This report presents findings of the fourth monthly toxicity bioassessment (Experiment 7) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas) and daphnids (Ceriodaphnia dubia). Tests conducted using diffuser discharge samples collected from January 14-20 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnows. Enriched culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution and control for the daphnids.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning January 14, 1993 (Isco composite initiated January 13), and continuing through January 20, 1993. Diffuser and intake collections were made between the hours of 0600 and 1030 EST.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were not continuous during the study (Appendix A). Dilution factors ranged from 2047-2500.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

2.7 Sample Transport: Samples collected and shipped on 01/14, 01/15, 01/19, and 01/20 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 01/16, 01/17, and 01/18 were transported the same day via private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test and enriched medium for the daphnid test). Intake samples were run at 100 percent in both tests. In addition, Tennessee River water, collected from the ARL experimental channels, was run at 100 percent only for the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: January 10, 1993

3.1.6 Hatch Date/Time: January 14, 1993/1130 CST - January 15, 1993/0630 CST

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.06 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

The temperature measured in one replicate beaker of the DSN001-25 percent treatment on day 5 was 23.8°C which is outside the prescribed range of 25°C ± 1°C. This deviation is not judged to have affected test results since all treatments had similar means and ranges and no toxic effects were demonstrated at higher or lower concentrations.

4.1.2 Date/Time Test Initiated: January 15, 1993/0955 CST

4.1.3 Date/Time Test Terminated: January 22, 1993/1000 CST

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old 3 times daily *ad libitum*.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

Temperatures measured in three of ten nontest cups on day 1 ranged from 23.6° to 23.7°C which was outside the prescribed range of 25°C ± 1°C. These deviations from the test protocol are not judged to have adversely affected test results. Since all cups were randomly arranged, comparison between treatments and controls was not impacted.

4.2.2 Date/Time Test Initiated: January 15, 1993/1015 CST

4.2.3 Date/Time Test Terminated: January 21, 1993/1030 CST

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: enriched culture medium (see Section 2.2)

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)
Daphnids--7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched medium (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and Ceriodaphnia Reproduction - Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

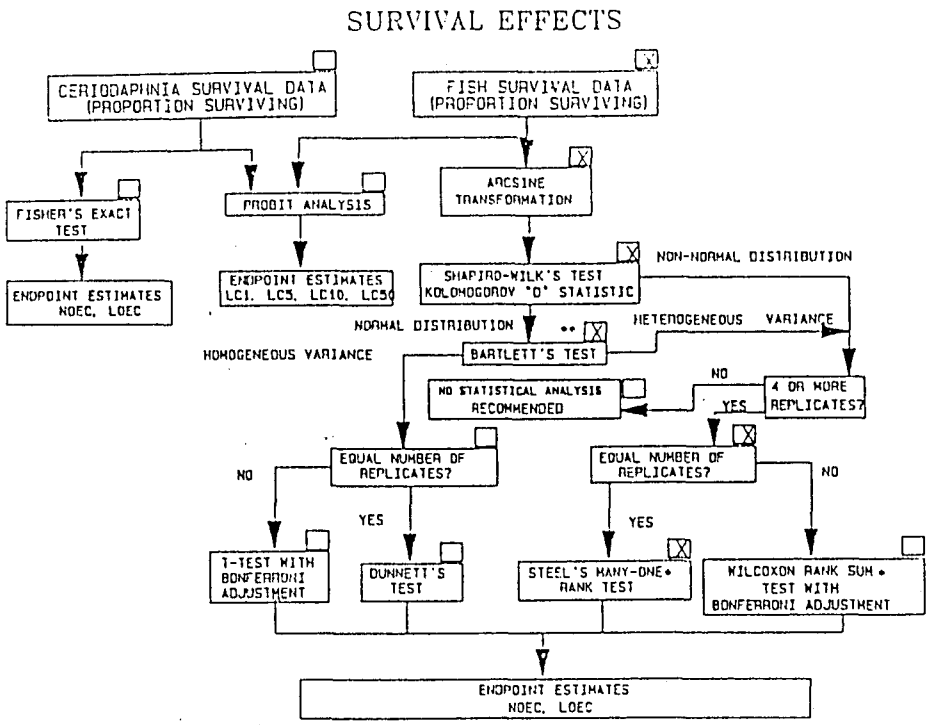
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from January 14-20, 1993.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 7, January 15-22, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	98	98	98	98
Discharge-50.0%	98	98	98	98	98	98	98
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	98	98	95	95

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 7, January 15-22, 1993.



* Test requires 4 replicates/treatment

** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 7, January 15-22, 1993.

Survival (%) Data

	Replicate											
Group	1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	100	90							98	
Discharge-50.0%	100	100	100	90							98	
Discharge-100%	100	100	100	100							100	
Intake	80	100	100	100							95	

*Indicates values significantly less than control (Medium).

IC_{25} >100% DSN101.

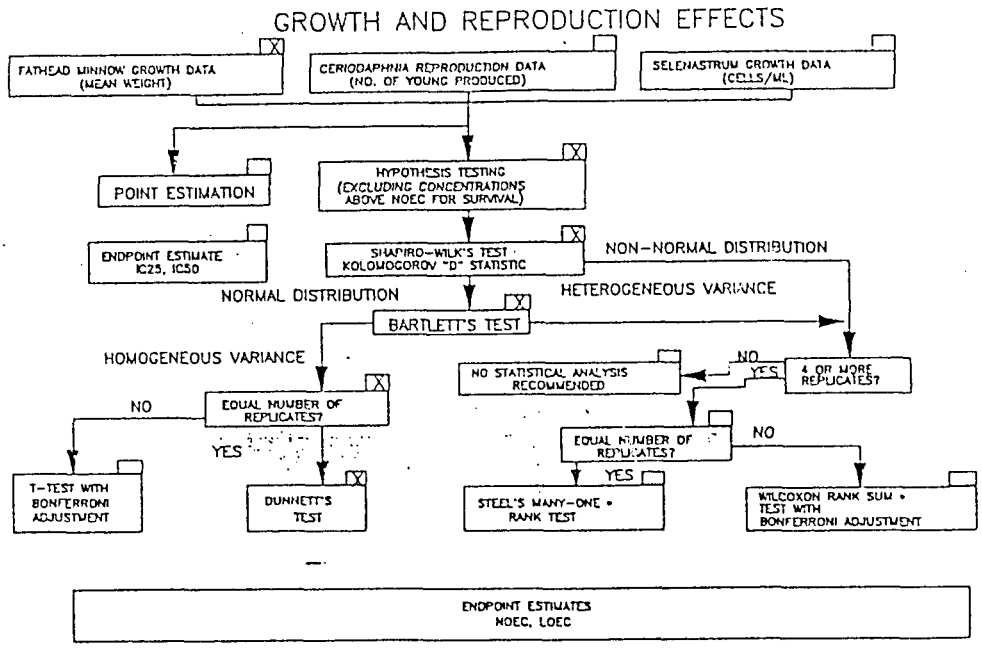
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	16
Discharge-50.0%	4	10	16
Discharge-100%	4	10	18
Intake	4	10	16

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 7, January 15-22, 1993.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 7, Watts Bar Nuclear Plant, January 15-22, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.06.

This represents a 15.02% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	0.44	0.43	0.37	0.42							0.41	
Discharge-2.0%	0.48	0.45	0.44	0.34							0.43	
Discharge-25.0%	0.41	0.43	0.49	0.45							0.45	
Discharge-50.0%	0.45	0.49	0.48	0.42							0.46	
Discharge-100%	0.43	0.46	0.42	0.45							0.44	
Intake	0.43	0.40	0.42	0.47							0.43	

*Indicates values significantly less than control (Medium).
EMS = 0.001. IC_{25} = >100% DSN101.

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from January 14-20, 1993.

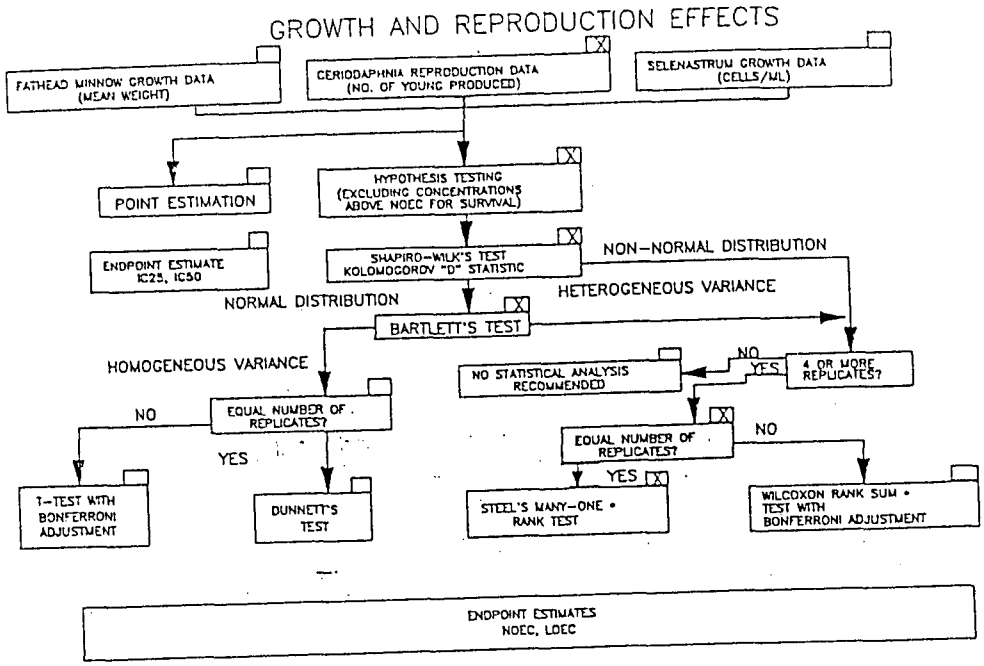
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 7, January 15-21, 1993.

Treatment	Total Daily % Survival 1	2	3	4	5	6
Medium	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100
Intake	100	100	100	100	100	100
TR	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 7, January 15-21, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 7, Watts Bar Nuclear Plant, January 15-21, 1993.

Reproduction (# young/female/6 days) Data

	Replicate											
Treatment	1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	20	26	19	25	26	9	20	29	27	19	22.00	
Discharge-2.0%	25	26	24	14	26	23	18	23	18	24	22.10	
Discharge-25.0%	21	20	15	6	27	22	20	21	22	21	19.50	
Discharge-50.0%	27	14	24	28	24	15	25	22	12	25	21.60	
Discharge-100%	25	22	27	22	30	11	25	26	24	24	23.60	
Intake	27	21	29	31	30	29	30	35	22	33	28.70	
TR	24	27	26	27	33	31	26	30	30	34	28.80	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101

Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	10	74	97.5
Discharge-25.0%	10	74	97.5
Discharge-50.0%	10	74	101.5
Discharge-100%	10	74	112.5
Intake	10	74	142.5
TR	10	74	141.0

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: Mean = 25.1°C (23.8°-26.0°)

6.3.1.2 Ceriodaphnia: Mean = 24.7°C (23.6°-25.7°)

6.3.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 7, January 15-22, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc, Total Phosphorus, and Clamtrol, January 13-20, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 7, January 15-22, 1993

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 7 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.4.2 Fathead Minnows

6.4.2.1 Chronic Tests

6.4.2.1.1 Date/Time of Most Recent Test:
January 5, 1993/1020 CST to January 12, 1993/1000 CST

6.4.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.006 mg Cu/L

6.4.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 39

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.006-0.0109 mg Cu/L

6.4.3 Ceriodaphnia

6.4.3.1 Chronic Tests

6.4.3.1.1 Date/Time of Most Recent Test:
January 5, 1993/0915 CST to January 11, 1993/0915 CST

6.4.3.1.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC_{25}: 0.012 mg Cu/L

6.4.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 37

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.153 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from January 14-20 showed no toxicity (survival or growth or reproduction effects) to fathead minnows or daphnids.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLARC501-1270

Appendix A

Dilution Factors for Watts Bar Nuclear Plant (WBN) DSN101 Based On Watts Bar (WB) Hydro Plant Releases, January 13-20, 1993.

Date	Zero Release (Hours)	WB Hydro Hourly Average Flow (cfs)	WBN DSN101 Daily Flow* (cfs)	Dilution (WBN:WB Hydro)
January 13	0	57125	22.9	1:2495
January 14	12	57263	22.9	1:2500
January 15	9	54188	22.9	1:2366
January 16	0	50950	22.9	1:2225
January 17	0	50825	22.9	1:2220
January 18	0	50813	22.9	1:2219
January 19	6	48846	22.9	1:2133
January 20	0	46875	22.9	1:2047
Average				1:2275

*Estimated daily flows.

PLARC501-1271(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc, Total Phosphorus, and Clamtrol, January 13-20, 1993

1993 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)	CLAMTROL (mg/L)
January 13	Grab*	0.111	-	-
January 14	Grab	0.096	<0.10	<0.2
January 15	Grab	0.121	0.12	<0.2
January 14-15	Composite*	0.124	<0.10	<0.2
January 15-16	Composite	-	-	<0.2
January 17	Grab	0.122	0.14	-
January 18	Grab	0.116	-	-
January 19	Grab	0.125	<0.10	-
January 19-20	Composite	0.114	0.10	<0.2
January 20	Grab	0.111	0.14	-

*Grab samples were at various times of the day and are not representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1271(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 7, January 15-22, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	25.0 (24.1-25.7)	-	8.4 (8.3-8.4)	6.4 (5.4-7.0)	-	8.2 (8.1-8.3)	7.7 (7.4-7.9)	-	346 (341-352)	349 (343-354)	63 (54-67)	96.4 (92.3-109.4)	-
Dschg 2.0% w/F.Med.	25.2 (24.4-25.8)	-	8.3 (8.2-8.4)	6.2 (5.1-6.8)	-	8.2 (8.1-8.3)	7.8 (7.6-7.9)	-	343 (338-349)	347 (340-357)	-	-	-
Dschg 25.0% w/F.Med.	25.1 (23.8-25.7)	-	8.2 (8.1-8.3)	6.2 (4.1-6.9)	-	8.1 (8.1-8.2)	7.8 (7.6-7.9)	-	303 (299-311)	307 (301-315)	-	-	-
Dschg 50.0% w/F.Med.	25.1 (24.1-25.8)	-	8.3 (8.1-8.3)	6.2 (5.5-6.8)	-	8.1 (8.0-8.1)	7.8 (7.6-8.0)	-	261 (256-270)	265 (258-276)	-	-	-
Enriched Medium	-	24.7 (23.6-25.7)	8.4 (8.3-8.4)	-	7.8 (7.6-8.1)	8.3 (8.2-8.3)	-	8.0 (7.9-8.1)	329 (323-340)	-	62 (61-63)	88.4 (87.2-88.9)	-
Dschg 2.0% w/E.Med.	-	24.7 (23.6-25.7)	8.3 (8.3-8.4)	-	7.8 (7.6-8.1)	8.1 (8.0-8.2)	-	8.0 (7.7-8.1)	322 (317-330)	-	-	-	-
Dschg 25.0% w/E.Med.	-	24.7 (23.6-25.7)	8.3 (8.2-8.3)	-	7.8 (7.6-8.1)	8.1 (8.1-8.2)	-	8.0 (7.7-8.1)	289 (284-292)	-	-	-	-
Dschg 50.0% w/E.Med.	-	24.7 (23.6-25.7)	8.2 (8.1-8.3)	-	7.8 (7.6-8.1)	8.1 (8.0-8.2)	-	8.0 (7.7-8.1)	251 (244-256)	-	-	-	-
Discharge 100%	25.1 (24.1-26.0)	24.7 (23.6-25.7)	8.3 (8.2-8.4)	6.1 (4.3-6.8)	7.8 (7.6-8.2)	8.0 (7.9-8.1)	7.8 (7.6-7.9)	8.0 (7.6-8.1)	173 (163-189)	175 (158-192)	57 (53-60)	67.9 (63.3-71.8)	<0.10 (<0.10-<0.1
Intake 100%	25.0 (24.0-25.8)	24.7 (23.6-25.7)	8.3 (8.2-8.4)	6.0 (4.4-6.8)	7.8 (7.6-8.1)	7.9 (7.8-7.9)	7.6 (7.4-7.9)	8.0 (7.7-8.1)	163 (153-171)	165 (155-177)	55 (51-58)	65.2 (61.6-68.4)	<0.10 (<0.10-<0.1
TR	-	24.7 (23.6-25.7)	8.2 (8.2-8.3)	-	7.8 (7.6-8.1)	8.0 (7.9-8.1)	-	8.0 (7.8-8.0)	144 (141-148)	-	51 (47-58)	59.2 (54.7-65.0)	-

*mg/L as CaCO3

PLARC501-127

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 8

Principle Investigator: Cynthia L. Russell

Starting Date: February 11, 1993

Ending Date: February 18, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. Compliance monitoring for this discharge was initiated in October 1992.

This report presents findings of the fifth monthly toxicity bioassessment (Experiment 8) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and/or subchronic responses of three trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas), daphnids (Ceriodaphnia dubia), and a unicellular alga (Selenastrum capricornutum). Tests conducted using samples collected from February 10-16, 1993, showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significant reduction in algal growth was demonstrated in 100, 50, and 25 percent concentrations DSN101 samples. No reduction in growth resulted from exposure to 2 percent sample. Maximum allowable toxicity based on dilution available at WBN during this study was a NOEC $\geq$0.06 percent effluent ($\bar{x}$ value for study period; see Appendix A). These data indicate the EPA recommended chronic instream criterion was not exceeded.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnow test. Enriched

culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution and control for the daphnid test. Tennessee River water (TR) from ARL (TRM 293) was used for dilution water and control for the algal test, and algal medium was run as a laboratory reference.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning February 10, 1993 (Isco composite initiated February 9), and continuing through February 16, 1993. Diffuser and intake collections were made between the hours of 0930 and 1200 EST.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were continuous during the study (Appendix A). Dilution factors ranged from 831 to 2552.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

Note: On day 0 of the test, the sample arrival temperatures were 5.0°C for discharge and 5.2°C for intake. These temperatures deviated from sample storage protocol calling for sample temperatures of 4°C or less upon arrival. Samples were well iced and apparently had not had adequate time for complete cooling.

2.7 Sample Transport: Samples collected and shipped on 02/10, 02/11, and 02/12 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 02/13, 02/14, 02/15, and 02/16 were transported the same day via

private courier (airplane or automobile) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to near 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test, enriched medium for the daphnid test, and TR for the algal test). Intake samples were run at 100 percent only in all tests, and Tennessee River water, collected from the ARL experimental channels, was run as an additional control in the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced

as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: February 6, 1993

3.1.6 Hatch Date/Time: February 10, 1993/1150 CST - February 11, 1993/0615 CST

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.07 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

3.3 Species: Selenastrum capricornutum, alga

3.3.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory, obtained from EPA, Newtown, Ohio, August 25, 1992.

3.3.2 Culture water: Culture medium consists of reconstituted water following EPA methods for the Algal Growth Test. [1] Culture medium pH is adjusted to 7.5 (7.4-7.6, acceptable range) when necessary using 0.1 N NaOH or 0.1 N HCl.

3.3.3 Temperature of Culture: 25°C ± 1°C

3.3.4 General Maintenance: Cultures are maintained in 250-500 mL flasks containing 100-250 mL algal medium in light and temperature controlled incubators. New cultures are started each week by inoculating flasks with a single colony from incubated agar plates containing macro- and micronutrients or by transferring 1 mL of inoculant from a culture flask into fresh autoclaved and filtered medium using aseptic techniques (see Section 3.3.2). Agar plates are prepared as needed. One half of the plates are stored at 4°C for contingency use, the remainder are incubated at 24°C ± 2°C. Medium used in algae sterile culture is vacuum-filtered through 0.45 µm membrane filters.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: February 11, 1993/0915 CST

4.1.3 Date/Time Test Terminated: February 18, 1993/0815 CST

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.2.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: February 11, 1993/0925 CST

4.2.3 Date/Time Test Terminated: February 17, 1993/1000 CST

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: enriched culture medium (see Section 2.2)

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50×10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.2.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

4.3 Algal, Selenastrum capricornutum, Growth Test, EPA Test Method 1003.0. [1]

4.3.1 Modification/Deviations to Method 1003.0: Mean initial cell density of three samples checked was 12,892 cells/mL, determined by Sedgwick-Rafter cell counts. The EPA methods call for an initial cell density of 10,000 cells/mL.

4.3.2 Date/Time Test Initiated: February 12, 1993/0925 CST

4.3.3 Date/Time Test Terminated: February 16, 1993/0840 CST

4.3.4 Test Chamber: 250-mL borosilicate Erlenmeyer flasks
Volume per Chamber: 100 mL

4.3.5 Cell Enumeration Method: Coulter Counter Model ZM with Sedgwick-Rafter Cell counts used for QA verification.

4.3.6 Initial Cell Density: 12,892 cells/mL (Cell density based on Sedgwick-Rafter counts).

4.3.7 Number of Replicates per Treatment: 4 (In addition an uninoculated sample was prepared for each treatment to serve as a blank for final cell counts.)

4.3.8 Dilution Water: Tennessee River water (TRM 293.0; see Section 2.2.)

4.3.9 Test Control: Tennessee River water (TRM 293.0; see Section 2.2.)

4.3.10 Test Temperature: 25°C ± 1°C

4.3.11 Photoperiod: Continuous illumination

4.3.12 Renewal Period: None. This test method is a 96-h static bioassay. Flasks were randomly placed into the incubator at test initiation, removed and hand swirled daily, and randomly replaced into the incubator (New Brunswick Scientific Model G25). The incubator provided constant shaking at a rate of 100 rpm.

4.3.13 Physical and Chemical Parameters Measured: Parameters measured for all test samples and algal medium at test initiation were temperature, DO, pH, conductivity, alkalinity, hardness, and total residual chlorine. Results are reported in Section 6.4.2. Temperatures were measured two or three

times daily in extra flasks placed randomly in the incubator. Since some variation was seen at different locations in the incubator, test flasks were randomly rearranged in the incubator daily when flask contents were swirled.

4.3.14 Statistics: Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [1] Decision processes followed for testing algal growth response are shown in Section 6.3.2. Based on tests for normally distributed data and homogenous variances, the statistical test used for growth endpoint determination was the Dunnett's Test with log transformation.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish - 7-day chronic (NOEC)
Daphnids - 7-day chronic (NOEC)
Algal - 4-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish, daphnids, algal
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic, enriched medium (see Section 3.2.2) for daphnid chronic, and algal medium without EDTA for algal chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth, Ceriodaphnia Reproduction, and Algal Growth - NOEC Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

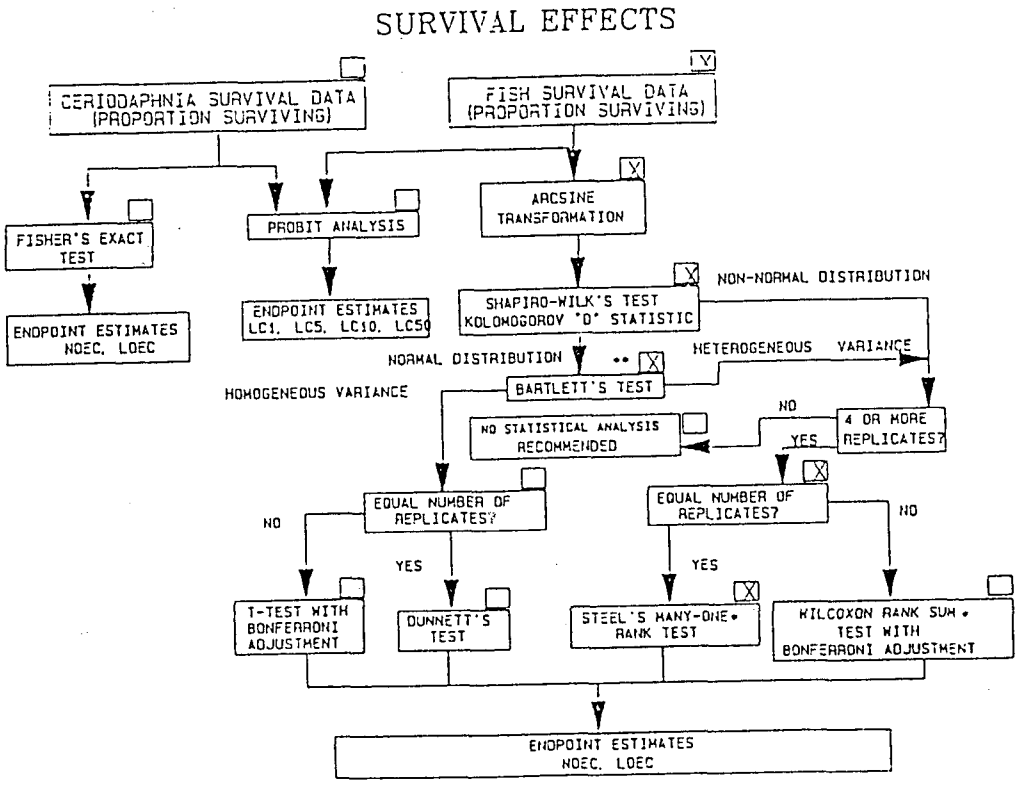
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from February 10-16, 1993.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 8, February 11-18, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	98	98	98
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	98	98
Intake	100	100	100	95	95	95	95

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 8, February 11-18, 1993.



* Test requires 4 replicates/treatment
** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 8, February 11-18, 1993.

Survival (%) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	100	100	100	100							100	
Discharge-2.0%	100	90	100	100							98	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	100	100	100							100	
Discharge-100%	100	100	90	100							98	
Intake	80	100	100	100							95	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101.

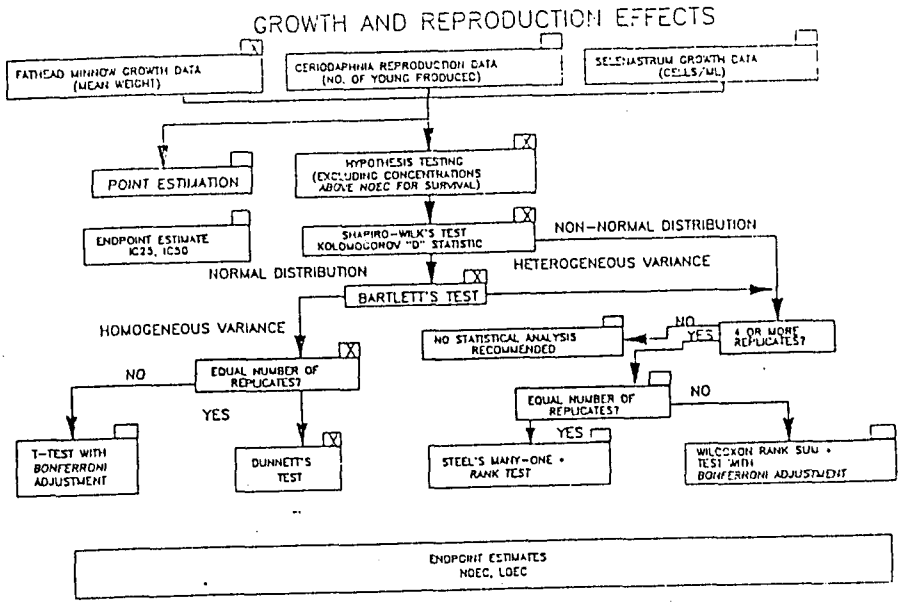
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	16
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	18
Discharge-100%	4	10	16
Intake	4	10	16

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 8, February 11-18, 1993.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 8, Watts Bar Nuclear Plant, February 11-18, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.08.

This represents a 11.63% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

	Replicate										
Group	1	2	3	4	5	6	7	8	9	10	Mean S*
Medium	0.64	0.67	0.71	0.57							0.65
Discharge-2.0%	0.66	0.61	0.58	0.60							0.61
Discharge-25.0%	0.55	0.64	0.65	0.61							0.61
Discharge-50.0%	0.68	0.66	0.58	0.68							0.65
Discharge-100%	0.66	0.68	0.75	0.63							0.68
Intake	0.66	0.63	0.64	0.65							0.65

*Indicates values significantly less than control (Medium).
EMS = 0.002. IC_{25} = >100% DSN101.

6.2 *Ceriodaphnia* Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from February 10-15, 1993.

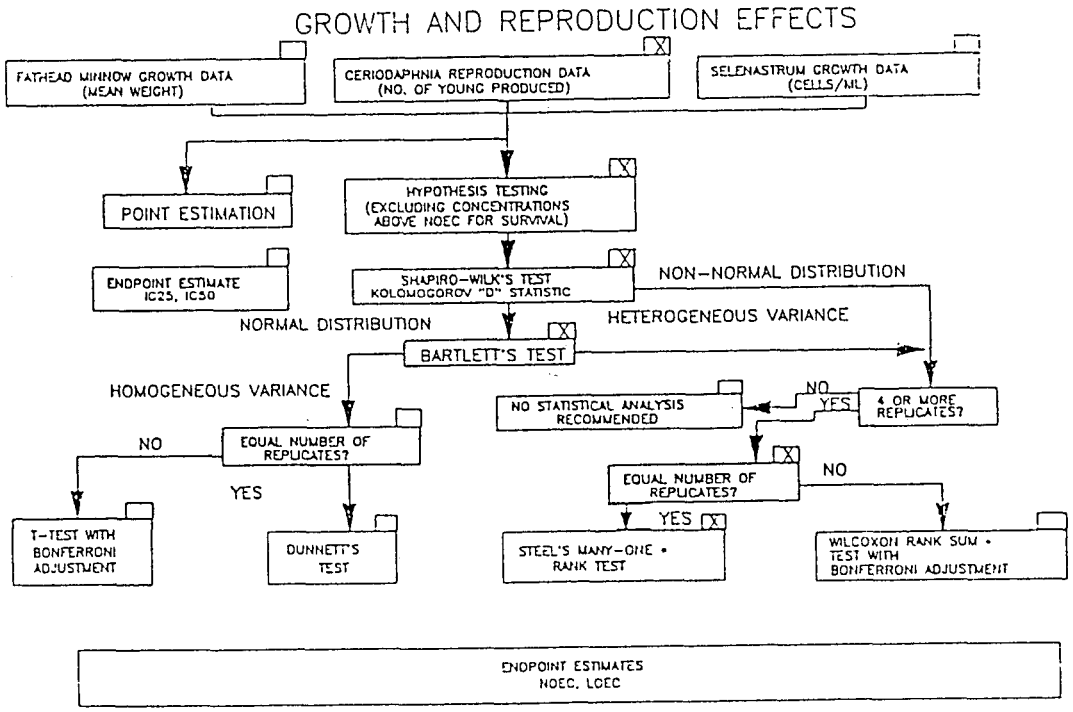
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for *Ceriodaphnia* Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 8, February 11-17, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Medium	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100
Intake	100	100	100	100	100	100
TR	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of *Ceriodaphnia* to Watts Bar Nuclear Plant Effluent, Experiment 8, February 11-17, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 8, Watts Bar Nuclear Plant, February 11-17, 1993.

Reproduction (# young/female/6 days) Data

Treatment	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	29	26	24	25	26	19	16	20	24	23	23.20	
Discharge-2.0%	24	27	17	23	17	16	17	16	9	17	18.30	
Discharge-25.0%	29	30	21	26	21	17	12	19	19	19	21.30	
Discharge-50.0%	21	30	21	17	18	6	6	14	17	16	16.60	
Discharge-100%	25	28	21	28	23	23	25	24	22	23	24.20	
Intake	29	27	27	28	25	26	22	25	24	26	25.90	
TR	24	24	27	27	26	22	28	27	25	26	25.60	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101

Steel's Many-one Rank Test

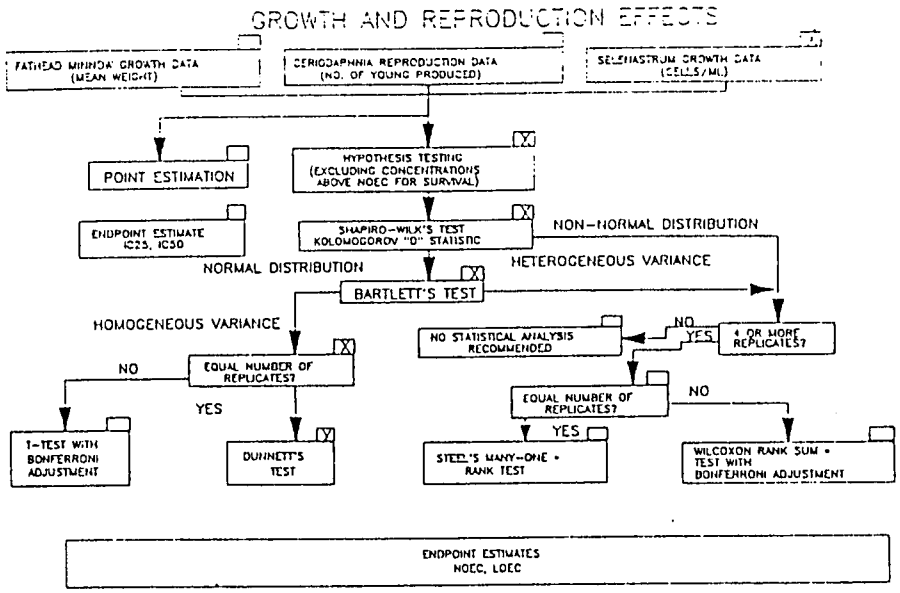
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	10	74	77.5
Discharge-25.0%	10	74	94.0
Discharge-50.0%	10	74	74.5
Discharge-100%	10	74	107.5
Intake	10	74	128.5
TR	10	74	126.5

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.3 Algal, Selenastrum capricornutum, Growth Test

6.3.1 Summary of Results: Diffuser discharge DSN101, collected February 11, 1993, demonstrated significant growth effects at 100, 50, and 25 percent concentrations. No reduction in growth occurred in the 2 percent concentration. The IC_{25} for DSN101 was 3.75 percent. No growth effects were demonstrated in the intake sample. Medium demonstrated a growth effect (likely due to absence of EDTA, EPA test medium recipe) and was excluded from the data analysis.

6.3.2 Statistical Decision Process for Determining Toxicity Endpoints for 4-Day Exposure of the Alga, Selenastrum capricornutum, to Watts Bar Nuclear Plant DSN101 Discharge, Experiment 8, February 12-16, 1993.



* Test requires 4 replicates/treatment

6.3.3 Analysis of Growth Data Using Dunnett's Test for Algal 4-Day Growth Test, Watts Bar Nuclear Plant, Experiment 8, February 12-16, 1993.

Log transformation was applied before data analysis. For this set of data, the minimum significant difference is 1004 x 10^3.

This represents a 38.74% reduction in No. cells/mL x 10^3
T = 2.41 Alpha = 0.05 (one-tailed test)

Algal Growth Data (No. cell/mL x 10^3)

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S
TR	2167	2544	2631	3105							2612	
Discharge-2.0%	1753	1769	2879	1775							2044	
Discharge-25.0%	816	771	1444	670							925	*
Discharge-50.0%	344	269	396	352							340	*
Discharge-100%	166	137	128	363							199	*
Intake	2158	1634	1410	1362							1641	

*Indicates values significantly less than the control (TR). EMS = 0.016
IC_{25} = 3.75%

6.4 Physical/Chemical Parameters

6.4.1 Overall Test Temperature

6.4.1.1 Fathead Minnow: Mean = 24.9°C (24.1°-25.5°)

6.4.1.2 Ceriodaphnia: Mean = 25.3°C (24.8°-25.5°)

6.4.1.3 Algae: Mean = 25.1°C (24.4°-25.7°)

6.4.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 8, February 11-18, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, February 9-16, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 8, February 11-18, 1993

Appendix D Initial Water Chemistry Values for Algal Growth Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 8, February 12, 1993

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 8 showed chronic results consistent with ARL control chart ranges for fathead minnows, daphnids and algae.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test:
January 26, 1993/0920 CST to February 2, 1993/0845 CST

6.5.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.004 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 40

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.011 mg Cu/L

6.5.3 Ceriodaphnia

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of Most Recent Test:
January 26, 1993/1030 CST to February 1, 1993/1030 CST

6.5.3.1.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC_{25}: 0.019 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 38

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.153 mg Cu/L

6.5.4 *Selenastrum capricornutum*

6.5.4.1 Chronic Tests

6.5.4.1.1 Date/Time of most recent test: January 11, 1993/0815 CST to January 15, 1993/0800

6.5.4.1.2 LOEC: 0.015 mg Cu/L
NOEC: 0.0008 mg Cu/L
IC_{25}: 0.0010 mg Cu/L

6.5.4.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 13

LOEC Range: 0.0004-0.003 mg Cu/L (four concentration increments)
NOEC Range: 0.0004-0.0015 mg Cu/L (three concentration increments)
IC_{25} Range: 0.0006-0.0017 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from February 10-16 showed no toxicity (survival or growth or reproduction effects) to fathead minnows or daphnids. A reduction in algal growth was demonstrated in diffuser discharge, collected February 11, 1993, at 100, 50, and 25 percent concentrations (NOEC = 2%, IC_{25} = 3.75%). Based on dilution available at WBN during this study, the EPA recommended instream criterion for chronic toxicity was not exceeded.

8.0 REFERENCES

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2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.
3. U.S. EPA. Quality Criteria for Water, 1986. EPA/440/5-86-001, May 1, 1986.

PLARC501-1387

Appendix A

Calculated Allowable* Toxicity Based Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, February 9-16, 1993.

Date	Zero Release (Hours)	WB Hydro Hourly Average Flow (cfs)	WBN DSN101 Daily Flow† (cfs)	Dilution (WBN:WB Hydro)	Allowable Toxicity TUc‡	Allowable Toxicity NOEC (% Effluent)
February 9	0	29092	11.4	1:2552	≤2553	≥0.04
February 10	0	26950	11.4	1:2364	≤2365	≥0.04
February 11	0	23883	11.4	1:2095	≤2096	≥0.05
February 12	0	16046	11.4	1:1408	≤1409	≥0.07
February 13	0	16008	11.4	1:1404	≤1405	≥0.07
February 14	0	16017	11.4	1:1405	≤1406	≥0.07
February 15	0	27792	11.4	1:2438	≤2439	≥0.04
February 16	0	19013	22.9	1:831	≤831	≥0.12
Average				1:1812	≤1813	≥0.06

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream concentration of an effluent to which organisms can be exposed indefinitely without causing unacceptable effect. [3]

†Estimated daily flows.

‡TUc = 100/NOEC.

PLARC501-1388(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, February 9-16, 1993

1993 Month	Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
February	9	Grab*	0.127	-
February	9-10	Composite*	0.145	0.10
February	10	Grab	0.150	-
February	11	Grab	0.100	-
February	14	Grab	0.135	0.13
February	15	Grab	0.122	0.13
February	16	Grab	0.087	-

*Grab samples were at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1388(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 8, February 11-18, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	24.7 (24.1-25.1)	-	8.2 (8.2-8.3)	6.0 (5.0-6.7)	-	8.2 (8.1-8.2)	7.6 (7.5-7.8)	-	337 (330-342)	341 (333-348)	64 (63-65)	92.8 (90.6-97.5)	-
Dschg 2.0% w/F.Med.	24.9 (24.3-25.4)	-	8.2 (8.2-8.3)	5.7 (4.7-6.4)	-	8.2 (8.1-8.2)	7.7 (7.5-7.8)	-	334 (330-339)	338 (330-345)	-	-	-
Dschg 25.0% w/F.Med.	24.9 (24.3-25.4)	-	8.3 (8.2-8.3)	5.7 (5.0-6.5)	-	8.0 (7.9-8.1)	7.7 (7.5-7.8)	-	297 (290-302)	301 (296-307)	-	-	-
Dschg 50.0% w/F.Med.	25.0 (24.4-25.5)	-	8.3 (8.3-8.3)	5.6 (4.2-6.4)	-	7.9 (7.6-8.0)	7.7 (7.5-7.7)	-	256 (247-261)	260 (252-267)	-	-	-
Enriched Medium	-	25.3 (24.8-25.5)	8.2 (8.2-8.3)	-	7.4 (7.2-7.5)	8.2 (8.1-8.2)	-	8.0 (7.8-8.1)	318 (315-323)	-	62 (61-62)	90.6 (87.2-94.1)	-
Dschg 2.0% w/E.Med.	-	25.3 (24.8-25.5)	8.2 (8.2-8.3)	-	7.5 (7.2-7.6)	8.1 (8.1-8.2)	-	8.1 (7.9-8.2)	315 (312-320)	-	-	-	-
Dschg 25.0% w/E.Med.	-	25.3 (24.8-25.5)	8.3 (8.2-8.3)	-	7.4 (7.2-7.5)	8.0 (7.9-8.1)	-	8.0 (7.9-8.1)	282 (278-285)	-	-	-	-
Dschg 50.0% w/E.Med.	-	25.3 (24.8-25.5)	8.3 (8.2-8.3)	-	7.4 (7.2-7.5)	7.9 (7.6-8.0)	-	8.0 (7.9-8.1)	246 (243-250)	-	-	-	-
Discharge 100%	25.0 (24.4-25.5)	25.3 (24.8-25.5)	8.3 (8.3-8.3)	5.8 (4.8-6.4)	7.4 (7.2-7.5)	7.8 (7.3-7.9)	7.7 (7.5-7.7)	8.0 (7.9-8.1)	174 (165-182)	179 (169-195)	59 (57-61)	71.3 (66.7-75.2)	<0.1 (<0.1-<0.1
Intake 100%	24.9 (24.2-25.4)	25.3 (24.8-25.5)	8.3 (8.3-8.4)	5.6 (4.9-6.3)	7.4 (7.2-7.5)	7.7 (7.3-7.9)	7.6 (7.4-7.6)	8.0 (7.8-8.1)	152 (150-155)	156 (153-165)	55 (53-56)	65.7 (61.6-70.1)	<0.1 (<0.1-<0.1
TR	-	25.3 (24.8-25.5)	8.3 (8.2-8.3)	-	7.4 (7.2-7.5)	8.0 (7.8-8.0)	-	8.0 (7.8-8.0)	156 (130-202)	-	52 (45-55)	62.1 (54.7-68.4)	<0.1 (<0.1-<0.1

*mg/L as $CaCO_3$

PLARC501-1389

Appendix D

Initial Water Chemistry Values for Algal Growth Test,
Watts Bar Nuclear Plant Biomonitoring, Test 8, February 12-16, 1993

Treatment	Temp.	DO	pH	Cond.	Hard.	Alk.	Total Residual Chlorine
	(°C)	(mg/L)	(S.U.)	(umhos)	*	*	(mg/L)
TR	24.7	8.9	7.9	245	71.8	63	-
Discharge-2.0%	24.7	9.1	7.9	248	78.7	65	-
Discharge-25.0%	24.7	8.9	7.9	251	80.4	65	-
Discharge-50.0%	25.3	8.9	7.7	256	80.4	66	-
Discharge-100%	24.8	8.7	7.5	264	83.8	67	<0.1
Intake	24.8	9.1	7.5	238	77.0	63	<0.1
Medium	24.7	6.8	7.4	90	17.1	10	-

*mg/L as $CaCO_3$

PLARC501-1388(3)

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 9

Principle Investigator: Cynthia L. Russell

Starting Date: March 19, 1993

Ending Date: March 26, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. Compliance monitoring for this discharge was initiated in October 1992.

This report presents findings of the sixth monthly toxicity bioassessment (Experiment 9) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and/or subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas) and daphnids (Ceriodaphnia dubia). Tests conducted using composite samples collected from March 17-24, 1993, showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnow test. Enriched

culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution and control for the daphnid test.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning March 18, 1993 (Isco composite initiated March 17), and continuing through March 24, 1993. Diffuser and intake collections were made between the hours of 0950 and 1100 EST.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were continuous during the study (Appendix A). Dilution factors ranged from 509 to 2674.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

Note: On day 1 of the test, the sample arrival temperature was 5.2°C for intake. This temperature deviated from sample storage protocol calling for sample temperatures of 4°C or less upon arrival. Sample was well iced and apparently had not had adequate time for complete cooling.

2.7 Sample Transport: Samples collected and shipped on 03/18, 03/19, 03/22, 03/23, and 03/24 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 03/20 and 03/21 were transported the same day via private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to near 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test and enriched medium for the daphnid test). Intake samples were run at 100 percent only in both tests, and Tennessee River water, collected from the ARL experimental channels, was run as an alternate control in the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: March 13-14, 1993

3.1.6 Hatch Date/Time: March 18, 1993/1045 CST - March 19, 1993/0615 CST

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.07 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal

culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: March 19, 1993/0845 CST

4.1.3 Date/Time Test Terminated: March 26, 1993/0748 CST

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old 3 times daily *ad libitum*.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.3.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. No statistical analysis of survival was necessary for survival data due to 100 percent survival in all test treatments. Decision processes followed for testing sublethal (growth) effects are shown in Section 6.1.3.1. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow growth endpoint determination was Steel's Many-one Rank Test.

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-micron mesh nylon screen was used to filter samples rather than a 60-micron mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: March 19, 1993/0900 CST

4.2.3 Date/Time Test Terminated: March 25, 1993/0905 CST

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: enriched culture medium (see Section 2.2)

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50×10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.2.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion'Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish - 7-day chronic (NOEC)
Daphnids - 7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish, daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched medium (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and <u>Ceriodaphnia</u> Reproduction -
NOEC Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
<u>Ceriodaphnia</u> Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from March 17-24, 1993. Significant reduction in fish growth occurred in the 25 percent DSN101 treatments based on the non-parameter test required for data analysis. This is judged not to indicate toxicity since mean weights for all treatments were very close and both lower and higher concentrations showed no toxicity.

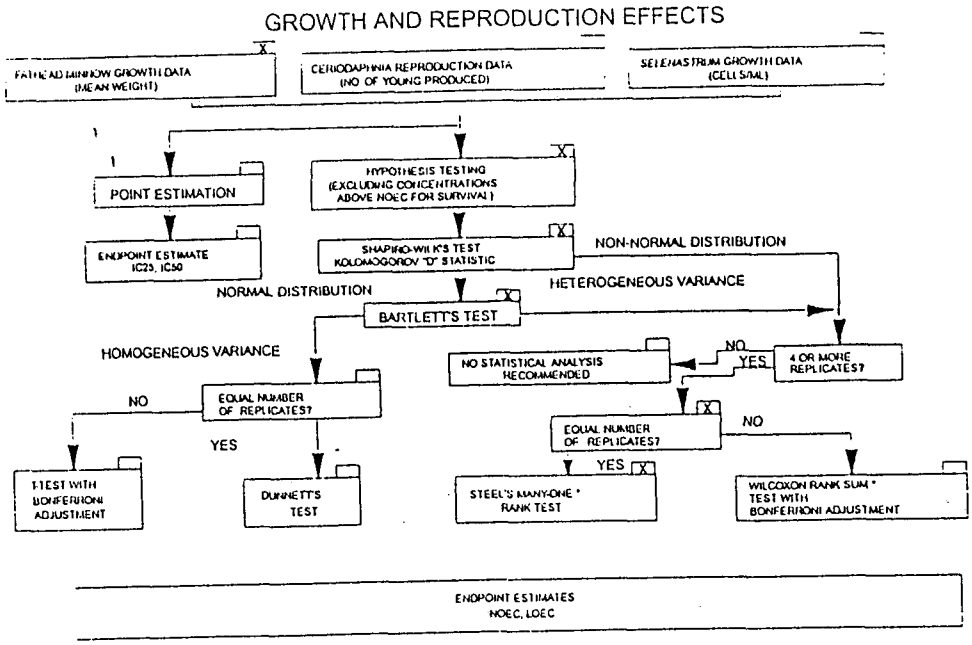
6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 9, March 19-26, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	100	100	100	100

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 9, March 19-26, 1993.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 9, Watts Bar Nuclear Plant, March 19-26, 1993.

	Dry Weight (mg) Data											
	Replicate											
Group	1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	.68	.70	.68	.75							.70	
Discharge--2.0%	.66	.66	.81	.65							.70	
Discharge--25.0%	.66	.63	.66	.64							.65	*
Discharge--50.0%	.69	.74	.67	.68							.70	
Discharge-100%	.69	.64	.65	.66							.66	
Intake	.72	.66	.69	.69							.69	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101.

Steel's Many-one Rank Test			
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	14
Discharge-25.0%	4	10	10 *
Discharge-50.0%	4	10	16
Discharge-100%	4	10	12
Intake	4	10	17

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.2 *Ceriodaphnia* Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from March 17-24, 1993.

6.2.2 Results, Survival Data:

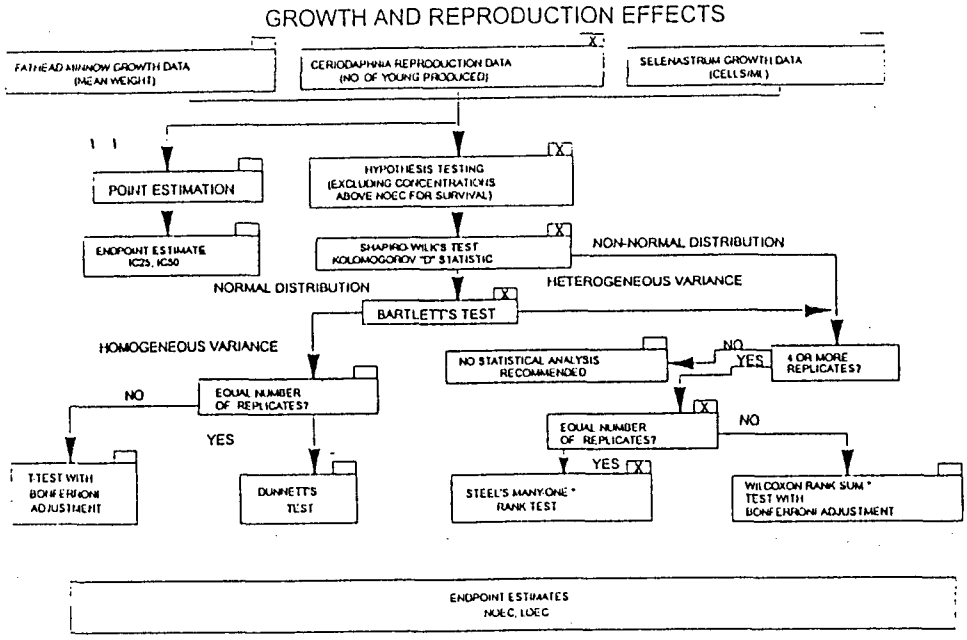
6.2.2.1 Daily percent survival summary for *Ceriodaphnia* Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 9, March 19-25, 1993.

	Total Daily % Survival						
Treatment	1	2	3	4	5	6	S*
Medium	100	100	100	100	90	90	
Discharge-2.0%	100	100	100	100	100	100	
Discharge-25.0%	100	100	100	100	100	100	
Discharge-50.0%	100	100	100	100	90	90	
Discharge-100%	100	100	100	100	100	100	
Intake	100	100	100	100	100	100	
TR	100	100	100	100	90	80	

*Indicates values significantly less than control (Medium) based on Fisher's Exact Test.

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 9, March 19-25, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 9, Watts Bar Nuclear Plant, March 19-25, 1993.

Reproduction (# young/female/6 days) Data

	Replicate											
Treatment	1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	28	24	30	31	24	20	29	11	28	12	23.70	
Discharge-2.0%	20	28	27	29	22	26	28	12	19	12	22.30	
Discharge-25.0%	33	28	29	32	25	29	28	12	28	28	27.20	
Discharge-50.0%	3	29	26	27	26	31	26	13	27	15	22.30	
Discharge-100%	29	29	29	27	30	27	14	14	33	30	26.20	
Intake	27	30	28	22	30	27	31	12	30	10	24.70	
TR	28	27	28	14	11	27	29	7	28	28	22.70	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101

Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	10	74	95.0
Discharge-25.0%	10	74	120.5
Discharge-50.0%	10	74	101.0
Discharge-100%	10	74	118.5
Intake	10	74	110.5
TR	10	74	99.0

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.4 Physical/Chemical Parameters

6.4.1 Overall Test Temperature

6.4.1.1 Fathead Minnow: Mean = 25.1°C (24.5°-25.6°)

6.4.1.2 Ceriodaphnia: Mean = 25.0°C (24.2°-25.6°)

6.4.2 Initial Alkalinity, Conductivity, Hardness

6.4.2.1 Low range values for TR caused by diultion of water in outdoor channels by a heavy rainfall event on Day 5 of the test. Mean values were similarly affected.

6.4.3 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 9, March 11-18, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, March 17-24, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 9, March 19-26, 1993

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 9 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test:
February 24, 1993/1000 CST to March 3, 1993/0900 CST

6.5.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.005 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 41

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.011 mg Cu/L

6.5.3 Ceriodaphnia

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of Most Recent Test:
February 24, 1993/1045 CST to March 6, 1993/0950 CST

6.5.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.02 mg Cu/L
IC_{25}: 0.026 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 39

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.028 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from March 17-24 showed no toxicity (survival or growth or reproduction effects) to fathead minnows or daphnids.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLARC501-1412

Appendix A

Dilution Factors for Watts Bar Nuclear Plant (WBN) DSN101 and Watts Bar (WB) Hydro Plant Releases, March 17-24, 1993.

Date	Zero Release (Hours)	WB Hydro Hourly Average Flow (cfs)	WBN DSN101 Daily Flow† (cfs)	Dilution (WBN:WB Hydro)
March 17	0	43321	16.2	1:2674
March 18	0	34617	34.3	1:1009
March 19	0	29492	34.3	1:860
March 20	0	22833	34.3	1:666
March 21	0	17446	34.3	1:509
March 22	0	22071	34.3	1:644
March 23	0	41742	34.3	1:1217
March 24	0	44204	34.3	1:1289
Average				1:1812

PLARC501-1413(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, March 17-24, 1993

1993 Month	Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
March	17	Grab*	0.070	0.12
March	17-18	Composite*	0.075	0.15
March	18	Grab	0.094	0.15
March	19	Grab	0.120	-
March	21	Grab	0.118	0.20
March	22	Grab	0.059	0.20
March	23	Grab	0.098	0.19
March	23-24	Composite	0.059	0.18
March	24	Grab	0.102	-

*Grab samples were at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1413(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 9, March 19-26, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	25.1 (24.6-25.6)	-	8.4 (8.3-8.4)	6.1 (4.6-7.0)	-	8.3 (8.2-8.3)	7.7 (7.5-7.9)	-	351 (348-355)	356 (352-362)	67 (66-68)	96.2 (94.1-99.2)	<0.1 (<0.1-<0.1)
Dschg 2.0% w/F.Med.	25.1 (24.5-25.5)	-	8.4 (8.3-8.4)	6.1 (5.3-7.0)	-	8.2 (8.2-8.3)	7.7 (7.6-7.9)	-	349 (345-356)	353 (348-358)	-	-	-
Dschg 25.0% w/F.Med.	25.2 (24.5-25.6)	-	8.3 (8.3-8.4)	6.1 (5.1-6.9)	-	8.1 (8.1-8.2)	7.7 (7.6-7.9)	-	309 (306-315)	314 (309-319)	-	-	-
Dschg 50.0% w/F.Med.	25.2 (24.5-25.6)	-	8.4 (8.3-8.4)	5.9 (5.0-6.7)	-	8.1 (8.0-8.2)	7.7 (7.6-7.9)	-	267 (262-273)	271 (266-281)	-	-	-
Enriched Medium	-	25.0 (24.2-25.6)	8.4 (8.3-8.4)	-	7.7 (7.5-7.8)	8.3 (8.2-8.3)	-	7.9 (7.7-8.0)	328 (320-332)	-	64 (63-66)	93.5 (90.6-95.8)	-
Dschg 2.0% w/E.Med.	-	25.0 (24.2-25.6)	8.4 (8.3-8.4)	-	7.7 (7.6-7.9)	8.2 (8.2-8.3)	-	8.0 (8.0-8.1)	325 (318-329)	-	-	-	-
Dschg 25.0% w/E.Med.	-	25.0 (24.2-25.6)	8.3 (8.3-8.3)	-	7.7 (7.6-7.9)	8.1 (8.1-8.2)	-	8.0 (7.9-8.1)	292 (286-296)	-	-	-	-
Dschg 50.0% w/E.Med.	-	25.0 (24.2-25.6)	8.3 (8.3-8.4)	-	7.7 (7.6-7.8)	8.2 (8.0-8.3)	-	8.0 (8.0-8.1)	257 (254-262)	-	-	-	-
Discharge 100%	25.2 (24.6-25.5)	25.0 (24.2-25.6)	8.4 (8.4-8.4)	5.9 (4.9-6.8)	7.7 (7.6-7.8)	8.0 (7.9-8.2)	7.7 (7.6-7.9)	8.0 (8.0-8.1)	182 (176-190)	229 (180-493)	63 (60-65)	75.2 (71.8-82.1)	<0.1 (<0.1-<0.1)
Intake 100%	25.0 (24.6-25.5)	25.0 (24.2-25.6)	8.4 (8.3-8.4)	6.1 (4.8-6.8)	7.7 (7.6-7.8)	8.0 (8.0-8.1)	7.7 (7.5-7.8)	8.0 (8.0-8.1)	176 (172-179)	180 (177-182)	62 (60-64)	74.3 (70.1-77.0)	<0.1 (<0.1-<0.1)
TR	-	25.0 (24.2-25.6)	8.3 (8.3-8.4)	-	7.7 (7.6-7.9)	8.1 (7.7-8.2)	-	8.0 (8.0-8.1)	141 (88-160)	-	48 (26-57)	59.3 (34.2-68.4)	<0.1 (<0.1-<0.1)

*mg/L as $CaCO_3$

PLARC501-1414

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 10

Principle Investigator: Cynthia L. Russell

Starting Date: April 16, 1993

Ending Date: April 23, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. This report presents findings of the seventh monthly toxicity bioassessment (Experiment 10) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas) and daphnids (Ceriodaphnia dubia).

Tests conducted using diffuser discharge and intake samples collected from April 15-21 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significantly reduced reproduction was demonstrated by daphnids in the 2 percent concentration of DSN101. This effect was attributed to the culture medium used for dilution which failed to meet control test acceptability criteria of minimum number of young.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnows. Enriched culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution for the daphnids. Intake was used as control for statistical analysis of data from the daphnid test due to failure of enriched medium to meet EPA's control acceptability criteria. [1]

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning April 15, 1993 (Isco composite initiated April 14), and continuing through April 21, 1993. Diffuser and intake collections were made between the hours of 0630 and 1045 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were not continuous during the study (Appendix A). Dilution factors ranged from 727-1027.

WBN DSN101 diffuser discharge was suspended for several hours on April 15, during the 30 minute injection of Copper-Trol into WBN's cooling water systems. Copper-Trol was not detectable (<0.05 ppm as tolyltriazole) in the WBN DSN101 composite sample.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

Note: On Days 1, 2 and 4 of the test, the sample arrival temperatures for discharge were 5.4°C, 4.7°C and 4.3°C, respectively. Temperatures for intake on Days 1 and 2 were 4.1°C. These temperatures deviated from sample storage protocol calling for sample temperatures of 4°C or less upon arrival. Samples were well iced and apparently had not had adequate time for complete cooling.

2.7 Sample Transport: Samples collected and shipped on 04/15, 04/16, 04/19, 04/20, and 04/21 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 04/17 and 04/18 were transported the same day via

private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100-µm nylon mesh filter into 2000-mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test and enriched medium for the daphnid test). Intake samples were run at 100 percent in both tests. In addition, Tennessee River water, collected from the ARL experimental channels, was run at 100 percent only for the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: April 11, 1993

3.1.6 Hatch Date/Time: April 15, 1993/1055 CDT - April 16, 1993/0650 CDT

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.06 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: April 16, 1993/0845 CDT

4.1.3 Date/Time Test Terminated: April 23, 1993/0815 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: April 16, 1993/0915 CDT

4.2.3 Date/Time Test Terminated: April 23, 1993/0940 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Dilution water was enriched culture medium (see Section 2.2) but failed to meet EPA criteria as a control. Intake was used as the control.

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Dunnett's Test. [1]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)
Daphnids--7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched medium (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and Ceriodaphnia Reproduction - Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

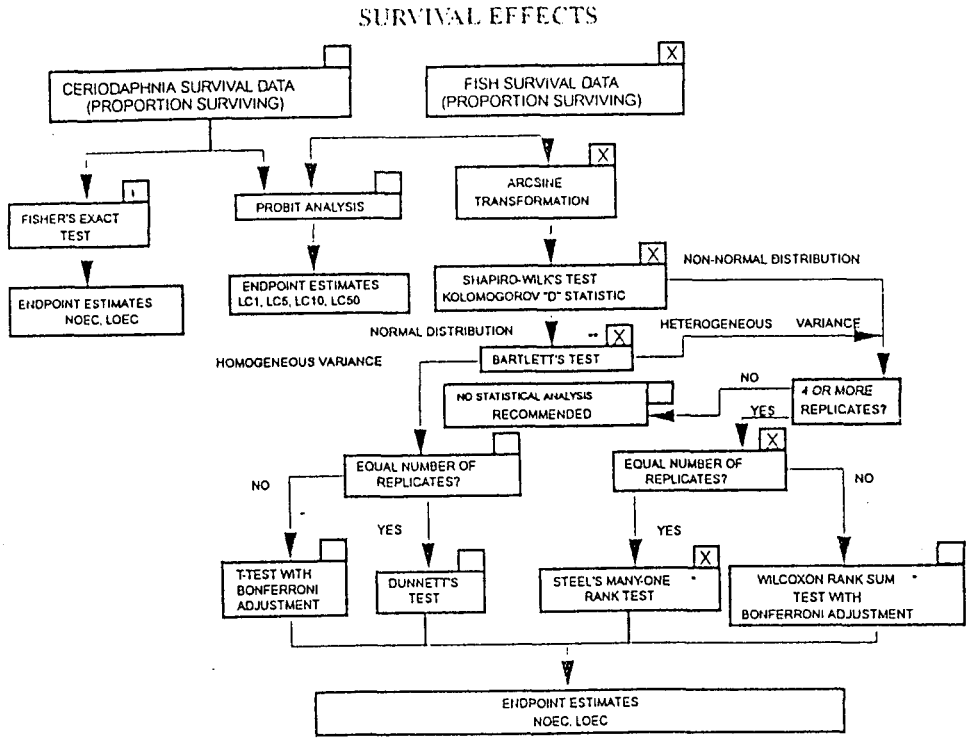
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from April 15-21, 1993.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 10, April 16-23, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	98	98	98	98	98	98
Discharge-50.0%	100	100	100	98	98	98	95
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	98	98	98	98	98

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 10, April 16-23, 1993.



6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 10, April 16-23, 1993.

	Survival (%) Data											
	Replicate											
Group	1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	90	100							98	
Discharge-50.0%	90	90	100	100							95	
Discharge-100%	100	100	100	100							100	
Intake	100	100	100	90							98	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101.

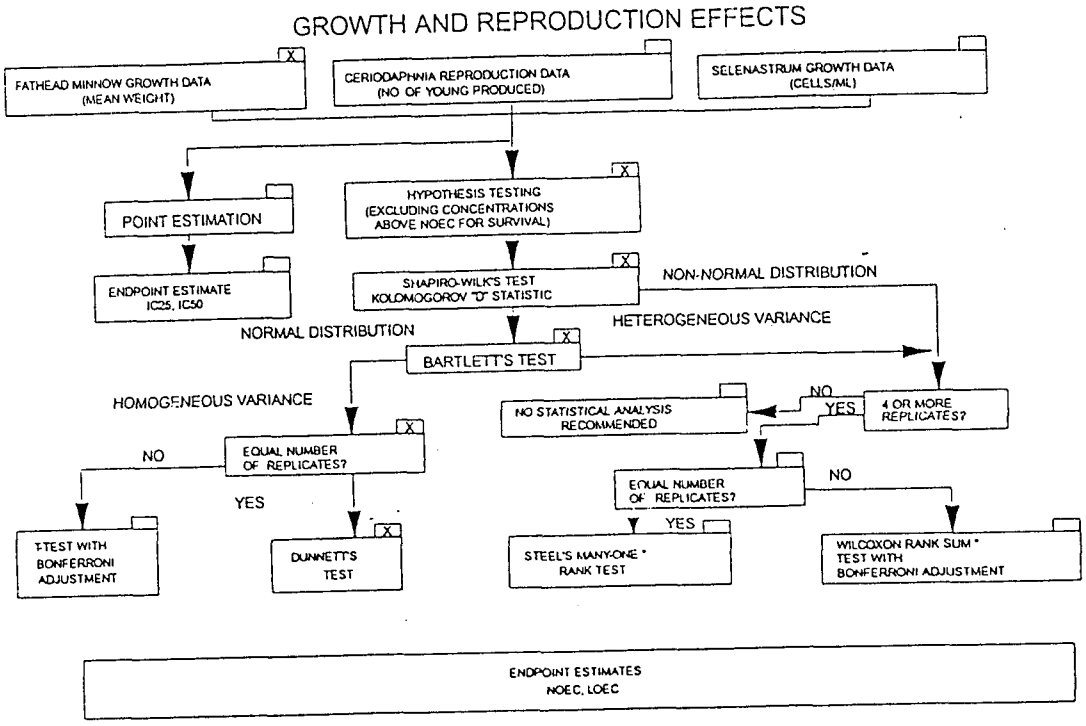
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	16
Discharge-50.0%	4	10	14
Discharge-100%	4	10	18
Intake	4	10	16

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 10, April 16-23, 1993.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 10, Watts Bar Nuclear Plant, April 16-23, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.06.

This represents a 11.90% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

	Dry Weight (mg) Data										
	Replicate										
Group	1	2	3	4	5	6	7	8	9	10	Mean S*
Medium	0.49	0.46	0.54	0.53							0.51
Discharge-2.0%	0.49	0.52	0.44	0.48							0.48
Discharge-25.0%	0.50	0.53	0.48	0.48							0.50
Discharge-50.0%	0.51	0.41	0.44	0.47							0.46
Discharge-100%	0.45	0.49	0.49	0.53							0.49
Intake	0.49	0.47	0.49	0.56							0.50

*Indicates values significantly less than control (Medium).
EMS = 0.001. IC_{25} = >100% DSN101.

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No toxicity was demonstrated in diffuser discharge or intake samples collected from April 15-20, 1993. Significant reduction in reproduction occurred in the 2 percent concentration of DSN101. This effect was attributed to the culture medium used for sample dilution which failed to meet minimum test acceptability ($\bar{x}$ young in medium = 9.2; EPA required = 15.0). Intake was used as the control for statistical analyses.

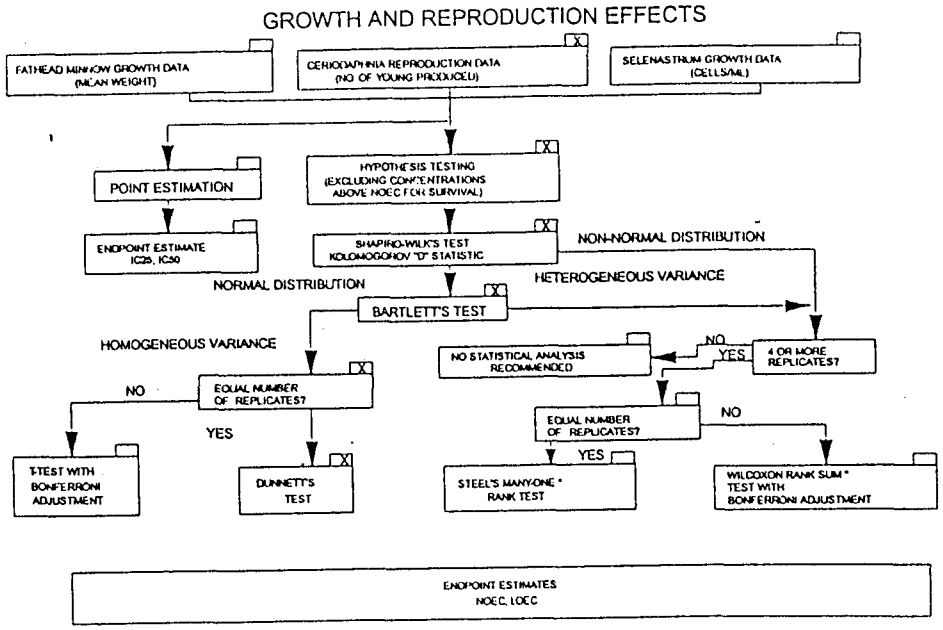
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 10, April 16-22, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Intake	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100
TR	100	100	100	100	100	100
Medium	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 10, April 16-22, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Dunnett's Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 10, Watts Bar Nuclear Plant, April 16-22, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 5.55.

This represents a 20.47% reduction in Reproduction (# young/female/6 days). T = 2.29 Alpha = 0.05 (one-tailed test)

Reproduction (# young/female/6 days) Data

Treatment	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Intake	29	29	26	32	36	27	18	31	17	26	27.10	
Discharge-2.0%	20	11	9	22	5	5	9	11	16	9	11.70	*
Discharge-25.0%	24	26	26	24	25	26	23	22	21	10	22.70	
Discharge-50.0%	15	29	29	27	27	26	25	29	28	29	26.40	
Discharge-100%	29	20	30	26	32	32	26	33	28	33	28.90	
TR	15	28	21	30	31	21	19	27	9	26	22.70	
Medium	11	3	11	11	10	2	3	7	18	16	9.20†	

*Indicates values significantly less than control (Intake).
EMS = 29.34. IC_{25} >100% DSN101
†Medium was not included in the data analysis because it did not meet EPA's control acceptability criteria.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: Mean = 24.9°C (24.3°-25.9°)

6.3.1.2 Ceriodaphnia: Mean = 24.8°C (24.0°-25.9°)

6.3.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 10, April 16-23, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc, Total Phosphorus, and Clamtrol, April 15-22, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 10, April 16-23, 1993

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 10 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.4.2 Fathead Minnows

6.4.2.1 Chronic Tests

6.4.2.1.1 Date/Time of Most Recent Test:
March 23, 1993/1010 CST to March 30, 1993/0930 CST

6.4.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.006 mg Cu/L

6.4.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 42

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.011 mg Cu/L

6.4.3 Ceriodaphnia

6.4.3.1 Chronic Tests

6.4.3.1.1 Date/Time of Most Recent Test:
March 23, 1993/0930 CST to March 30, 1993/0830 CST

6.4.3.1.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC_{25}: 0.012 mg Cu/L

6.4.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 40

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.028 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from April 15-21 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significantly reduced reproduction was demonstrated in the 2 percent concentration of DSN101 by daphnids. This effect was attributed to the culture medium used for dilution which failed to meet control test acceptability criteria of minimum number of young.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.
3. U.S. EPA. Quality Criteria for Water, 1986. EPA/440/5-86-001, May 1, 1986.

PLARC501-1467

Appendix A

Dilution Factors for Watts Bar Nuclear Plant (WBN) DSN101 and Watts Bar (WB) Hydro Plant Releases, April 15-22, 1993.

Date	Zero Release	WB Hydro Hourly Average Flow	WBN DSN101 Daily Flow*	Dilution
	(Hours)	(cfs)	(cfs)	(WBN:WB Hydro)
April 15	0	34275	34.3†	1:999
April 16	0	35225	34.3	1:1027
April 17	2	28441	34.3	1:829
April 18	4	24930	34.3	1:727
April 19	0	28196	34.3	1:822
April 20	0	27838	34.3	1:812
April 21	0	27954	34.3	1:815
April 22	0	30396	34.3	1:886
Average				1:864

*Estimated daily flows. Actual flows become considerably less as hours of zero release increase.

†Diffuser discharge suspended from 1140 to 1700 EDT on April 15 during 30-minute coppertrol injection.

PLARC501-1468(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, April 15-22, 1993

1993 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
April 15	Grab*	0.067	-
April 15-16	Composite*	0.135	0.25
April 16	Grab	0.130	-
April 18	Grab	0.092	0.175
April 19	Grab	0.120	0.16
April 20	Grab	0.081	-
April 20-21	Composite*	0.101	0.20
April 21	Grab	0.100	-
April 22	Grab	0.088	-

*Grab samples were at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1468(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 10, April 16-23, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	24.8 (24.3-25.2)	-	8.2 (8.0-8.4)	6.0 (4.9-6.7)	-	8.2 (8.2-8.3)	7.7 (7.6-7.8)	-	349 (338-358)	354 (342-366)	67 (61-71)	93.6 (92.3-94.1)	-
Dschg 2.0% w/F.Med.	24.9 (24.4-25.2)	-	8.2 (8.0-8.4)	5.8 (4.4-6.7)	-	8.2 (8.2-8.3)	7.8 (7.6-7.9)	-	346 (334-354)	351 (342-360)	-	-	-
Dschg 25.0% w/F.Med.	25.0 (24.6-25.3)	-	8.2 (8.2-8.3)	5.6 (4.5-6.5)	-	8.1 (8.1-8.2)	7.7 (7.5-7.8)	-	307 (298-315)	311 (304-317)	-	-	-
Dschg 50.0% w/F.Med.	24.9 (24.5-25.9)	-	8.3 (8.2-8.3)	5.7 (4.4-6.8)	-	8.1 (8.0-8.2)	7.7 (7.6-7.8)	-	265 (260-269)	269 (264-272)	-	-	-
Enriched Medium	-	24.8 (24.0-25.9)	8.2 (8.0-8.3)	-	7.7 (7.7-7.9)	8.3 (8.2-8.3)	-	8.1 (7.9-8.2)	327 (316-339)	-	66 (61-70)	93.2 (92.3-94.1)	-
Dschg 2.0% w/E.Med.	-	24.8 (24.0-25.9)	8.2 (8.0-8.3)	-	7.7 (7.6-7.9)	8.2 (8.2-8.3)	-	8.1 (8.0-8.2)	322 (311-335)	-	-	-	-
Dschg 25.0% w/E.Med.	-	24.8 (24.0-25.9)	8.2 (8.0-8.3)	-	7.7 (7.6-7.9)	8.2 (8.1-8.2)	-	8.1 (8.0-8.2)	290 (283-300)	-	-	-	-
Dschg 50.0% w/E.Med.	-	24.8 (24.0-25.9)	8.3 (8.2-8.3)	-	7.7 (7.6-7.9)	8.1 (8.0-8.1)	-	8.1 (8.0-8.2)	255 (248-264)	-	-	-	-
Discharge 100%	24.8 (24.3-25.2)	24.8 (24.0-25.9)	8.3 (8.2-8.3)	5.7 (4.6-6.4)	7.7 (7.6-7.9)	8.0 (8.0-8.1)	7.6 (7.6-7.7)	8.1 (8.0-8.2)	182 (175-190)	186 (180-193)	64 (62-67)	78.4 (77.0-83.8)	<0.10 (<0.10-<0.10)
Intake 100%	24.9 (24.6-25.2)	24.8 (24.0-25.9)	8.3 (8.2-8.4)	5.6 (4.1-6.4)	7.7 (7.6-7.8)	8.0 (7.9-8.0)	7.7 (7.5-7.7)	8.1 (7.9-8.1)	177 (172-183)	181 (175-185)	63 (62-66)	75.7 (71.8-80.4)	<0.10 (<0.10-<0.10)
TR	-	24.8 (24.0-25.9)	8.2 (8.1-8.3)	-	7.7 (7.6-7.9)	8.0 (7.9-8.1)	-	8.0 (7.9-8.1)	151 (149-152)	-	52 (51-54)	63.5 (61.6-66.7)	<0.10 (<0.10-<0.10)

*mg/L as $CaCO_3$

PLARC501-1469

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnow test. Enriched culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution for the daphnid test. Tennessee River water (TR) from ARL (TRM 293) was used for dilution water for the algal test. Because of the failure of enriched medium to meet EPA's control acceptability criteria, Intake was used as control for the daphnid test. Although TR met EPA's control acceptability criteria in the algal test, Intake was used as the control because of lower variability among replicates (11 percent versus Tennessee River water 14 percent) and higher algal growth (1762 cells/mL x 10^3 vs. 1245 cells/mL x 10^3). In addition to the controls listed above, filtered TR was run with the daphnid test and algal medium containing EDTA was run with the algal test as extra laboratory controls to use in the event both the dilution water and intake samples were unacceptable for control purposes.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning May 11, 1993 (Isco composite initiated May 10), and continuing through May 17, 1993. Diffuser and intake collections were made between the hours of 0800 and 1000 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations during the study are summarized in Appendix A. Dilution factors ranged from 159 to 468.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

Note: On Days 2, 4 and 6 of the test, the discharge sample arrival temperatures were 4.9°C, 5.0°C and 5.0°C, respectively. These temperatures deviated from sample storage protocol calling for sample temperatures of 4°C or less upon arrival. Samples were well iced and apparently had not had adequate time for complete cooling.

2.7 Sample Transport: Samples collected and shipped on 05/11, 05/12, 05/13, 05/14 and 05/17 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 05/15 and 05/16 were transported the same day via private courier (airplane or automobile) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to near 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test, enriched medium for the daphnid test, and TR for the algal test). Intake samples were run at 100 percent only in all tests, and Tennessee River water, collected from the ARL experimental channels, was run as an additional control in the daphnid test. Samples collected May 13 were used in the algal test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per

hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: May 7, 1993

3.1.6 Hatch Date/Time: May 11, 1993/0800-1045 CDT

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.05 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

3.3 Species: Selenastrum capricornutum, alga

3.3.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory, obtained from EPA, Newtown, Ohio, March 10, 1993.

3.3.2 Culture water: Culture medium consists of reconstituted water following EPA methods for the Algal Growth Test. [1] Culture medium pH is adjusted to 7.5 (7.4-7.6, acceptable range) when necessary using 0.1 N NaOH or 0.1 N HCl.

3.3.3 Temperature of Culture: 25°C ± 1°C

3.3.4 General Maintenance: Cultures are maintained in 250-500 mL flasks containing 100-250 mL algal medium in light and temperature controlled incubators. New cultures are started each week by inoculating flasks with a single colony from incubated agar plates containing macro- and micronutrients or by transferring 1 mL of inoculant from a culture flask into fresh autoclaved and filtered medium using aseptic techniques (see Section 3.3.2). Agar plates are prepared as needed. One half of the plates are stored at 4°C for contingency use, the remainder are incubated at 24°C ± 2°C. Medium used in algae sterile culture is vacuum-filtered through 0.45 µm membrane filters.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: May 12, 1993/0730 CDT

4.1.3 Date/Time Test Terminated: May 19, 1993/0730 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old 3 times daily *ad libitum*.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.2.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal

distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: May 12, 1993/0830 CDT

4.2.3 Date/Time Test Terminated: May 18, 1993/0830 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Enriched culture medium (see Section 2.2) was used for dilution water but failed to meet EPA minimum acceptability criteria for controls. Intake was used as the control for statistical purposes.

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.2.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

4.3 Algal, Selenastrum capricornutum, Growth Test, EPA Test Method 1003.0. [1]

4.3.1 Modification/Deviations to Method 1003.0: Mean initial cell density of three samples checked was 10,782 cells/mL, determined by Sedgwick-Rafter cell counts. The EPA methods call for an initial cell density of 10,000 cells/mL.

4.3.2 Date/Time Test Initiated: May 14, 1993/0900 CDT

4.3.3 Date/Time Test Terminated: May 18, 1993/0805 CDT

4.3.4 Test Chamber: 250-mL borosilicate Erlenmeyer flasks
Volume per Chamber: 100 mL

4.3.5 Cell Enumeration Method: Coulter Counter Model ZM with Sedgwick-Rafter Cell counts used for QA verification.

4.3.6 Initial Cell Density: 10,782 cells/mL (Cell density based on Sedgwick-Rafter counts).

4.3.7 Number of Replicates per Treatment: 4 (In addition an uninoculated sample was prepared for each treatment to serve as a blank for final cell counts.)

4.3.8 Dilution Water: Tennessee River water (TRM 293.0; see Section 2.2.)

4.3.9 Test Control: Intake

4.3.10 Test Temperature: 25°C ± 1°C

4.3.11 Photoperiod: Continuous illumination

4.3.12 Renewal Period: None. This test method is a 96-h static bioassay. Flasks were randomly placed into the incubator at test initiation, removed and hand swirled daily, and randomly replaced into the incubator (New Brunswick Scientific Model G25). The incubator provided constant shaking at a rate of 100 rpm.

4.3.13 Physical and Chemical Parameters Measured: Parameters measured for all test samples and algal medium at test initiation were temperature, DO, pH, conductivity, alkalinity, hardness, and total residual chlorine. Results are reported in Section 6.4.2. Temperatures were measured two or three times daily in extra flasks placed randomly in the incubator. Since some variation was seen at different locations in the incubator, test flasks were randomly rearranged in the incubator daily when flask contents were swirled.

4.3.14 Statistics: Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [1] Decision processes followed for testing algal growth response are shown in Section 6.3.2. Based on tests for normally distributed data and homogenous variances, the statistical test used for growth endpoint determination was the Dunnett's Test.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish - 7-day chronic (NOEC)
Daphnids - 7-day chronic (NOEC)
Algal - 4-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish, daphnids, algal
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic, enriched medium (see Section 3.2.2) for daphnid chronic, and algal medium without EDTA for algal chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth, <u>Ceriodaphnia</u> Reproduction, and Algal Growth - NOEC Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
<u>Ceriodaphnia</u> Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

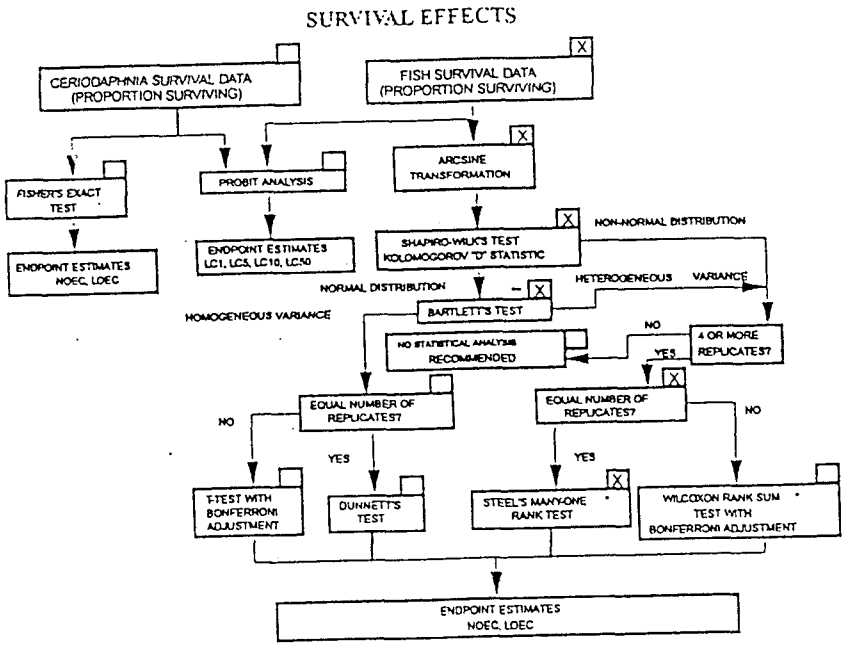
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from May 10-17, 1993. Although one replicate in the Intake sample had 40 percent survival, the overall reduction was not statistically significant. This mortality does not appear to have been treatment related since survival was 100 percent in the other three replicates. The cause of the mortality is not known.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 11, May 12-19, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	98	98	95	88	85

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 11, May 12-19, 1993.



* Test requires 4 replicates/treatment

** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 11, May 12-19, 1993.

Survival (%) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	100	100	100							100	
Discharge-100%	100	100	100	100							100	
Intake	100	40	100	100							85	

*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101.

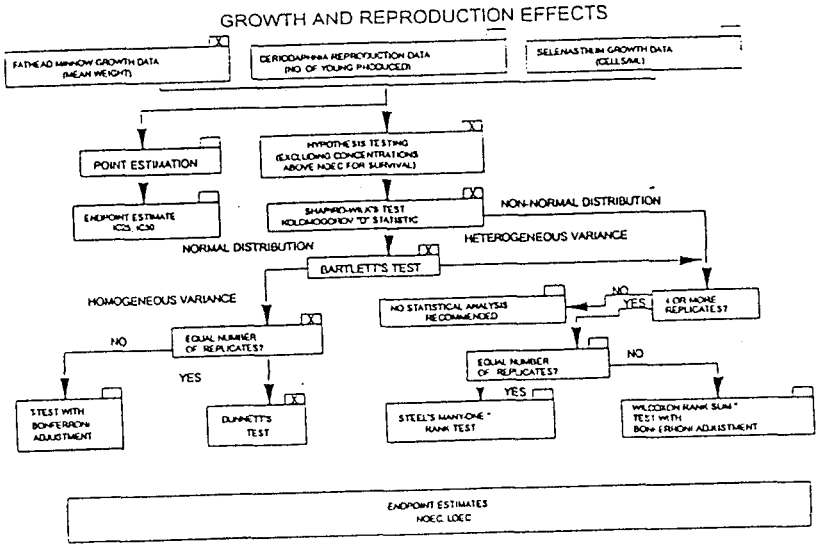
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	18
Discharge-100%	4	10	18
Intake	4	10	16

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 11, May 12-19, 1993.



* Test requires 4 replicates/treatment

6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 11, Watts Bar Nuclear Plant, May 12-19, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.05.

This represents a 14.62% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

	Dry Weight (mg) Data										
	Replicate										
Group	1	2	3	4	5	6	7	8	9	10	Mean S*
Medium	0.35	0.31	0.34	0.33							0.33
Discharge-2.0%	0.32	0.34	0.34	0.35							0.34
Discharge-25.0%	0.33	0.35	0.34	0.37							0.35
Discharge-50.0%	0.38	0.41	0.31	0.35							0.36
Discharge-100%	0.38	0.32	0.33	0.34							0.34
Intake	0.31	0.38	0.30	0.36							0.34

*Indicates values significantly less than control (Medium).
EMS = 0.001. IC_{25} = >100% DSN101.

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from May 10-16, 1993. Significant reduction in reproduction occured in the 2 percent concentration of DSN101. This effect appeared to be related to culture medium used for sample dilution which failed to meet minimum test acceptability (50 percent of adults had three broods; EPA requires 60 percent).

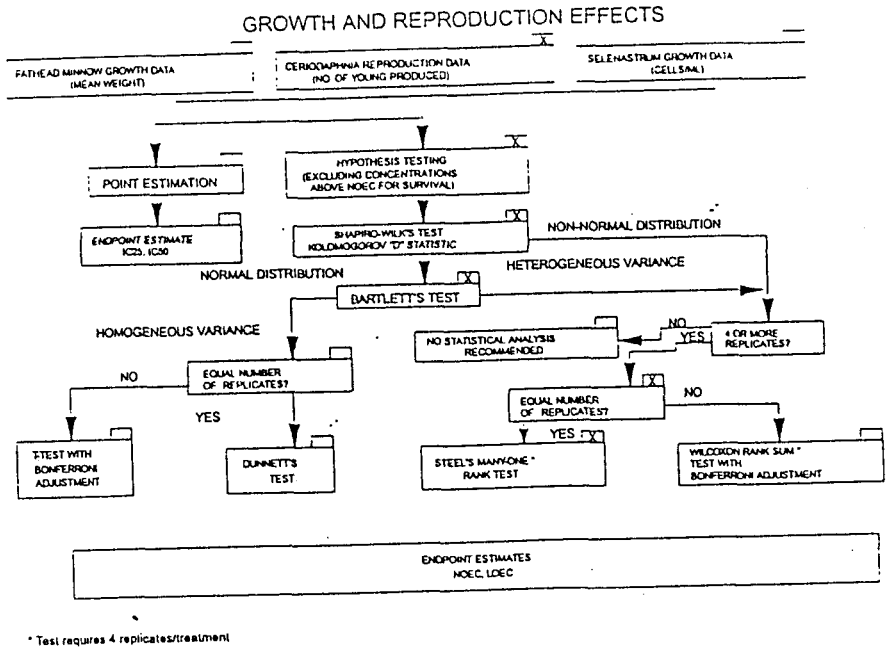
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 11, May 12-18, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Intake	100	100	100	100	100	100
Discharge-2.0%	100	90	90	90	90	90
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	80
TR	100	100	100	100	100	90

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 11, May 12-18, 1993.



6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 11, Watts Bar Nuclear Plant, May 12-18, 1993.

Reproduction (# young/female/6 days) Data

Treatment	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Intake	4	24	26	12	24	12	28	12	25	23	19.00	
Discharge-2.0%	3	10	8	0	6	17	8	9	8	3	7.20	*
Discharge-25.0%	23	21	9	25	27	18	6	9	12	22	17.20	
Discharge-50.0%	28	14	11	12	25	25	22	12	28	10	18.70	
Discharge-100%	22	25	31	26	25	29	20	11	27	27	24.30	
TR	22	24	27	23	27	28	29	12	18	12	22.20	
Medium†	11	15	16	17	13	18	19	24	25	10	16.80	

*Indicates values significantly less than control (Intake).
IC_{25} >100% DSN101
†Data shown for information only; not included in statistical analysis.

Steel's Many-one Rank Test

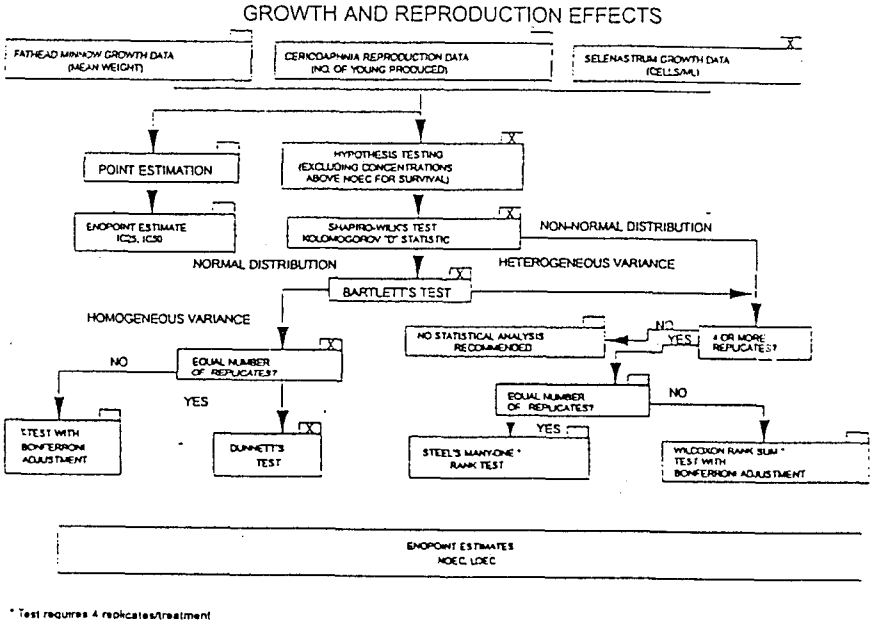
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	10	75	65.0 *
Discharge-25.0%	10	75	93.5
Discharge-50.0%	10	75	104.0
Discharge-100%	10	75	125.5
TR	10	75	116.0

*Values less than Critical Rank Sum are significantly less than the control (Intake).

6.3 Algal, Selenastrum capricornutum, Growth Test

6.3.1 Summary of Results: Diffuser discharge DSN101, collected May 13, 1993, demonstrated significant growth effects at 100, 50, and 25 percent concentrations compared to intake controls. No reduction in growth occurred in the 2 percent concentration. The IC_{25} for DSN101 was 4.42 percent.

6.3.2 Statistical Decision Process for Determining Toxicity Endpoints for 4-Day Exposure of the Alga, Selenastrum capricornutum, to Watts Bar Nuclear Plant DSN101 Discharge, Experiment 11, May 14-18, 1993.



6.3.3 Analysis of Growth Data Using Dunnett's Test for Algal 4-Day Growth Test, Watts Bar Nuclear Plant, Experiment 11, May 14-18, 1993.

No transformation was applied before data analysis. For this set of data, the minimum significant difference is 459.3 x 10^3.

This represents a 26.07% reduction in No. cells/mL x 10^3
T = 2.45 Alpha = 0.05 (one-tailed test)

Algal Growth Data (No. cell/mL x 10^3)

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Intake	1828	1748	2053	1419							1762.00	
Discharge-2.0%	1014	1108	1708	1575							1351.25	
Discharge-25.0%	1009	1071	1110	1083							1068.25	*
Discharge-50.0%	1046	1332	584	895							964.25	*
Discharge-100%	366	661	454	310							447.75	*
TR	1575	1101	721	1586							1245.75	*
Medium (w/EDTA)	1748	1976	1874	1945							1885.75	

*Indicates values significantly less than the control (Intake). EMS = 70302
IC_{25} = 4.42%

6.4 Physical/Chemical Parameters

6.4.1 Overall Test Temperature

6.4.1.1 Fathead Minnow: Mean = 24.8°C (24.4°-25.3°)

6.4.1.2 Ceriodaphnia: Mean = 24.7°C (24.2°-25.3°)

6.4.1.3 Algae: Mean = 25.3°C (24.7°-25.8°)

6.4.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 11, May 12-19, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, May 10-17, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 11, May 12-19, 1993

Appendix D Initial Water Chemistry Values for Algal Growth Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 11, May 14, 1993

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 11, showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids. A satisfactory control chart has not been established for the algae, but data is included for information purposes.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test:
April 26, 1993/0900 CDT to May 3, 1993/0945 CDT

6.5.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.006 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 43

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.011 mg Cu/L

6.5.3 <u>Ceriodaphnia</u>

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of Most Recent Test:
April 27, 1993/0845 CDT to May 3, 1993/0845 CDT

6.5.3.1.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC_{25}: 0.014 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 41

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.028 mg Cu/L

6.5.4 Selenastrum capricornutum

6.5.4.1 Chronic Tests

6.5.4.1.1 Date/Time of most recent test: May 3, 1993/0800 CDT to May 7, 1993/0730 CDT

6.5.4.1.2 LOEC: >0.003 mg Cu/L
NOEC: >0.003 mg Cu/L
IC_{25}: 0.0031 mg Cu/L

6.5.4.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 14

LOEC Range: 0.0004->0.003 mg Cu/L (four concentration increments)
NOEC Range: 0.0004->0.003 mg Cu/L (four concentration increments)
IC_{25} Range: >0.0003-0.0017 mg Cu/L

7.0 CONCLUSION

Tests conducted using samples collected from May 10-17, 1993, showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significantly reduced reproduction was demonstrated by daphnids in the 2 percent concentration of DSN101, but this effect appeared to be related to culture medium used for dilution which failed to meet control test acceptability criteria of minimum number of adults having three broods. Significant reduction in algal growth was demonstrated in 100, 50, and 25 percent concentrations DSN101 samples as compared to Intake. No reduction in growth resulted from exposure to 2 percent sample. Maximum allowable toxicity based on dilution available at WBN during this study was a NOEC $\geq$0.37 percent effluent ($\bar{x}$ value for study period; see Appendix A). These data indicate the EPA recommended chronic instream criterion was not exceeded.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLARC501-1592

Appendix A

Calculated Allowable* Toxicity Based Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, May 10-17, 1993.

	WB Hydro		WBN DSN101		Allowable Toxicity	
Date	Zero Release (Hours)	Hourly Average Flow (cfs)	Daily Flow† (cfs)	Dilution (WBN:WB Hydro)	TUc‡	NOEC (% Effluent)
May 10	10	35357	75.5	1:468	≤468	≥0.21
May 11	6	28367	75.5	1:376	≤376	≥0.27
May 12	0	14225	75.5	1:188	≤189	≥0.53
May 13	11	26838	75.5	1:356	≤356	≥0.28
May 14	12	25767	75.5	1:341	≤342	≥0.29
May 15	5	18458	75.5	1:244	≤245	≥0.41
May 16	7	11971	75.5	1:159	≤160	≥0.63
May 17	8	19881	65.6	1:303	≤304	≥0.33
Average				1:304	≤305	≥0.37

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream effluent concentration to which organisms can be exposed indefinitely without causing unacceptable effect.

†Estimated daily flows.

‡TUc = 100/NOEC.

PLARC501-1593(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, May 10-17, 1993

1993 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
May 10	Grab*	0.097	<0.10
May 11	Grab	0.085	-
May 12	Grab	0.062	-
May 12-13	Composite*	0.078	0.145
May 13	Grab	0.083	-
May 16	Grab	0.135	0.145
May 16-17	Composite	0.068	0.180
May 17	Grab	0.103	0.142

*Grab samples were at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1593(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and *Ceriodaphnia* Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 11, May 12-19, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	24.8 (24.5-25.3)	-	8.3 (8.2-8.3)	6.5 (5.1-7.3)	-	8.2 (8.2-8.3)	7.8 (7.6-8.0)	-	335 (333-339)	337 (333-341)	62 (59-64)	93.4 (90.6-97.5)	-
Dschg 2.0% w/F.Med.	24.7 (24.4-25.2)	-	8.3 (8.2-8.3)	6.6 (5.7-7.2)	-	8.2 (8.2-8.3)	7.8 (7.7-8.0)	-	332 (329-335)	335 (331-342)	-	-	-
Dschg 25.0% w/F.Med.	24.8 (24.4-25.2)	-	8.3 (8.3-8.3)	6.3 (5.0-7.2)	-	8.1 (8.1-8.2)	7.8 (7.7-7.9)	-	296 (293-300)	301 (297-305)	-	-	-
Dschg 50.0% w/F.Med.	24.9 (24.6-25.2)	-	8.3 (8.3-8.3)	6.2 (4.9-7.0)	-	8.1 (8.0-8.2)	7.8 (7.6-7.9)	-	258 (255-263)	276 (260-358)	-	-	-
Enriched Medium	-	24.7 (24.2-25.3)	8.3 (8.2-8.3)	-	7.7 (7.5-7.8)	8.2 (8.2-8.3)	-	8.1 (8.0-8.2)	321 (314-335)	-	63 (60-70)	91.8 (88.9-95.8)	-
Dschg 2.0% w/E.Med.	-	24.7 (24.2-25.3)	8.3 (8.2-8.3)	-	7.7 (7.7-7.9)	8.2 (8.2-8.2)	-	8.1 (8.1-8.2)	319 (313-332)	-	-	-	-
Dschg 25.0% w/E.Med.	-	24.7 (24.2-25.3)	8.3 (8.2-8.3)	-	7.7 (7.6-7.7)	8.1 (8.1-8.2)	-	8.1 (8.1-8.2)	286 (280-297)	-	-	-	-
Dschg 50.0% w/E.Med.	-	24.7 (24.2-25.3)	8.3 (8.2-8.3)	-	7.6 (7.6-7.7)	8.1 (8.0-8.1)	-	8.1 (8.1-8.2)	251 (246-261)	-	-	-	-
Discharge 100%	24.9 (24.5-25.2)	24.7 (24.2-25.3)	8.3 (8.3-8.3)	6.2 (5.6-6.9)	7.6 (7.5-7.7)	8.0 (7.9-8.1)	7.8 (7.7-7.9)	8.1 (8.0-8.2)	182 (177-188)	199 (179-280)	66 (64-68)	79.1 (77.0-80.4)	<0.1 (<0.1-<0.1)
Intake 100%	24.8 (24.5-25.3)	24.7 (24.2-25.3)	8.3 (8.3-8.3)	6.3 (5.4-7.0)	7.7 (7.6-7.8)	8.0 (7.9-8.1)	7.8 (7.8-7.9)	8.1 (8.1-8.2)	178 (173-182)	177 (158-189)	64 (63-66)	76.2 (71.8-80.4)	<0.1 (<0.1-<0.1)
TR	-	24.7 (24.2-25.3)	7.4 (7.2-7.6)	-	7.7 (7.6-7.8)	7.7 (7.6-7.8)	-	8.1 (8.0-8.2)	159 (152-165)	-	57 (55-61)	66.9 (63.3-71.8)	-

PLARC501-1594

*mg/L as $CaCO_3$

Appendix D

Initial Water Chemistry Values for Algal Growth Test,
Watts Bar Nuclear Plant Biomonitoring, Test 11, May 14-18, 1993

Treatment	Temp. (°C)	DO (mg/L)	pH (S.U.)	Cond. (umhos)	Hard. *	Alk. *	Total Residual Chlorine (mg/L)
Intake	24.9	8.8	7.8	264	88.9	72	-
Discharge-2.0%	24.8	9.0	8.0	246	-	-	-
Discharge-25.0%	24.8	8.9	8.0	251	-	-	-
Discharge-50.0%	25.1	8.7	8.0	257	-	-	-
Discharge-100%	25.0	8.6	8.0	268	90.6	74	<0.1
TR	24.8	9.0	8.0	246	78.7	65	<0.1
Medium	24.8	8.0	7.5	92	13.7	9	-

*mg/L as $CaCO_3$

PLARC501-1593(3)

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 12

Principle Investigator: Cynthia L. Russell

Starting Date: June 9, 1993

Ending Date: June 16, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. This report presents findings of the ninth monthly toxicity bioassessment (Experiment 12) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas) and daphnids (Ceriodaphnia dubia).

Tests conducted using diffuser discharge and intake samples collected from June 7-14 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significantly reduced reproduction was demonstrated by daphnids in the 2 percent concentration of DSN101. This effect was attributed to the culture medium used for dilution which failed to meet control test acceptability criteria for minimum number of young.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnows. Enriched culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River water (TR), was used for dilution for the daphnids; however, intake was used as control in the daphnid test due to failure of enriched medium to meet EPA's control acceptability criteria. [1] In addition, filtered TR was run as an additional control in the daphnid test to use in the event both dilution water and intake samples were unacceptable for control purposes.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning June 8, 1993 (Isco composite initiated June 7), and continuing through June 14, 1993. Diffuser and intake collections were made between the hours of 0815 and 0930 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were not continuous during the study (Appendix A). Dilution factors ranged from 880-1603.

WBN DSN101 diffuser discharge remained suspended for two additional hours after Watts Bar Hydro began operating on the morning of June 10, during the 11 minute injection of Copper-Trol (tolyltriazole) into WBN's cooling water systems. Copper-Trol was not detected in the WBN DSN101 discharge samples (Appendix B).

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

Note: On Days 1 and 2 of the test, the sample arrival temperatures for discharge were 4.5°C and 4.7°C, respectively. Temperatures for intake on Days 1 and 2 were 4.1°C. These temperatures deviated from sample storage protocol calling for sample temperatures of 4°C or less upon arrival. Samples were well iced and apparently had not had adequate time for complete cooling.

2.7 Sample Transport: Samples collected and shipped on 06/08, 06/09, 06/10, 06/11, and 06/14 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 06/12 and 06/13 were transported the same day via

private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100-µm nylon mesh filter into 2000-mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test and enriched medium for the daphnid test). Intake samples were run at 100 percent in both tests. In addition, Tennessee River (TR) water, collected from the Browns Ferry Nuclear Plant intake, was run as an additional control in the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced

as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: June 4, 1993

3.1.6 Hatch Date/Time: June 8, 1993/1010 CDT - June 9, 1993/0700 CDT

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.07 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Medium used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL medium are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

One temperature measured in the DSN101-25% on Day 1 was 26.1°C, which exceeds the EPA test range of 25°C ± 1°C. This slight deviation was a one time occurrence and had no bearing on test results.

4.1.2 Date/Time Test Initiated: June 9, 1993/0850 CDT

4.1.3 Date/Time Test Terminated: June 16, 1993/0800 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: June 9, 1993/1030 CDT

4.2.3 Date/Time Test Terminated: June 15, 1993/0945 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Dilution water was enriched culture medium (see Section 2.2) but failed to meet EPA criteria as a control. Intake was used as the control for statistical purposes.

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)
Daphnids--7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched medium (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and Ceriodaphnia Reproduction - Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

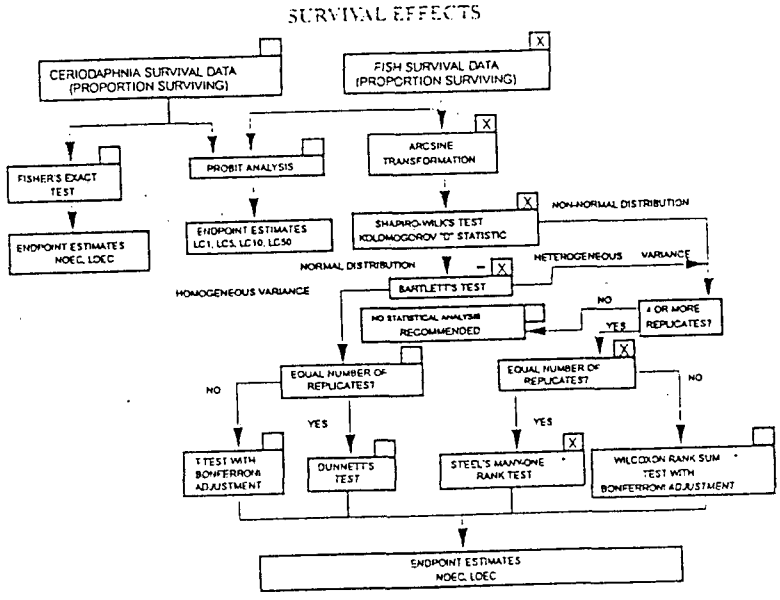
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from June 7-14, 1993.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 12, June 9-16, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	100	100	98	98

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 12, June 9-16, 1993.



* Test requires 4 replicates/treatment
** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 12, June 9-16, 1993.

	Survival (%) Data											
	Replicate											
Group	1	2	3	4	5	6	7	8	9	10	Mean	S *
Medium	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	100	100	100							100	
Discharge-100%	100	100	100	100							100	
Intake	90	100	100	100							98	

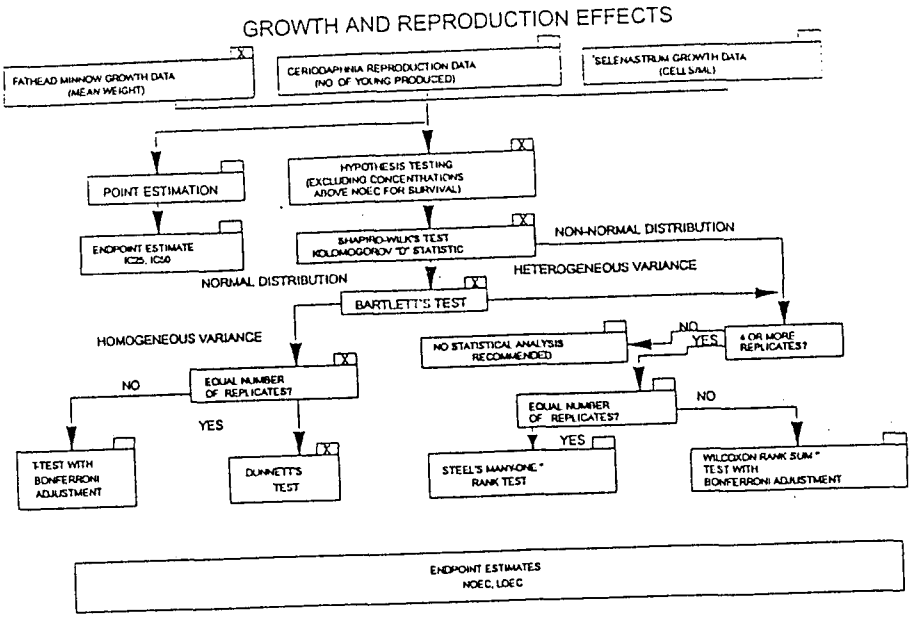
*Indicates values significantly less than control (Medium).
IC_{25} >100% DSN101.

Steel's Many-one Rank Test			
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	18
Discharge-100%	4	10	18
Intake	4	10	16

*Values less than Critical Rank Sum are significantly less than the control (Medium).

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 12, June 9-16, 1993.



6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 12, Watts Bar Nuclear Plant, June 9-16, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.10.

This represents a 16.97% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean S*
Medium	0.65	0.51	0.52	0.67							0.59
Discharge-2.0%	0.64	0.59	0.53	0.58							0.58
Discharge-25.0%	0.58	0.61	0.64	0.62							0.61
Discharge-50.0%	0.57	0.45	0.64	0.63							0.57
Discharge-100%	0.60	0.50	0.54	0.60							0.56
Intake	0.51	0.50	0.56	0.54							0.53

*Indicates values significantly less than control (Medium).
EMS = 0.003. IC_{25} = >100% DSN101.

6.2 *Ceriodaphnia* Survival and Reproduction Test

6.2.1 Summary of Results: No toxicity was demonstrated in diffuser discharge or intake samples collected from June 7-13, 1993. Significant reduction in reproduction occurred in the 2 percent concentration of DSN101. This effect was attributed to the culture medium used for sample dilution which failed to meet minimum test acceptability ($\bar{x}$ young in medium = 4.0; EPA required = 15.0). Intake was used as the control for statistical analyses.

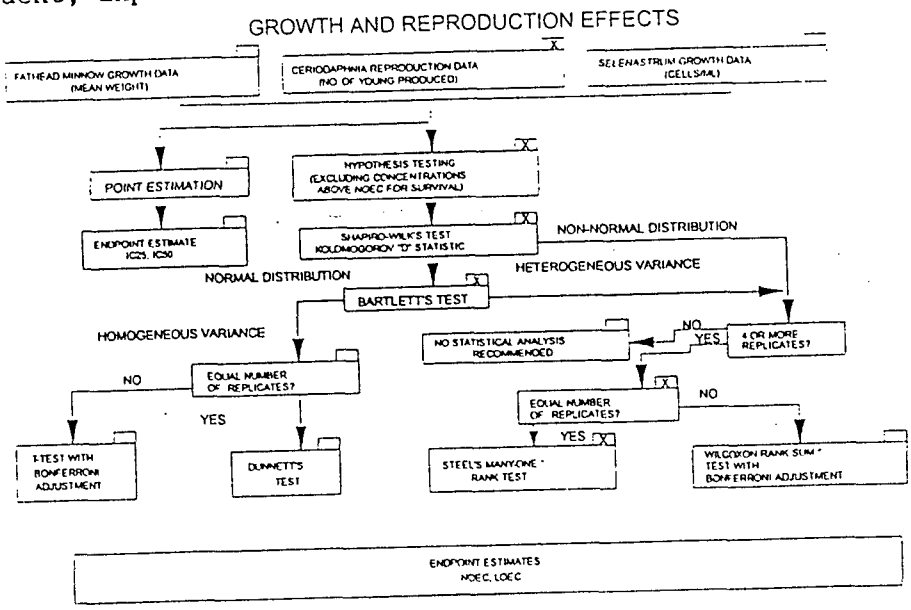
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for *Ceriodaphnia* Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 12, June 9-15, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Intake	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	90	80
TR	100	100	100	100	100	100
Medium	100	100	100	90	90	90

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of *Ceriodaphnia* to Watts Bar Nuclear Plant Effluent, Experiment 12, June 9-15, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 12, Watts Bar Nuclear Plant, June 9-15, 1993.

	Reproduction (# young/female/6 days) Data											
	Replicate											
Treatment	1	2	3	4	5	6	7	8	9	10	Mean	S*
Intake	25	11	18	28	19	24	13	22	22	23	20.5	
Discharge-2.0%	10	2	10	6	8	0	6	12	6	6	6.6	*
Discharge-25.0%	9	25	19	22	26	21	21	21	23	24	21.1	
Discharge-50.0%	22	24	20	24	23	24	26	27	21	24	23.5	
Discharge-100%	21	11	25	22	20	22	13	26	22	23	20.5	
TR	14	30	27	22	26	23	23	15	24	25	22.9	
Medium†	10	0	4	8	3	0	4	1	2	8	4.0	

*Indicates values significantly less than control (Intake).
†Data shown for information only; not included in statistical analysis.

	Steel's Many-one Rank Test			
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum	S*
Discharge-2.0%	10	75	56.0	*
Discharge-25.0%	10	75	107.0	
Discharge-50.0%	10	75	122.5	
Discharge-100%	10	75	104.0	
TR	10	75	121.0	

*Values less than Critical Rank Sum are significantly less than the control (Intake). IC_{25} >100%.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: Mean = 25.2°C (24.4°-26.1°)

6.3.1.2 Ceriodaphnia: Mean = 24.9°C (24.1°-25.5°)

6.3.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 12, June 9-16, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc, Total Phosphorus, and Clamtrol, June 7-14, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 12, June 9-16, 1993

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 12 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.4.2 Fathead Minnows

6.4.2.1 Chronic Tests

6.4.2.1.1 Date/Time of Most Recent Test:
June 1, 1993/0845 CDT to June 8, 1993/0840 CDT

6.4.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.007 mg Cu/L

6.4.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 44

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.011 mg Cu/L

6.4.3 <u>Ceriodaphnia</u>

6.4.3.1 Chronic Tests

6.4.3.1.1 Date/Time of Most Recent Test:
June 1, 1993/0945 CDT to June 8, 1993/0940 CDT

6.4.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.002 mg Cu/L
IC_{25}: 0.030 mg Cu/L

6.4.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 42

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.030 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from June 7-14 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significantly reduced reproduction was demonstrated in the 2 percent concentration of DSN101 by daphnids. This effect was attributed to the culture medium used for dilution which failed to meet control test acceptability criteria of minimum number of young.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLARC501-1655

Appendix A

Calculated Allowable Toxicity Based Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, June 7-14, 1993.

	WB Hydro		WBN DSN101	
Date	Zero Release (Hours)	Hourly Average Flow (cfs)	Daily Flow* (cfs)	Dilution (WBN:WB Hydro)
June 7	9	33393	22.9	1:1458
June 8	4	29705	22.9	1:1297
June 9	8	36719	22.9	1:1603
June 10	8	33494	22.9	1:1463
June 11	9	31107	22.9	1:1358
June 12	9	33927	22.6	1:1501
June 13	11	20154	22.9	1:880
June 14	10	32236	22.9	1:1408
Average				1:1371

*Estimated daily flows.

PLARC501-1656(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, June 7-14, 1993

1993 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)	TOLYTRIAZOLE (mg/L)
June 8	Grab*	0.047	<0.10	-
June 9	Grab	0.067	0.15	-
June 8-9	Composite*	0.062	<0.10	-
June 10	Grab	0.114	-	<0.5
June 10-11	Composite	0.096	<0.10	<0.5
June 11	Grab	0.084	-	-
June 12	Grab	0.100	-	-
June 14	Grab	0.148	0.18	-

*Grab samples were at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1656(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and *Ceriodaphnia* Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 12, June 9-16, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	25.2 (24.4-25.7)	-	8.3 (8.3-8.3)	5.9 (5.0-6.4)	-	8.1 (8.0-8.2)	7.7 (7.6-7.7)	-	342 (337-346)	345 (339-351)	63 (62-65)	96.2 (94.1-99.2)	-
Dschg 2.0% w/F.Med.	25.3 (24.4-26.0)	-	8.3 (8.2-8.4)	5.5 (4.2-6.2)	-	8.1 (8.0-8.2)	7.6 (7.5-7.7)	-	338 (332-342)	342 (335-348)	-	-	-
Dschg 25.0% w/F.Med.	25.3 (24.7-26.1)	-	8.3 (8.3-8.3)	5.5 (4.7-6.1)	-	8.0 (7.9-8.0)	7.6 (7.6-7.7)	-	300 (296-304)	307 (301-314)	-	-	-
Dschg 50.0% w/F.Med.	25.3 (24.8-25.7)	-	8.3 (8.2-8.3)	5.4 (4.3-6.0)	-	7.9 (7.8-7.9)	7.6 (7.5-7.7)	-	260 (257-264)	265 (262-270)	-	-	-
Enriched Medium	-	24.9 (24.1-25.5)	8.3 (8.2-8.3)	-	7.8 (7.7-7.9)	8.1 (8.1-8.1)	-	8.1 (8.0-8.1)	323 (319-330)	-	63 (63-63)	93.2 (92.3-94.1)	-
Dschg 2.0% w/E.Med.	-	24.9 (24.1-25.5)	8.2 (8.0-8.3)	-	7.7 (7.6-7.8)	8.1 (8.1-8.1)	-	8.1 (8.0-8.1)	317 (313-325)	-	-	-	-
Dschg 25.0% w/E.Med.	-	24.9 (24.1-25.5)	8.3 (8.2-8.3)	-	7.8 (7.7-7.9)	8.0 (7.9-8.0)	-	8.1 (8.0-8.2)	286 (283-291)	-	-	-	-
Dschg 50.0% w/E.Med.	-	24.9 (24.1-25.5)	8.3 (8.2-8.3)	-	7.7 (7.6-7.8)	7.9 (7.8-8.0)	-	8.1 (8.0-8.2)	250 (244-255)	-	-	-	-
Discharge 100%	25.2 (24.6-25.7)	24.9 (24.1-25.5)	8.3 (8.3-8.3)	5.6 (4.9-6.2)	7.7 (7.6-7.8)	7.8 (7.6-7.9)	7.6 (7.6-7.7)	8.1 (8.0-8.2)	180 (179-180)	185 (183-186)	66 (65-68)	75.2 (73.5-77.0)	<0.1 (<0.1-<0.1)
Intake 100%	25.2 (24.7-25.6)	24.9 (24.1-25.5)	8.1 (6.9-8.4)	5.5 (4.8-6.3)	7.7 (7.6-7.8)	7.5 (7.4-7.7)	7.5 (7.1-7.7)	8.1 (8.0-8.2)	175 (173-178)	178 (176-182)	65 (63-66)	72.8 (71.8-73.5)	<0.1 (<0.1-<0.1)
TR	-	24.9 (24.1-25.5)	6.9 (6.6-7.1)	-	7.7 (7.6-7.8)	7.6 (7.6-7.7)	-	8.0 (8.0-8.1)	169 (165-174)	-	57 (54-60)	68.1 (65.0-70.1)	-

PLARC501-1657

*mg/L as $CaCO_3$

STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 13

Principle Investigator: Cynthia L. Russell

Starting Date: July 15, 1993

Ending Date: July 22, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. This report presents findings of the tenth monthly toxicity bioassessment (Experiment 13) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas) and daphnids (Ceriodaphnia dubia).

Tests conducted using diffuser discharge and intake samples collected from July 14-20 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture medium (moderately hard reconstituted water) was used for dilution water and control for the fathead minnows. Enriched culture medium, moderately hard reconstituted water containing ten percent filtered Tennessee River (TR) water, was used for dilution for the daphnids.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning July 14, 1993 (Isco composite initiated July 13), and continuing through July 20, 1993. Diffuser and intake collections were made between the hours of 0900 and 1130 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above with the exception of the discharge sample collected on July 18. Electricity to the sampler was off at the time of sample collection. The partial composite sample was combined with a grab sample for use in toxicity testing.

2.5 Pertinent Site Conditions: Watts Bar Hydro and WBN DSN101 operations were not continuous during the study (Appendix A). Dilution factors ranged from 585 to 712.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

2.7 Sample Transport: Samples collected and shipped on 07/14, 07/15, 07/16, 07/19, and 07/20 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 07/17 and 07/18 were transported the same day via private courier (airplane) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Aquatic Research Laboratory (ARL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100-μm nylon mesh filter into 2000-mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was run at 100, 50, 25, and 2 percent concentrations (diluted with culture medium for the fathead minnow test and enriched medium for the daphnid test). Intake samples were run at 100 percent in both tests. In addition, Tennessee River water, collected from the ARL experimental channels, was run at 100 percent only for the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: ARL inhouse culture

3.1.2 Culture Water: Culture medium consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture medium was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: July 10, 1993

3.1.6 Hatch Date/Time: July 14, 1993/1050 CDT - July 15, 1993/0640 CDT

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.05 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Aquatic Research Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation.

Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Food used for Ceriodaphnia cultures and during this study was obtained from a commercial source (Aquastar) and consisted of YCT and Selenastrum prepared according to EPA requirements. Individual animals contained in cups with 15 mL medium were fed 0.1 mL food and algal at renewal.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: July 15, 1993/0935 CDT

4.1.3 Date/Time Test Terminated: July 22, 1993/0850 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.3.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. No statistical test was used for fathead minnow survival endpoint determination since survival was 100 percent for all treatments. Decision processes followed for testing sublethal (growth) effects are shown in Section 6.1.3.1. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched culture medium (see Section 3.2.2)

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: July 15, 1993/1000 CDT

4.2.3 Date/Time Test Terminated: July 21, 1993/1000 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Enriched culture medium (see Section 2.2) was used for dilution and control water.

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.1 mL algae (30-35 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.3.

4.2.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. The decision process followed for testing sublethal (reproduction) effects is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1]

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All ARL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to ARL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure DS-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures DS-43.6 and DS-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures DS-43.7 and DS-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures DS-43.3 and DS-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure DS-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure DS-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish--7-day chronic (NOEC)
Daphnids--7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched medium (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and Ceriodaphnia Reproduction - Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from July 14-20, 1993.

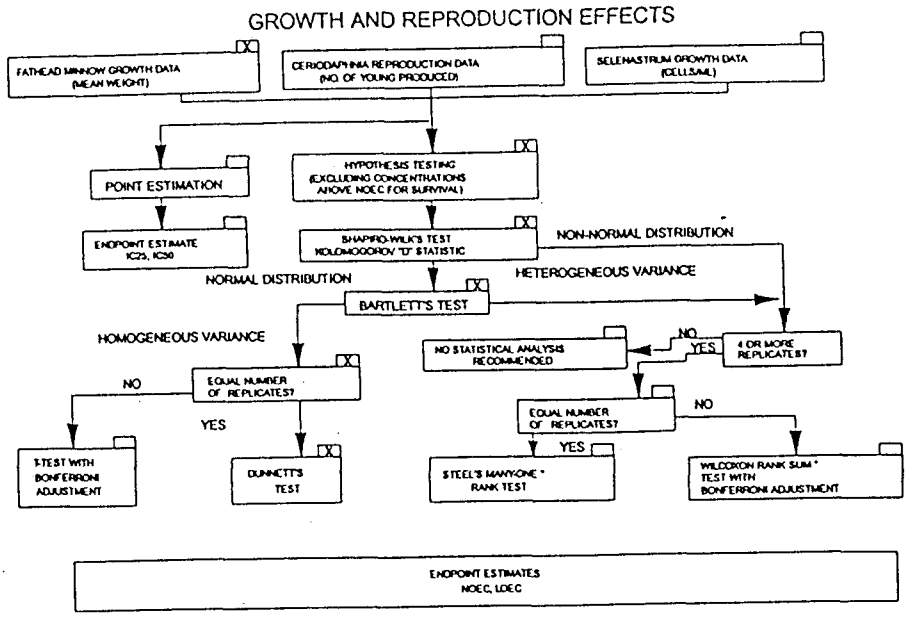
6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 13, July 15-22, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Medium	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	100	100	100	100

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 13, July 15-22, 1993.



6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 13, Watts Bar Nuclear Plant, July 15-22, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.06.

This represents a 11.89% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

Dry Weight (mg) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean S*
Medium	0.54	0.49	0.53	0.48							0.51
Discharge-2.0%	0.49	0.57	0.51	0.55							0.53
Discharge-25.0%	0.49	0.51	0.54	0.60							0.54
Discharge-50.0%	0.59	0.51	0.50	0.52							0.53
Discharge-100%	0.49	0.50	0.53	0.52							0.51
Intake	0.50	0.50	0.52	0.57							0.52

*Indicates values significantly less than control (Medium).
EMS = 0.001. IC_{25} = >100% DSN101.

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No toxicity was demonstrated in diffuser discharge or intake samples collected from July 14-19, 1993.

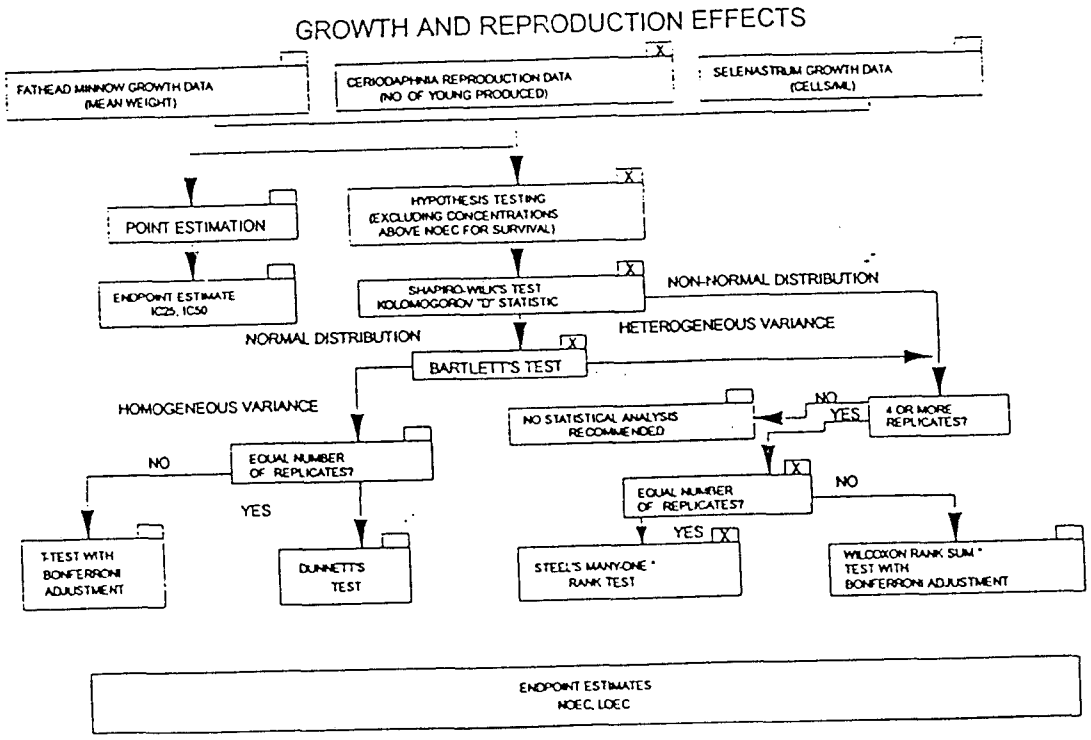
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 13, July 15-21, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Medium	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100
Intake	100	100	100	100	100	100
TR	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 13, July 15-21, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 13, Watts Bar Nuclear Plant, July 15-21, 1993.

Reproduction (# young/female/6 days) Data												
	Replicate											
Treatment	1	2	3	4	5	6	7	8	9	10	Mean	S*
Medium	22	17	17	20	25	19	12	18	25	12	18.7	
Discharge-2.0%	24	23	15	14	22	18	20	8	25	9	17.8	
Discharge-25.0%	19	7	19	19	20	21	19	23	18	21	18.6	
Discharge-50.0%	15	22	16	8	19	23	19	18	16	22	17.8	
Discharge-100%	20	10	19	23	19	22	22	11	17	28	19.1	
Intake	18	7	20	16	20	24	15	5	19	20	16.4	
TR	2	3	21	19	21	22	21	22	22	21	17.4	

*Indicates values significantly less than control (Medium).

Steel's Many-one Rank Test				
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum	S*
Discharge-2.0%	10	74	102.5	
Discharge-25.0%	10	74	110.0	
Discharge-50.0%	10	74	99.5	
Discharge-100%	10	74	108.5	
Intake	10	74	96.5	
TR	10	74	111.0	

*Values less than Critical Rank Sum are significantly less than the control (Medium). IC_{25} >100%.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: Mean = 25.1°C (24.4°-25.9°)

6.3.1.2 Ceriodaphnia: Mean = 25.3°C (24.8°-25.6°)

6.3.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 13, July 15-22, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, July 13-20, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 13, July 15-22, 1993

6.4 Reference Toxicant Tests

6.4.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 13 showed chronic results consistent with ARL control chart ranges for fathead minnows and daphnids.

6.4.2 Fathead Minnows

6.4.2.1 Chronic Tests

6.4.2.1.1 Date/Time of Most Recent Test:
July 6, 1993/1055 CDT to July 13, 1993/1000 CDT

6.4.2.1.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC_{25}: 0.012 mg Cu/L

6.4.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 45

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.012 mg Cu/L

6.4.3 Ceriodaphnia

6.4.3.1 Chronic Tests

6.4.3.1.1 Date/Time of Most Recent Test:
July 12, 1993/0935 CDT to July 18, 1993/1030 CDT

6.4.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.02 mg Cu/L
IC_{25}: 0.025 mg Cu/L

6.4.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 43

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.011-0.030 mg Cu/L

7.0 CONCLUSION

Tests conducted using diffuser discharge and intake samples collected from July 14-20 showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLARC501-1714

Appendix A

Dilution Factors for Watts Bar Nuclear Plant (WBN) DSN101 and Watts Bar (WB) Hydro Plant Releases, July 13-20, 1993.

Date	WB Hydro		WBN DSN101	Dilution
	Zero Release	Hourly Average Flow	Daily Flow*	
	(Hours)	(cfs)	(cfs)	(WBN:WB Hydro)
July 13	13	30436	49.2	1:619
July 14	14	34960	49.2	1:711
July 15	12	29058	49.2	1:591
July 16	13	31727	49.2	1:645
July 17	10	28779	49.2	1:585
July 18	11	30954	49.2	1:630
July 19	10	34014	49.2	1:692
July 20	8	34325	48.2	1:712
Average				1:648

*Estimated daily flows. Actual flows become considerably less as hours of zero release increase.

PLARC501-1715(1)

Appendix B

Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, July 13-20, 1993

1993 Month Date	SAMPLE TYPE*	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)
July 13	Grab	0.051	-
July 14	Grab	0.062	<0.10
July 14-15	Composite	0.052	0.18
July 15	Grab	0.134	-
July 16	Grab	0.042	-
July 19	Grab	0.039	0.19
July 18-19	Composite	0.088	0.17
July 20	Grab	0.039	0.24

*Grab samples were at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of composite samples used for the toxicity tests.

PLARC501-1715(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and *Ceriodaphnia* Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 13, July 15-22, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	25.1 (24.4-25.8)	-	8.3 (8.1-8.4)	5.8 (4.8-6.6)	-	8.2 (8.1-8.2)	7.7 (7.5-7.8)	-	341 (338-351)	345 (341-352)	61 (58-65)	93.2 (92.3-94.1)	-
Dschg 2.0% w/F.Med.	25.2 (24.6-25.8)	-	8.2 (8.1-8.3)	5.8 (5.0-6.4)	-	8.1 (7.8-8.2)	7.6 (7.5-7.7)	-	337 (326-348)	345 (339-350)	-	-	-
Dschg 25.0% w/F.Med.	25.1 (24.4-25.7)	-	8.3 (8.1-8.4)	5.6 (4.2-6.6)	-	8.0 (7.9-8.1)	7.7 (7.5-7.8)	-	303 (296-311)	310 (302-314)	-	-	-
Dschg 50.0% w/F.Med.	25.2 (24.4-25.9)	-	8.3 (8.2-8.3)	5.8 (4.7-6.4)	-	7.9 (7.8-7.9)	7.7 (7.6-7.8)	-	267 (261-273)	273 (268-278)	-	-	-
Enriched Medium	-	25.3 (24.8-25.6)	8.3 (8.2-8.4)	-	7.6 (7.4-7.6)	8.1 (8.1-8.2)	-	8.0 (7.9-8.0)	328 (326-332)	-	64 (62-65)	90.0 (88.9-90.6)	-
Dschg 2.0% w/E.Med.	-	25.3 (24.8-25.6)	8.3 (8.2-8.3)	-	7.6 (7.4-7.7)	8.1 (8.0-8.2)	-	8.0 (8.0-8.1)	325 (322-328)	-	-	-	-
Dschg 25.0% w/E.Med.	-	25.3 (24.8-25.6)	8.3 (8.3-8.3)	-	7.6 (7.4-7.7)	8.0 (7.9-8.0)	-	8.1 (8.0-8.1)	295 (293-297)	-	-	-	-
Dschg 50.0% w/E.Med.	-	25.3 (24.8-25.6)	8.3 (8.3-8.3)	-	7.5 (7.4-7.7)	7.9 (7.8-7.9)	-	8.0 (7.9-8.1)	262 (260-265)	-	-	-	-
Discharge 100%	25.1 (24.4-25.6)	25.3 (24.8-25.6)	8.3 (8.1-8.4)	5.7 (4.8-6.3)	7.6 (7.4-7.8)	7.7 (7.5-7.8)	7.7 (7.6-7.8)	8.0 (7.9-8.1)	196 (194-197)	201 (198-205)	70 (69-72)	80.4 (78.7-82.1)	<0.1 (<0.1-<0.1)
Intake 100%	25.1 (24.4-25.6)	25.3 (24.8-25.6)	7.5 (7.2-7.8)	5.7 (4.6-6.2)	7.5 (7.3-7.6)	7.5 (7.4-7.6)	7.7 (7.6-7.7)	8.0 (7.9-8.1)	193 (190-197)	196 (194-199)	68 (67-69)	79.4 (77.0-82.1)	<0.1 (<0.1-<0.1)
TR	-	25.3 (24.8-25.6)	6.9 (6.6-7.4)	-	7.5 (7.3-7.6)	7.6 (7.1-7.9)	-	7.9 (7.8-8.0)	159 (156-162)	-	49 (48-50)	60.5 (58.1-63.3)	-

PLARC501-1716

*mg/L as $CaCO_3$

TENNESSEE VALLEY AUTHORITY
TOXICITY TESTING LABORATORY
STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS), CERIODAPHNIA DUBIA (DAPHNIDS), SELENASTRUM CAPRICORNUTUM, (ALGAL)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 14

Principle Investigator: Cynthia L. Russell

Starting Date: August 19, 1993

Ending Date: August 26, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. Compliance monitoring for this discharge was initiated in October 1992. This report presents findings of the eleventh monthly toxicity bioassessment (Experiment 14) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and/or subchronic responses of three trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas), daphnids (Ceriodaphnia dubia), and a unicellular alga (Selenastrum capricornutum).

Tests conducted using samples collected from August 18-24, 1993, showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significant reduction in algal growth was demonstrated in 2.0. 25.0, 50.0, and 100 percent concentrations of DSN101 samples as compared to Intake. The NOEC based on IC_{25} calculation was 1.10 percent. Maximum allowable toxicity based on dilution available at WBN during this study was an NOEC $\geq$0.20 percent effluent ($\bar{x}$ value for study period; see Appendix A). Therefore, these data indicate the EPA recommended chronic instream criterion was not exceeded.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture water (moderately hard reconstituted water) was used for dilution water and control for the fathead minnow test. Enriched culture water, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution for the daphnid test. Tennessee River water (TR) from TTL (TRM 293) was used for dilution water for the algal test. Although TR met EPA's control acceptability criteria in the algal test, Intake was used as the control because of higher algal growth (2316 cells/mL x 10^3 vs. 1596 cells/mL x 10^3). In addition to the controls listed above, filtered TR was run with the daphnid test and algal culture water containing EDTA was run with the algal test as extra laboratory controls to use in the event both the dilution water and intake samples were unacceptable for control purposes.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning August 18, 1993 (Isco composite initiated August 17), and continuing through August 24, 1993. Diffuser and intake collections were made between the hours of 0900 and 1230 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above with the exception of the DSN101 sample collected August 24 and used in testing August 25. This sample was a mixture of a composite and a grab sample due to interruption of the discharge flow during the sample collection period. A grab sample was collected after the discharge was reopened to complete the volume requirement.

2.5 Pertinent Site Conditions:

2.5.1 Watts Bar Hydro and WBN DSN101 operations were not continuous during the study (Appendix A). Dilution factors ranged from 424 to 667.

2.5.2 WBN DSN101 diffuser discharge remained suspended for three additional hours after Watts Bar Hydro began operating on the morning of August 18, during the 10 minute injection of

CopperTrol (tolyltriazole) into the WBN cooling water systems. CopperTrol was not detected in the WBN DSN101 discharge samples (Appendix B).

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

Note: On Day 2 of the test, the intake sample arrival temperature was 5.1°C. This temperature deviated from sample storage protocol calling for sample temperatures of 4°C or less upon arrival. The sample was well iced and apparently had not had adequate time for complete cooling.

2.7 Sample Transport: Samples collected and shipped on 8/18, 08/19, 08/20, 08/23, and 08/24 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 08/21 and 08/22 were transported the same day via private courier (airplane or automobile) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Toxicity Testing Laboratory (TTL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to near 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser discharge was tested at 2.0, 25.0 50.0, and 100 percent concentrations (diluted with culture water for the fathead minnow test, enriched water for the daphnid test, and TR for the algal test). Intake samples were tested at 100 percent only in all tests, and Tennessee River water, collected from the TTL experimental channels, was run as an additional control in the daphnid test. Samples collected August 19 were used in the algal test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.1.2 Culture Water: Culture water consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser

to bring dissolved gases to saturation. Culture water was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: August 14-15, 1993

3.1.6 Hatch Date/Time: August 18, 1993/1105-August 19, 1993/0700.

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.08 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up

to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation. Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Water used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL water are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

3.3 Species: Selenastrum capricornutum, alga

3.3.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory, obtained from EPA, Newtown, Ohio, March 10, 1993.

3.3.2 Culture water: Culture water consists of reconstituted water following EPA methods for the Algal Growth Test. [1] Culture water pH is adjusted to 7.5 (7.4-7.6, acceptable range) when necessary using 0.1 N NaOH or 0.1 N HCl.

3.3.3 Temperature of Culture: 25°C ± 1°C

3.3.4 General Maintenance: Cultures are maintained in 250-500 mL flasks containing 100-250 mL algal culture water in light and temperature controlled incubators. New cultures are started each week by inoculating flasks with a single colony from incubated agar plates containing macro- and micronutrients or by transferring 1 mL of inoculant from a culture flask into fresh autoclaved and filtered culture water using aseptic techniques (see Section 3.3.2). Agar plates are prepared as needed. One half of the plates are stored at 4°C for contingency use, the remainder are incubated at 20°C ± 2°C. Water used in algae sterile culture is vacuum-filtered through 0.45 µm membrane filters.

4.0 TEST METHODS

4.1 Fathead Minnow (*Pimephales promelas*) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: August 19, 1993/0900 CDT

4.1.3 Date/Time Test Terminated: August 26, 1993/0845 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (*Artemia*) nauplii <24-h old 3 times daily *ad libitum*.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.2.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal

distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched water (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: August 19, 1993/0930 CDT

4.2.3 Date/Time Test Terminated: August 25, 1993/0920 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Enriched culture water (see Section 2.2) was used for dilution water and control.

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.2.

4.2.12 Statistics:

Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine sublethal (reproduction) toxicity endpoints. The decision process followed is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Dunnett's Test. [1]

4.3 Algal, Selenastrum capricornutum, Growth Test, EPA Test Method 1003.0. [1]

4.3.1 Modification/Deviations to Method 1003.0: Mean initial cell density of three samples checked was 10,245 cells/mL, determined by Sedgwick-Rafter cell counts. The EPA methods call for an initial cell density of 10,000 cells/mL.

4.3.2 Date/Time Test Initiated: August 20, 1993/0845 CDT

4.3.3 Date/Time Test Terminated: August 24, 1993/0750 CDT

4.3.4 Test Chamber: 250-mL borosilicate Erlenmeyer flasks
Volume per Chamber: 100 mL

4.3.5 Cell Enumeration Method: Coulter Counter Model ZM with Sedgwick-Rafter Cell counts used for QA verification.

4.3.6 Initial Cell Density: 10,245 cells/mL (Cell density based on Sedgwick-Rafter counts).

4.3.7 Number of Replicates per Treatment: 4 (In addition an uninoculated sample was prepared for each treatment to serve as a blank for final cell counts.)

4.3.8 Dilution Water: Tennessee River water (TRM 293.0; see Section 2.2.)

4.3.9 Test Control: Intake

4.3.10 Test Temperature: 25°C ± 1°C

4.3.11 Photoperiod: Continuous illumination

4.3.12 Renewal Period: None. This test method is a 96-h static bioassay. Flasks were randomly placed into the incubator at test initiation, removed and hand swirled daily, and randomly replaced into the incubator (New Brunswick Scientific Model G25). The incubator provided constant shaking at a rate of 100 rpm.

4.3.13 Physical and Chemical Parameters Measured: Parameters measured for all test samples and algal water at test initiation were temperature, DO, pH, conductivity, alkalinity, hardness, and total residual chlorine. Results are reported in Section 6.4.2. Temperatures were measured two or three times daily in extra flasks placed randomly in the incubator. Since some variation was seen at different locations in the incubator, test flasks were randomly rearranged in the incubator daily when flask contents were swirled.

4.3.14 Statistics: Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine toxicity endpoints. [1] Decision processes followed for testing algal growth response are shown in Section 6.3.2. Based on tests for normally distributed data and homogenous variances, the statistical test used for growth endpoint determination was the Dunnett's Test.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures ES-43.7 and ES-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures ES-43.3 and ES-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure ES-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish - 7-day chronic (NOEC)
Daphnids - 7-day chronic (NOEC)
Algal - 4-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish, daphnids, algal
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic, enriched water (see Section 3.2.2) for daphnid chronic, and algal water without EDTA for algal chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth, Ceriodaphnia Reproduction, and Algal Growth - NOEC Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

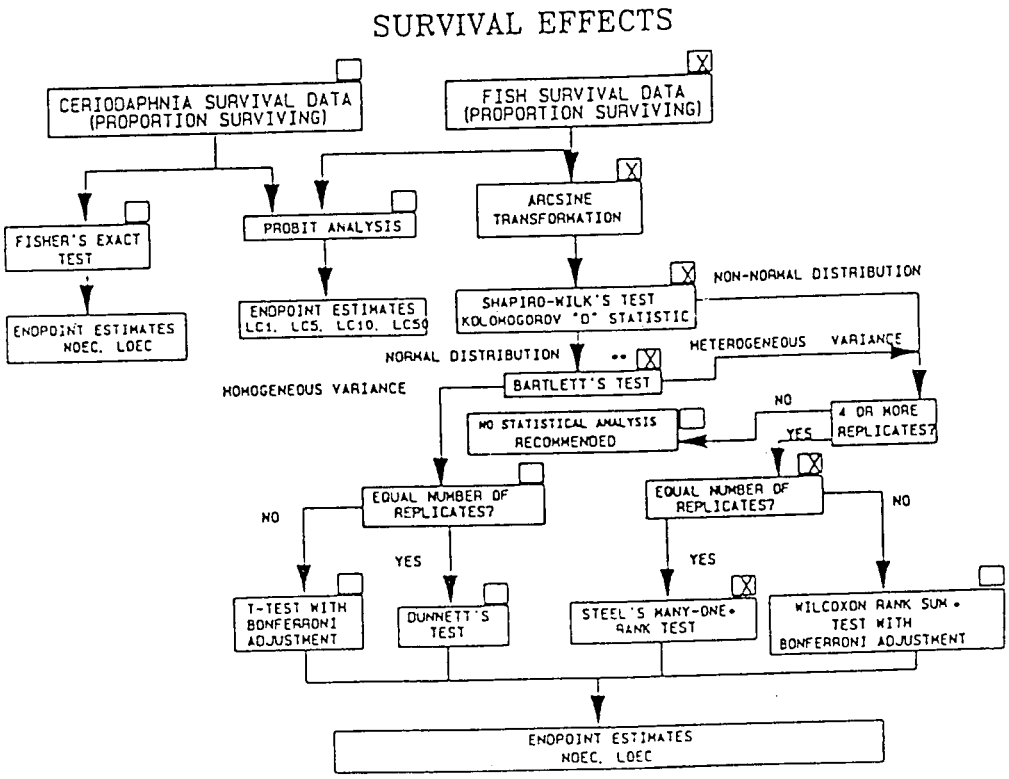
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for diffuser discharge or intake samples collected from August 18-24, 1993.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 14, August 19-26, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	98	98	98
Discharge-100%	100	100	100	100	100	100	100
Intake	100	100	100	100	100	100	100

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 14, August 19-26, 1993.



* Test requires 4 replicates/treatment

** Levene's Test used due to variance equal to zero for one or more treatments.

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 14, August 19-26, 1993.

Survival (%) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S *
Control	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	90	100	100							98	
Discharge-100%	100	100	100	100							100	
Intake	100	100	100	100							100	

*Indicates values significantly less than control.

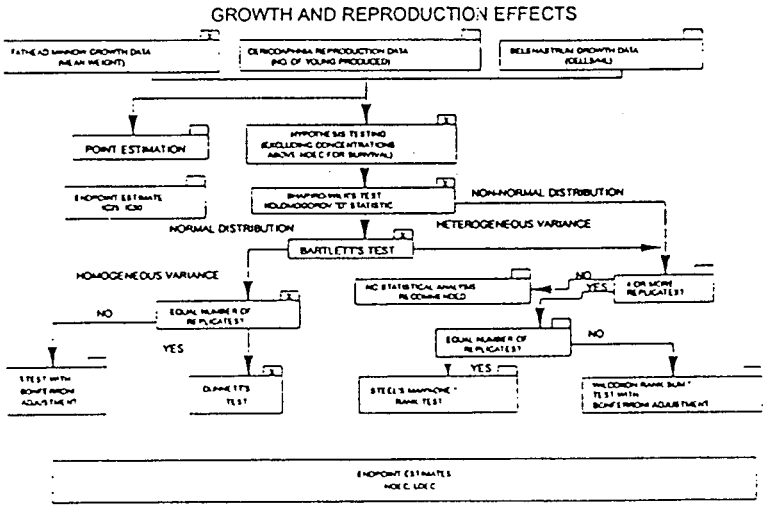
Steel's Many-one Rank Test

Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	16
Discharge-100%	4	10	18
Intake	4	10	18

*Values less than Critical Rank Sum are significantly less than the control.

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 14, August 19-26, 1993.



6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 14, Watts Bar Nuclear Plant, August 19-26, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.08.

This represents a 13.26% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

	Dry Weight (mg) Data										
	Replicate										
Group	1	2	3	4	5	6	7	8	9	10	Mean S*
Control	0.55	0.65	0.58	0.61							0.60
Discharge-2.0%	0.68	0.59	0.61	0.58							0.61
Discharge-25.0%	0.62	0.52	0.67	0.68							0.62
Discharge-50.0%	0.64	0.69	0.66	0.58							0.64
Discharge-100%	0.58	0.50	0.51	0.54							0.53
Intake	0.55	0.58	0.58	0.59							0.57

*Indicates values significantly less than control. EMS = 0.002.
IC_{25} = >100% DSN101.

6.2 Ceriodaphnia Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from August 18-23, 1993.

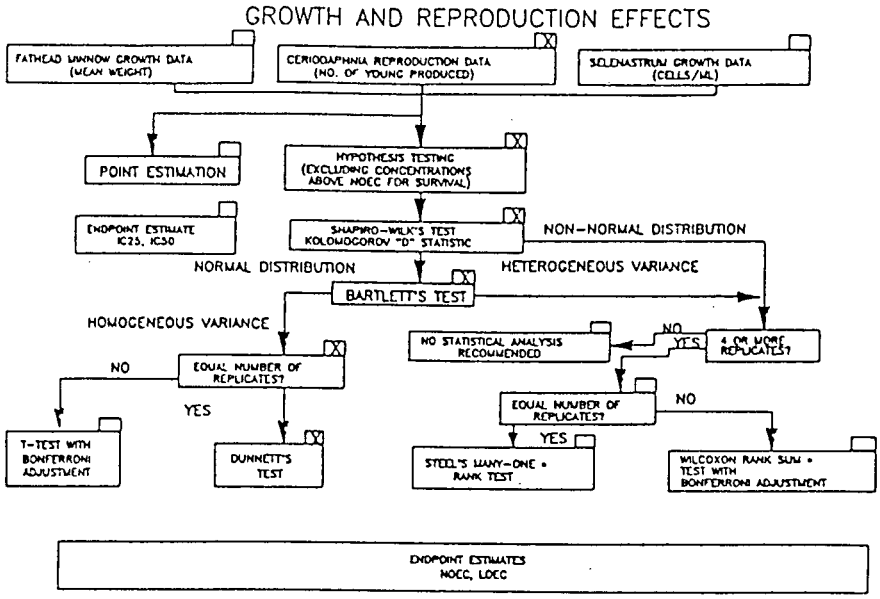
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for Ceriodaphnia Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 14, August 19-25, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Control	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	100	100
Intake	100	100	100	100	100	100
TR	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 14, August 19-25, 1993.



* Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Dunnett's Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 14, Watts Bar Nuclear Plant, August 19-25, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 5.03.

This represents a 29.58% reduction in Reproduction (# Young/Female/6 Days).

T = 2.35 Alpha = 0.05 (one-tailed test)

Reproduction (# Young/Female/6 Days) Data

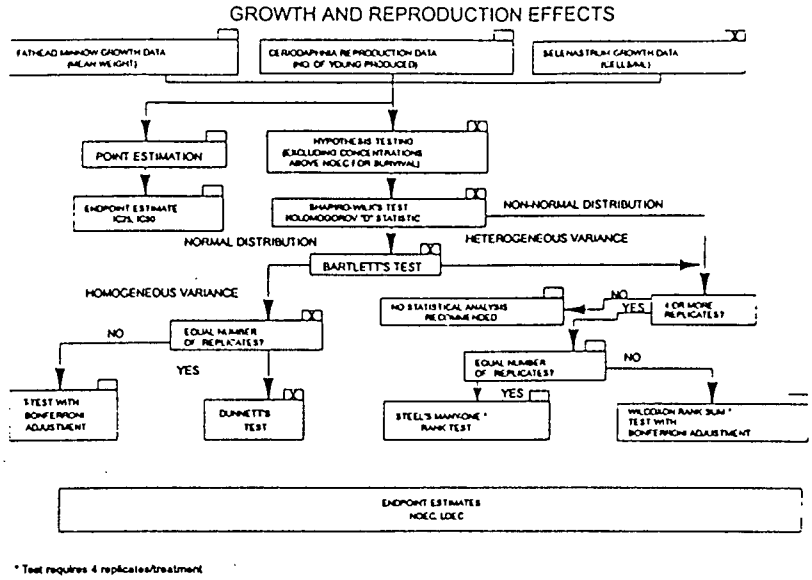
Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean S*
Control	22	14	12	11	8	25	19	24	17	18	17.00
Discharge-2.0%	20	6	13	21	9	16	15	18	2	9	12.90
Discharge-25.0%	19	23	22	26	19	18	23	24	22	21	21.70
Discharge-50.0%	22	22	20	24	23	26	24	29	27	21	23.80
Discharge-100%	20	22	24	22	23	23	6	26	23	11	20.00
Intake	14	7	17	21	12	16	15	17	21	19	15.90
TR	15	23	25	19	25	25	22	26	25	29	23.40

*Indicates values significantly less than control. EMS = 22.89.
IC_{25} = >100% DSN101.

6.3 Algal, Selenastrum capricornutum, Growth Test

6.3.1 Summary of Results: Diffuser discharge DSN101, collected August 19, 1993, demonstrated significant growth effects at 2.0, 25.0, 50.0, and 100 percent concentrations compared to intake controls. The IC_{25} for DSN101 was 1.10 percent.

6.3.2 Statistical Decision Process for Determining Toxicity Endpoints for 4-Day Exposure of the Alga, Selenastrum capricornutum, to Watts Bar Nuclear Plant DSN101 Discharge, Experiment 14, August 20-24, 1993.



6.3.3 Analysis of Growth Data Using Dunnett's Test for Algal 4-Day Growth Test, Watts Bar Nuclear Plant, Experiment 14, August 20-24, 1993.

No transformation was applied before data analysis. For this set of data, the minimum significant difference is 238 x 10^3.

This represents a 10.26% reduction in No. cells/mL x 10^3
T = 2.45 Alpha = 0.05 (one-tailed test)

Algal Growth Data (No. cell/mL x 10^3)

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean	S*
Intake	2370	2525	2255	2115							2316.25	
TR	1717	1394	1558	1717							1596.50	*
Discharge-2.0%	1216	1146	1393	1289							1261.00	*
Discharge-25.0%	851	1004	762	790							851.75	*
Discharge-50.0%	559	499	576	501							533.75	*
Discharge-100%	161	170	137	161							157.25	*
Culture Water (w/EDTA)	2343	2701	2189	2244							2369.25	

*Indicates values significantly less than the control. EMS = 18808
IC_{25} = 1.10%

6.4 Physical/Chemical Parameters

6.4.1 Overall Test Temperature

6.4.1.1 Fathead Minnow: Mean = 24.7°C (24.3°C-25.0°C)

6.4.1.2 Ceriodaphnia: Mean = 25.2°C (24.8°C-25.5°C)

6.4.1.3 Algae: Mean = 24.8°C (24.2°C-25.4°C)

6.4.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 14, August 19-26, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, August 17-24, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 14, August 19-26, 1993

Appendix D Initial Water Chemistry Values for Algal Growth Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 14, August 20, 1993

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 14, showed chronic results consistent with TTL control chart ranges for fathead minnows and daphnids. A satisfactory control chart has not been established for the algae, but data is included for information purposes.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test:
August 4, 1993/0905 CDT to August 11, 1993/0835 CDT

6.5.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.005 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 46

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.011 mg Cu/L

6.5.3 Ceriodaphnia

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of Most Recent Test:
August 19-27, 1993/0930 CDT

6.5.3.1.2 LOEC: 0.02 mg Cu/L
NOEC: 0.008 mg Cu/L
IC_{25}: 0.006 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 45

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.006-0.028 mg Cu/L

6.5.4 *Selenastrum capricornutum*

6.5.4.1 Chronic Tests

6.5.4.1.1 Date/Time of Most Recent Test:
August 9, 1993/0745 CDT to August 13, 1993/0645 CDT

6.5.4.1.2 LOEC: 0.0004 mg Cu/L
NOEC: 0.0002 mg Cu/L
IC_{25}: 0.0003 mg Cu/L

6.5.4.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 14

LOEC Range: 0.0004->0.003 mg Cu/L (four concentration increments)
NOEC Range: 0.0002-0.003 mg Cu/L (four concentration increments)
IC_{25} Range: 0.0003-0.0017 mg Cu/L

7.0 CONCLUSION

Tests conducted using samples collected from August 18-24, 1993, showed no toxicity (survival, growth, or reproduction) to fathead minnows or daphnids. Significant reduction in algal growth was demonstrated in 2.0. 25.0, 50.0, and 100 percent concentrations of DSN101 samples as compared to Intake. The NOEC based on IC_{25} calculation was 1.10 percent. Maximum allowable toxicity based on dilution available at WBN during this study was a NOEC $\geq$0.20 percent effluent ($\bar{x}$ value for study period; see Appendix A). Therefore, these data indicate the EPA recommended chronic instream criterion was not exceeded.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

PLARC501-1812

Appendix A

Calculated Allowable* Toxicity Based on Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, August 17-24, 1993.

	WB Hydro		WBN DSN101		Allowable Toxicity	
Date	Zero Release	Hourly Average Flow	Daily Flow†	Dilution	TUc‡	NOEC
	(Hours)	(cfs)	(cfs)	(WBN:WB Hydro)		(% Effluent)
August 17	4	32820	49.2	1:667	≤668	≥0.15
August 18	4§	32720	55.9	1:585	≤586	≥0.17
August 19	5	34532	75.5	1:457	≤458	≥0.22
August 20	7	35029	75.5	1:464	≤464	≥0.22
August 21	10	32029	75.5	1:424	≤425	≥0.24
August 22	11	36385	75.5	1:482	≤483	≥0.21
August 23	8	37775	75.5	1:500	≤500	≥0.20
August 24	9	35087	75.5	1:465	≤465	≥0.22
Average				1:505	≤506	≥0.20

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream effluent concentration to which organisms can be exposed indefinitely without causing unacceptable effect.

†Estimated daily flows. Actual flows become considerably less as hours of zero release increase.

‡TUc = 100/NOEC.

§The WBN diffuser discharge was suspended for 7 hours.

PLARC501-1813(1)

Appendix B

Watts Bar Nuclear Plant Biomonitoring
Diffuser Discharge Sample Analyses
August 17-24, 1993

1993 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/l)	TOTAL PHOSPHORUS (mg/l)	TOLYLTRIAZOLE (mg/l)
August 17	Grab	<0.020	0.171	
August 18	Grab	<0.020	--	
August 18-19	Composite*	0.048	0.19	<0.5
August 19	Grab	<0.020	--	
August 22-23	Composite*	0.101	0.215	
August 23	Grab*	<0.020	0.234	
August 24	Grab	<0.020	0.228	

*Grab samples were collected at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test. Composite samples were aliquots of the composite samples used for the toxicity tests.

PLARC501-1813(2)

Appendix C

Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 14, August 19-26, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Control	24.7 (24.4-24.9)	-	8.3 (8.3-8.4)	6.2 (5.6-7.0)	-	8.2 (8.2-8.3)	7.7 (7.6-7.8)	-	348 (334-356)	351 (336-359)	65 (61-67)	95.3 (92.3-97.5)	-
Dschg 2.0% w/F.Ctrl.	24.7 (24.4-25.0)	-	8.3 (8.2-8.4)	5.9 (4.7-6.8)	-	8.2 (8.1-8.2)	7.6 (7.4-7.8)	-	345 (330-354)	348 (332-358)	-	-	-
Dschg 25.0% w/F.Ctrl.	24.7 (24.5-24.9)	-	8.3 (8.3-8.3)	5.8 (4.8-6.7)	-	8.1 (8.1-8.2)	7.7 (7.6-7.7)	-	313 (301-320)	318 (304-325)	-	-	-
Dschg 50.0% w/F.Ctrl.	24.7 (24.4-25.0)	-	8.3 (8.2-8.3)	5.8 (5.0-6.7)	-	8.1 (8.0-8.1)	7.6 (7.5-7.8)	-	278 (269-282)	282 (272-289)	-	-	-
Cerio Control	-	25.2 (24.8-25.5)	8.3 (8.3-8.4)	-	7.7 (7.6-7.8)	8.2 (8.1-8.2)	-	8.1 (8.0-8.1)	324 (315-336)	-	67 (64-69)	89.8 (85.5-94.1)	-
Dschg 2.0% w/C.Ctrl.	-	25.2 (24.8-25.5)	8.3 (8.3-8.4)	-	7.8 (7.7-7.9)	8.2 (8.1-8.2)	-	8.1 (8.0-8.1)	323 (310-337)	-	-	-	-
Dschg 25.0% w/C.Ctrl.	-	25.2 (24.8-25.5)	8.3 (8.2-8.3)	-	7.7 (7.6-7.8)	8.2 (8.1-8.2)	-	8.1 (8.0-8.1)	295 (285-304)	-	-	-	-
Dschg 50.0% w/C.Ctrl.	-	25.2 (24.8-25.5)	8.3 (8.3-8.3)	-	7.7 (7.6-7.8)	8.1 (8.0-8.1)	-	8.1 (8.0-8.1)	265 (260-272)	-	-	-	-
Discharge 100%	24.6 (24.3-24.9)	25.2 (24.8-25.5)	8.3 (8.2-8.3)	6.1 (5.2-6.7)	7.7 (7.6-7.8)	8.0 (7.9-8.1)	7.7 (7.7-7.8)	8.1 (8.0-8.1)	206 (204-209)	211 (207-218)	77 (75-80)	83.8 (82.1-85.5)	<0.1 (<0.1-<0.1)
Intake 100%	24.7 (24.4-24.9)	25.2 (24.8-25.5)	8.3 (8.3-8.3)	5.8 (4.8-6.4)	7.7 (7.6-7.8)	8.0 (7.9-8.1)	7.7 (7.6-7.7)	8.1 (8.0-8.1)	201 (198-203)	204 (201-211)	73 (68-75)	83.1 (80.4-85.5)	<0.1 (<0.1-<0.1)
TR	-	25.2 (24.8-25.5)	6.6 (6.5-6.7)	-	7.7 (7.6-7.8)	7.9 (7.8-8.0)	-	8.0 (7.9-8.0)	175 (171-179)	-	59 (58-60)	65.6 (63.3-68.4)	<0.1 (<0.1-<0.1)

*mg/L as $CaCO_3$

PLARC501-1814

Appendix D

Initial Water Chemistry Values for Algal Growth Test,
Watts Bar Nuclear Plant Biomonitoring, Test 14, August 20-24, 1993

Treatment	Temp.	DO	pH	Cond.	Hard.	Alk.	Total Residual Chlorine
	(°C)	(mg/L)	(S.U.)	(µmhos)	*	*	(mg/L)
Intake	25.2	6.1	8.3	281	94.1	81	-
Discharge-2.0%	25.0	9.0	8.0	257	-	-	-
Discharge-25.0%	24.9	8.4	8.1	267	-	-	-
Discharge-50.0%	24.8	7.8	8.1	274	-	-	-
Discharge-100%	24.9	6.9	8.1	291	95.8	83	<0.1
TR	25.1	8.5	8.0	258	77.0	68	<0.1
Culture Water	24.9	6.7	7.6	94	13.7	10	-

*mg/L as $CaCO_3$

PLARC501-1813(3)

TENNESSEE VALLEY AUTHORITY
TOXICITY TESTING LABORATORY
STANDARD REPORT FORM

STATIC RENEWAL TESTS USING PIMEPHALES PROMELAS (FATHEAD MINNOWS) AND CERIODAPHNIA DUBIA (DAPHNIDS)

Test Title: Watts Bar Nuclear Plant (WBN), Experiment 15

Principle Investigator: Cynthia L. Russell

Starting Date: September 25, 1993

Ending Date: October 2, 1993

1.0 EXECUTIVE SUMMARY

Baseline toxicological assessments of the Watts Bar Nuclear Plant (WBN) DSN101 were conducted in January 1991, April 1992, and June 1992. Compliance monitoring for this discharge was initiated in October 1992. This report presents findings of the twelfth monthly toxicity bioassessment (Experiment 15) conducted during operation of an antifouling injector system at Watts Bar Nuclear Plant (WBN). This study evaluates acute and/or subchronic responses of two trophic levels to the diffuser discharge complex effluent. Organisms used for testing were larval fathead minnows (Pimephales promelas) and daphnids (Ceriodaphnia dubia).

Tests conducted using samples collected from September 24-30, 1993, showed no effect in fathead minnow survival, but there was a significant reduction in fathead minnow growth at 25 and 50 percent concentrations DSN101 samples as compared to control. Daphnid reproduction was significantly reduced only at 2 percent concentration. This effect was attributed to the culture medium used for dilution. Maximum allowable toxicity based on dilution available at WBN during this study was an NOEC $\geq$0.19 percent effluent ($\bar{x}$ value for study period; see Appendix A). These data indicate the EPA recommended chronic instream criterion was not exceeded.

2.0 SAMPLE COLLECTION/TREATMENTS

2.1 Test Sample Identification (Chemical/Effluent/Elutriate, etc.): The test sample selected for biomonitoring was the WBN diffuser discharge DSN101. Samples were collected from the diffuser pipe manhole. In addition, samples were collected from the south bank of the intake channel for comparison with diffuser samples to determine plant wastewater contributions.

2.2 Control and/or Dilution Water: Laboratory culture water (moderately hard reconstituted water) was used for dilution water and control for the fathead minnow test. Enriched culture water, moderately hard reconstituted water containing ten percent filtered Tennessee River water, was used for dilution for the daphnid test.

2.3 Sample Dates and Times: Test samples were collected daily from each station by TVA Field Engineering personnel from Chattanooga, TN, beginning September 24, 1993 (Isco composite initiated September 23), and continuing through September 30, 1993. Diffuser and intake collections were made between the hours of 0900 and 1000 EDT.

2.4 Sampling Method: Diffuser outfall and Intake samples were collected using Isco samplers programmed to collect 625 mL per hour during each 24-h sample period. Samples were caught in composite bottles which were surrounded by ice within the sampler housing and poured into thoroughly rinsed Nalgene cubitainers. Contingency grab samples were also collected at each of these stations at the times the samplers were reset. These samples were stored in cubitainers on ice until the following day. In the event of sampler malfunction another grab sample would be collected at the end of the 24-h period to be combined with the first to yield a composite grab sample for use in the toxicity tests.

All discharge and intake samples used in testing were Isco samples collected as described above with the exception of the DSN101 sample collected September 26 and used in testing September 27. This sample was a mixture of a composite and a grab sample due to interruption of the discharge flow during the sample collection period. A grab sample was collected after the discharge was reopened to complete the volume requirements.

2.5 Pertinent Site Conditions:

2.5.1 Watts Bar Hydro and WBN DSN101 operations were not continuous during the study (Appendix A). Dilution factors ranged from 241 to 751.

2.5.2 WBN DSN101 diffuser discharge was suspended for 47 hours after Watts Bar Hydro began operating on the morning of September 24, during and after the injection of ClamTrol into WBN's cooling water systems. ClamTrol was not detected in a grab sample of the WBN DSN101 discharge collected in the morning of September 26, after the diffusers were opened (Appendix B).

The injection of antifouling, metal corrosion inhibitors was not operational during the first 3 days of this toxicity test.

2.6 Sample Storage/Handling: All samples (Isco and grab) were placed on ice in ice chests after excess air was pressed from the cubitainers. All samples remained on ice during shipping and were stored on ice or in refrigerators at 4°C until used in tests. Contingency grab samples not used were discarded.

2.7 Sample Transport: Samples collected and shipped on 09/24, 09/27, 09/28, 09/29, and 09/30 were transported to Browns Ferry Nuclear Plant by overnight TVA mail courier. Samples collected 09/25 and 09/26 were transported the same day via private courier (airplane or automobile) to Pryor Field, Decatur, Alabama, where they were picked up by project personnel and taken to TVA's Toxicity Testing Laboratory (TTL) at Browns Ferry Nuclear Plant site, near Athens, Alabama.

2.8 Sample Pretreatment: Sample temperature was raised to 25°C in a warm water bath and samples were aerated as necessary to bring DO levels down to near 100 percent saturation. Adequate water for use in test initiation or daily renewal was filtered through a 100 µm nylon mesh filter into 2000 mL beakers, and appropriate dilutions were prepared where applicable.

2.9 Test Treatments: Diffuser outfall (discharge) was tested at 2.0, 25.0, 50.0, and 100 percent concentrations (diluted with culture water for the fathead minnow test and enriched water for the daphnid test). Intake samples were tested at 100 percent only in all tests, and Tennessee River water, collected from the TTL experimental channels, was run as an additional control in the daphnid test.

3.0 TEST ORGANISMS/CULTURING CONDITIONS

3.1 Species: *Pimephales promelas*, Fathead minnow

3.1.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.1.2 Culture Water: Culture water consisted of 50 percent dechlorinated tap water and 50 percent moderately hard reconstituted water. Dechlorination was achieved by activated carbon filtration and verified by DPD titration. Reagents for reconstituted water were added to reverse osmosis product water. Both waters were passed through a pack column degasser to bring dissolved gases to saturation. Culture water was continuously aerated to help ensure aseptic conditions. Total hardness was approximately 95 mg/L as $CaCO_3$.

3.1.3 Temperature of Culture: 25°C ± 1°C

3.1.4 General Maintenance: Adult fathead minnows are maintained in glass aquaria in a flow through recirculating system. Flow rate to aquaria used for spawning is one aquarium volume per hour. Approximately 20 percent of the water volume is replaced twice weekly. Adults are fed three times daily. Sexually mature fish are placed in 21-L glass aquaria (one male, four females) and reproduction is checked and recorded daily. Spawns are removed from aquaria and incubated in 1-L glass beakers under aeration to the proper stage of development for the target test.

Fish health is monitored regularly and corrective action is taken if necessary. Spawning frequency from individual aquaria is tracked and sexually spent individuals are replaced as necessary. Every 4-6 months a group of the same age fish from at least three spawns are reared to adults for replacement spawners.

3.1.5 Spawn Date: September 19-20, 1993

3.1.6 Hatch Date/Time: September 24, 1993/1055-September 25, 1993/0855.

3.1.7 Acclimation Water: Moderately hard reconstituted water

3.1.8 Acclimation Temperature: 25°C ± 1°C

3.1.9 Mean Dry Weight at Start of Test: 0.12 mg

3.1.10 Diseases and Treatment: None

3.1.11 Food and Feeding: Larvae were fed brine shrimp (Artemia) nauplii <24-h old twice daily beginning after hatching to ensure food availability if larvae began feeding prior to test initiation.

3.2 Species: Ceriodaphnia dubia, daphnid

3.2.1 Source: Inhouse culture, TVA, Toxicity Testing Laboratory

3.2.2 Culture Water: Enriched moderately hard reconstituted water containing ten percent filtered Tennessee River water and 1 ppb selenium.

3.2.3 Temperature of Culture: 25°C ± 1°C

3.2.4 General Maintenance: Adults used to produce neonates for test initiation are typically selected as neonates on 2 or 3 consecutive days 6-8 days prior to test initiation (adults up to 14 days old may be used for neonate production). These animals are raised individually (a brood may be raised as a group until Day 3 at which time animals are transferred to individual cups) and a record is made of their reproduction. Their fourth brood is generally the second brood with 8 or more young and is the brood preferred for test initiation. Mass cultures may be maintained in 8-L glass battery jars in light and temperature controlled incubators. New cultures are started weekly (7-10 days) with approximately 50 neonates. These neonates are selected from the third or fourth brood of the adult, from broods containing 8 or more young.

3.2.5 Food and Feeding: Ceriodaphnia are fed food made according to methods modified from EPA/600/4-89/001 with tropical fish food substituted for trout chow and alfalfa substituted for Cerophyll. [1]

In addition to the yeast/alfalfa/fish food recipe, the alga Selenastrum capricornutum concentrated to 50 x 10^6 cells/mL is also fed as part of the regular diet. Water used for algal culture contains approximately 1.0 ppb selenium. The feeding rate for mass cultures is 5 mL prepared food and 5 mL algae concentrate at culture initiation and 5 mL prepared food only every other day thereafter. Individual animals contained in cups with 15 mL water are fed 0.1 mL of food and 0.2 mL of algae at renewal and 0.1 mL food recipe only on intermediate days.

4.0 TEST METHODS

4.1 Fathead Minnow (Pimephales promelas) Larval Survival and Growth Test, EPA Test Method 1000.0. [1]

4.1.1 Modification/Amendments to Method 1000.0:

A 100-µm mesh nylon screen was used to filter samples rather than a 60-µm mesh as described in EPA methods.

4.1.2 Date/Time Test Initiated: September 25, 1993/0947 CDT

4.1.3 Date/Time Test Terminated: October 2, 1993/0845 CDT

4.1.4 Test Chamber: 600 mL borosilicate glass beakers
Volume per chamber: 350 mL

4.1.5 Number of Test Organisms per Chamber: 10

4.1.6 Number of Replicates per Treatment: 4

4.1.7 Dilution Water/Control Water: moderately hard reconstituted water

4.1.8 Renewal Period: 24-h

4.1.9 Test Temperature: 25°C ± 1°C

4.1.10 Feeding Regime During Test: Fathead larvae were fed brine shrimp (Artemia) nauplii <24-h old 3 times daily ad libitum.

4.1.11 Physical and Chemical Parameters Measured: Parameters measured daily (initially) on fresh samples were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chlorine.

Final measurements taken daily before renewal were temperature and DO in every test beaker and pH and conductivity in one replicate per treatment. Mean values and ranges are reported in Section 6.4.2.

4.1.12 Statistics:

Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific test method to determine toxicity endpoints. Decision processes followed for testing survival and sublethal (growth) effects are shown in Sections 6.1.2.2 and 6.1.3.1, respectively. Based on tests for normal distribution and homogenous variances, the statistical test used for fathead minnow survival endpoint determination was Steel's Many-one Rank Test. Fathead minnow growth data showed normal distribution and homogeneous variance making Dunnett's Test the appropriate statistical test used for endpoint determination. [1]

4.2 Ceriodaphnia Survival and Reproduction Test, EPA Test Method 1002.0. [1]

4.2.1 Modifications/Deviations to Method 1002.0:

Enriched water (see Section 3.2.2)

Prepared food recipe (see Section 3.2.5)

Algal cell density (see Section 3.2.5)

Feeding regime (see Section 4.2.10)

A 100-mm mesh nylon screen was used to filter samples rather than a 60-mm mesh as described in EPA methods.

4.2.2 Date/Time Test Initiated: September 25, 1993/1035 CDT

4.2.3 Date/Time Test Terminated: October 2, 1993/0945 CDT

4.2.4 Test Chamber: 1-ounce plastic cups (Plastics, Inc., #P.I.-1)
Volume per Chamber: 15 mL

4.2.5 Number of test organisms per chamber: 1

4.2.6 Number of replicates per treatment: 10

4.2.7 Dilution Water/Control Water: Enriched culture water (see Section 2.2) was used for dilution water. Intake was used as control for statistical purposes.

4.2.8 Renewal Period: 24 hours

4.2.9 Test Temperature: 25°C ± 1°C

4.2.10 Feeding Regime During Test: Each organism was fed 0.1 mL of prepared food and 0.2 mL algae (50 x 10^6 cells/mL) concentrate daily (added to renewal water before introduction of test organism).

4.2.11 Physical and Chemical Parameters Measured: Parameters measured daily ("initial") on fresh test solutions and control waters were temperature (temperature was adjusted to equal "final" temperature before renewal), DO, pH, conductivity, alkalinity, hardness, and total residual chorine.

"Final" measurements of temperature were made in 10 randomly selected cups when the tray was removed from the incubator. DO and pH were measured daily in 1 cup per treatment following renewal. Mean values and ranges are reported in Section 6.4.2.

4.2.12 Statistics:

Fishers Exact Test was used to evaluate Ceriodaphnia survival effects. Revised statistical procedures contained in the second edition of EPA's short-term chronic toxicity methods require a decision process for testing statistical assumptions before selecting a specific statistical test to determine sublethal (reproduction) toxicity endpoints. The decision process followed is shown in Section 6.2.3.1. Based on tests for normally distributed data and homogeneous variances, the statistical test used for endpoint determination was Steel's Many-one Rank Test. [1] Laboratory culture water failed to meet test acceptability criteria and was omitted from statistical analyses.

5.0 QUALITY ASSURANCE

5.1 Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage; glassware preparation; test organism culturing/acquisition and acclimation; test organism handling during test; and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA/600/4-89/001. [1] Any known deviations were noted during the study and are reported herein.

5.2 Physical and Chemical Methods

5.2.1 Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable). All TTL chemicals are recorded in a bound Laboratory Chemical Logbook and specific chemicals used were documented on a chemical record sheet contained in the study notebook.

5.2.2 Instruments: All identification, service, and standardization information pertaining to TTL laboratory instruments is contained in bound Laboratory Instrument Logbooks and specific instruments used were documented on an instrument record sheet contained in the study notebook.

5.2.3 Temperature was measured using mercury thermometers. The instrument was standardized and inspected with readings made according to TVA procedure ES-42.11. [2]

5.2.4 Dissolved oxygen was measured using a YSI Model 57 oxygen meter. The instrument was standardized (using the Winkler method) and readings were taken according to TVA procedures ES-43.6 and ES-4.24, respectively. [2]

5.2.5 The pH was measured using an Orion Model SA250 meter equipped with an Orion Ross combination electrode. The instrument was standardized and readings were made according to TVA procedures ES-43.7 and ES-42.8, respectively. [2]

5.2.6 Conductance was measured using a YSI Model 32 SCT meter. The instrument was standardized and readings were taken according to TVA procedures ES-43.3 and ES-42.3, respectively. [2]

5.2.7 Alkalinity was measured by titration of 100 mL samples with 0.02 N H_2SO_4 to an end point of 4.5 according to TVA procedure ES-42.1. [2]

5.2.8 Hardness was determined by titration of 50 mL samples with EDTA to a colorimetric endpoint using an indicator (Instructions provided by Reagent Manufacturer [Calgon]), Schwarzenbach Method.

5.2.9 Total residual chlorine (TRC) was determined using the DPD Titrimetric Method according to TVA procedure ES-42.9, Rev. 0. [2]

5.3 Reference Toxicant Tests

5.3.1 Test Type: Fish - 7-day chronic (NOEC)
Daphnids - 7-day chronic (NOEC)

5.3.2 Standard Toxicants Used:

Copper Sulfate Reference Toxicant Solution - fish and daphnids
Source/Brand: EPA

5.3.3 Dilution Water Used: Moderately hard reconstituted water for fish chronic and enriched water (see Section 3.2.2) for daphnid chronic.

5.3.4 Statistics: Chronic Tests, Fathead Minnow Survival/Growth and Ceriodaphnia Reproduction -
NOEC Dunnett's Test or Steel's Many-one Rank Test.
IC_{25} - EPA Bootstrap Procedure.
Ceriodaphnia Survival - Fisher's Exact Test.

6.0 RESULTS

6.1 Fathead Minnow Larval Survival and Growth Test

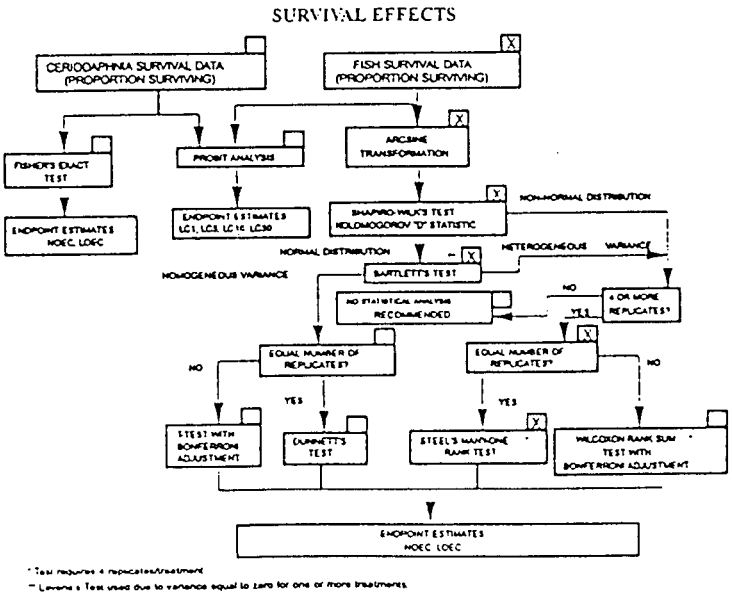
6.1.1 Summary of Results: No significant survival or growth effects were demonstrated for 100 percent diffuser discharge or intake samples collected from September 24-30, 1993; however, growth effects were demonstrated in 25.0 and 50.0 percent concentrations of DSN101.

6.1.2 Results, Survival Data:

6.1.2.1 Daily Percent Survival Summary for Fathead Minnow Larval Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 15, September 25-October 2, 1993.

Treatment	Total Daily % Survival						
Day	1	2	3	4	5	6	7
Control	100	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	100	100
Discharge-50.0%	100	100	100	100	100	100	100
Discharge-100%	100	100	98	98	98	98	98
Intake	100	100	100	100	100	100	100

6.1.2.2 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Survival of Fathead Minnows in Watts Bar Nuclear Plant Effluent, Experiment 15, September 25-October 2, 1993.



* Test requires 4 replicates/treatment
** Levene's Test used due to variance equal to zero for one or more treatments

6.1.2.3 Analysis of Survival Data Using Steel's Many-one Rank Test for Fathead Minnow 7-Day Larval Survival Test, Watts Bar Nuclear Plant, Experiment 15, September 25-October 2, 1993.

	Survival (%) Data											
	Replicate											
Group	1	2	3	4	5	6	7	8	9	10	Mean	S *
Control	100	100	100	100							100	
Discharge-2.0%	100	100	100	100							100	
Discharge-25.0%	100	100	100	100							100	
Discharge-50.0%	100	100	100	100							100	
Discharge-100%	100	100	90	100							98	
Intake	100	100	100	100							100	

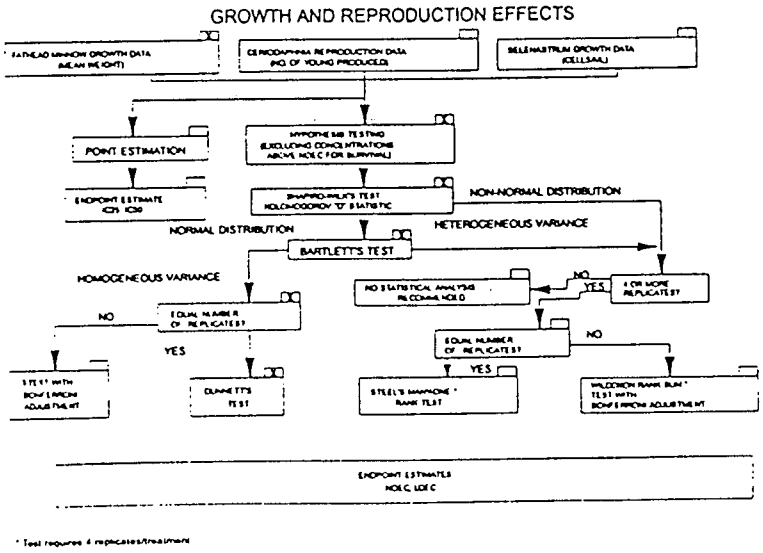
*Indicates values significantly less than control.

Steel's Many-one Rank Test			
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	4	10	18
Discharge-25.0%	4	10	18
Discharge-50.0%	4	10	18
Discharge-100%	4	10	16
Intake	4	10	18

*Values less than Critical Rank Sum are significantly less than the control.

6.1.3 Results, Growth Data:

6.1.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 7-Day Exposure of Fathead Minnows to Watts Bar Nuclear Plant Effluent, Experiment 15, September 25-October 2, 1993.



6.1.3.2 Analysis of Dry Weight (mg) Data Using Dunnett's Test for Fathead Minnow 7-Day Larval Growth Test, Experiment 15, Watts Bar Nuclear Plant, September 25-October 2, 1993.

No transformation applied before data analysis. For this set of data, the minimum significant difference is 0.08.

This represents a 12.33% reduction in Dry Weight (mg).
T = 2.41 Alpha = 0.05 (one-tailed test)

	Dry Weight (mg) Data										
	Replicate										
Group	1	2	3	4	5	6	7	8	9	10	Mean S*
Control	0.63	0.63	0.62	0.65							0.63
Discharge-2.0%	0.62	0.56	0.51	0.65							0.58
Discharge-25.0%	0.48	0.56	0.49	0.59							0.53 *
Discharge-50.0%	0.50	0.57	0.58	0.50							0.54 *
Discharge-100%	0.58	0.63	0.67	0.59							0.62
Intake	0.54	0.59	0.65	0.58							0.59

*Indicates values significantly less than control. EMS = 0.002.
IC_{25} = >100% DSN101.

6.2 *Ceriodaphnia* Survival and Reproduction Test

6.2.1 Summary of Results: No significant survival or reproduction effects were demonstrated for diffuser discharge or intake samples collected from September 24-29, 1993. Reproduction effects demonstrated in 2 percent concentration DSN101 were attributed to the culture water used for dilution.

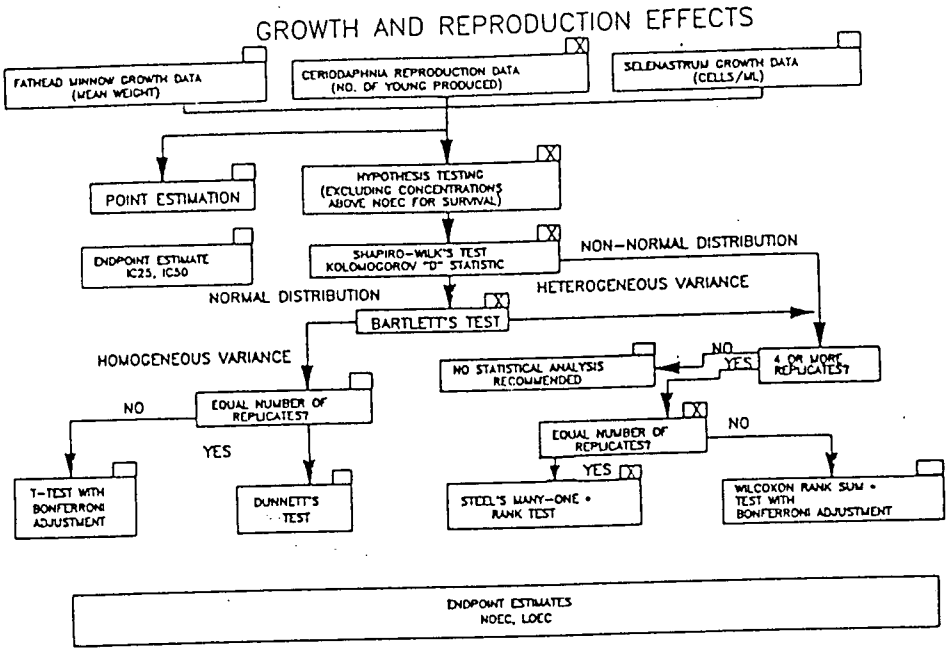
6.2.2 Results, Survival Data:

6.2.2.1 Daily percent survival summary for *Ceriodaphnia* Survival Test, Watts Bar Nuclear Plant Biomonitoring, Experiment 15, September 25-October 1, 1993.

	Total Daily % Survival					
Treatment	1	2	3	4	5	6
Intake	100	100	100	100	100	100
Discharge-2.0%	100	100	100	100	100	100
Discharge-25.0%	100	100	100	100	100	90
Discharge-50.0%	100	100	100	100	100	100
Discharge-100%	100	100	100	100	90	90
TR	100	100	100	100	100	100

6.2.3 Results, Reproduction Data:

6.2.3.1 Statistical Decision Process for Determining Toxicity Endpoints for 6-Day (3-Brood) Exposure of Ceriodaphnia to Watts Bar Nuclear Plant Effluent, Experiment 15, September 25-October 1, 1993.



• Test requires 4 replicates/treatment

6.2.3.2 Analysis of Reproduction (# young/female/6 days) Data Using Steel's Many-one Rank Test for Ceriodaphnia (6-Day) Reproduction Test, Experiment 15, Watts Bar Nuclear Plant, September 25-October 1, 1993.

Reproduction (# Young/Female/6 Days) Data

Group	Replicate 1	2	3	4	5	6	7	8	9	10	Mean S*
Intake	30	34	7	20	29	30	32	36	42	30	29.00
Discharge-2.0%	9	9	8	14	8	24	23	29	23	24	17.10*
Discharge-25.0%	3	29	13	10	9	15	30	31	27	30	19.70
Discharge-50.0%	14	16	37	11	13	38	32	33	31	33	25.80
Discharge-100%	15	13	32	22	16	15	33	36	35	21	23.80
TR	12	35	36	26	12	24	37	37	37	27	28.30

*Indicates values significantly less than control.

Steel's Many-one Rank Test			
Treatment	No. of Replicates	Critical Rank Sum	Rank Sum*
Discharge-2.0%	10	75	70.5 *
Discharge-25.0%	10	75	78.5
Discharge-50.0%	10	75	103.5
Discharge-100%	10	75	93.0
TR	10	75	106.5

*Values less than Critical Rank Sum are significantly less than the control. IC25 = >100% DSN101.

6.3 Physical/Chemical Parameters

6.3.1 Overall Test Temperature

6.3.1.1 Fathead Minnow: Mean = 25.0°C (24.3°C-25.9°C)

6.3.1.2 Ceriodaphnia: Mean = 25.0°C (24.0°C-25.9°C)

6.3.2 Toxicity test water chemistry summary for Watts Bar Nuclear Plant Biomonitoring, Experiment 15, September 25-October 2, 1993.

See: Appendix B Analysis of Watts Bar Nuclear Plant Diffuser (DSN101) Discharge Concentrations of Total Zinc and Total Phosphorus, September 23-30, 1993.

Appendix C Water Chemistry Mean Values and Ranges for Fathead Minnow and Ceriodaphnia Tests, Watts Bar Nuclear Plant Biomonitoring, Experiment 15, September 23-October 2, 1993

6.5 Reference Toxicant Tests

6.5.1 Summary of Results:

The most recent reference toxicant tests conducted prior to WBN, Experiment 15, showed chronic results consistent with TTL control chart ranges for fathead minnows and daphnids.

6.5.2 Fathead Minnows

6.5.2.1 Chronic Tests

6.5.2.1.1 Date/Time of Most Recent Test:
September 8, 1993/0930 CDT to September 15, 1993/0930 CDT

6.5.2.1.2 LOEC: 0.008 mg Cu/L
NOEC: 0.003 mg Cu/L
IC_{25}: 0.006 mg Cu/L

6.5.2.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 47

LOEC Range: 0.008-0.02 mg Cu/L (one concentration increment)
NOEC Range: 0.003-0.008 mg Cu/L (one concentration increment)
IC_{25} Range: 0.004-0.012 mg Cu/L

6.5.3 Ceriodaphnia

6.5.3.1 Chronic Tests

6.5.3.1.1 Date/Time of Most Recent Test:
September 20, 1993/1025 CDT to September 28, 1993/1040 CDT

6.5.3.1.2 LOEC: 0.05 mg Cu/L
NOEC: 0.002 mg Cu/L
IC_{25}: 0.011 mg Cu/L

6.5.3.1.3 Control Chart Information:

Number of standard tests completed by laboratory: 47

LOEC Range: 0.008-0.05 mg Cu/L (two concentration increments)
NOEC Range: 0.003-0.02 mg Cu/L (two concentration increments)
IC_{25} Range: 0.006-0.030 mg Cu/L

7.0 CONCLUSION

Tests conducted using samples collected from September 24-30, 1993, showed no effect in fathead minnow survival, but there was a significant reduction in fathead minnow growth at 25 and 50 percent concentrations DSN101 samples as compared to control. Daphnid reproduction was significantly reduced only at 2 percent concentration. This effect was attributed to the culture medium used for dilution. Maximum allowable toxicity based on dilution available at WBN during this study was an NOEC $\geq$0.19 percent effluent ($\bar{x}$ value for study period; see Appendix A). These data indicate the EPA recommended chronic instream criterion was not exceeded.

8.0 REFERENCES

1. Weber, C. I., W. H. Peltier, T. J. Norberg-King, W. B. Horning, F. A. Kessler, J. R. Menkdick, T. W. Neiheisel, P. A. Lewis, D. J. Klemm, Q. H. Pickering, F. L. Robinson, J. M. Lazorchak, L. J. Wymer, and R. W. Freyberg. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001 (March 1989) and EPA/600/4-89/001a (September 1989).
2. Field Operations Natural Resource Engineering Procedures Manual, Vol. 1, Division of Natural Resource Operations, Tennessee Valley Authority.

Appendix A

Calculated Allowable* Toxicity Based on Watts Bar Nuclear Plant (WBN) and Watts Bar (WB) Hydro Plant Releases, September 23-30, 1993.

Date	WB Hydro		WBN DSN101		Allowable Toxicity	
	Zero Release	Hourly Average Flow	Daily Flow†	Dilution	TUc‡	NOEC
	(Hours)	(cfs)	(cfs)	(WBN:WB Hydro)		(% Effluent)
September 23	2	36950	49.2	1:751	≤752	≥0.13
September 24	4§	29335	49.2	1:596	≤597	≥0.17
September 25	10§	26693	0.0	-	-	-
September 26	9§	23680	98.3	1:241	≤241	≥0.41
September 27	6	33022	49.2	1:671	≤672	≥0.15
September 28	6	35928	49.2	1:730	≤731	≥0.14
September 29	6	28000	49.7	1:563	≤564	≥0.18
September 30	6	27261	49.2	1:554	≤555	≥0.18
Average					≤514	≥0.19

*Based on achieving a Criteria Continuous Concentration (CCC) of 1.0 TUc and assuming complete mixing, where the CCC is the EPA national water quality criteria recommendation for the highest instream effluent concentration to which organisms can be exposed indefinitely without causing unacceptable effect.

†Estimated daily flows. Actual flows become considerably less as hours of zero release increase.

‡TUc = 100/NOEC.

§The WBN diffuser discharge was suspended for a total of 47 hours.

PLARC501-1913(1)

Appendix B

Watts Bar Nuclear Plant Biomonitoring
Diffuser Discharge Sample Analyses
September 23-30, 1993

1993 Month Date	SAMPLE TYPE	TOTAL ZINC (mg/L)	TOTAL PHOSPHORUS (mg/L)	CLAMTROL (mg/L)
September 26	Grab	0.088	0.250	<0.2
September 27	Grab	0.047	0.107	
September 28	Grab	0.060	--	
September 29	Grab*	0.065	--	
September 30	Grab	0.097	--	

*Grab samples were collected at various times of the day and are not necessarily representative of exposure concentrations that test organisms would have received during the test.

PLARC501-1913(2)

Appendix

Water Chemistry Mean Values and Ranges for Fathead Minnow and *Ceriodaphnia* Tests,
Watts Bar Nuclear Plant Biomonitoring, Experiment 15, September 25-October 2, 1993

Source	Temperature		Dissolved Oxygen			pH			Conductivity		Alk	Hardness	Chlorine
	Fish	Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Final Cerio	Initial	Final Fish	Initial	Initial	Initial
	(°C)	(°C)	(mg/L)	(mg/L)	(mg/L)	(S.U.)	(S.U.)	(S.U.)	(µmhos)	(µmhos)	*	*	(mg/L)
Fish Medium	25.0 (24.5-25.6)	-	8.2 (8.1-8.3)	6.0 (4.8-7.0)	-	8.2 (8.2-8.3)	7.8 (7.7-8.0)	-	328 (309-342)	331 (311-349)	59 (55-63)	92.9 (85.5-97.5)	-
Dschg 2.0% w/F.Med.	24.9 (24.4-25.5)	-	8.2 (8.1-8.3)	6.1 (4.8-7.0)	-	8.2 (8.2-8.2)	7.8 (7.7-8.0)	-	323 (306-340)	329 (309-344)	-	-	-
Dschg 25.0% w/F.Med.	25.1 (24.4-25.6)	-	8.3 (8.2-8.4)	6.0 (4.7-7.0)	-	8.1 (7.9-8.1)	7.8 (7.7-8.0)	-	296 (281-308)	301 (286-317)	-	-	-
Dschg 50.0% w/F.Med.	25.2 (24.6-25.9)	-	8.3 (8.2-8.4)	5.9 (4.6-6.9)	-	8.1 (8.0-8.1)	7.8 (7.7-8.0)	-	265 (254-272)	269 (260-278)	-	-	-
Enriched Medium	-	25.0 (24.0-25.9)	8.1 (8.0-8.2)	-	7.8 (7.7-8.0)	8.2 (8.1-8.3)	-	8.2 (8.1-8.2)	322 (320-323)	-	60 (60-60)	92.3 (92.3-92.3)	-
Dschg 2.0% w/E.Med.	-	25.0 (24.0-25.9)	8.1 (8.0-8.2)	-	7.6 (7.5-7.7)	8.2 (8.2-8.3)	-	8.2 (8.1-8.2)	320 (318-321)	-	-	-	-
Dschg 25.0% w/E.Med.	-	25.0 (24.0-25.9)	8.3 (8.3-8.4)	-	7.8 (7.7-7.9)	8.1 (8.0-8.2)	-	8.2 (8.1-8.3)	293 (291-294)	-	-	-	-
Dschg 50.0% w/E.Med.	-	25.0 (24.0-25.9)	8.3 (8.1-8.4)	-	7.7 (7.6-7.7)	8.1 (8.0-8.1)	-	8.2 (8.1-8.3)	264 (263-265)	-	-	-	-
Discharge 100%	25.1 (24.7-25.5)	25.0 (24.0-25.9)	8.3 (8.2-8.3)	5.9 (4.8-6.9)	7.8 (7.7-7.9)	8.0 (7.8-8.1)	7.8 (7.7-8.0)	8.2 (8.2-8.3)	204 (203-206)	208 (202-211)	72 (70-74)	84.5 (83.8-85.5)	<0.1 (<0.1-0.1)
Intake 100%	25.0 (24.3-25.9)	25.0 (24.0-25.9)	8.3 (8.3-8.3)	5.7 (4.1-6.4)	7.9 (7.7-8.0)	8.0 (7.9-8.1)	7.8 (7.7-7.9)	8.3 (8.2-8.3)	202 (201-202)	204 (202-208)	71 (70-72)	82.3 (82.1-83.8)	<0.1 (<0.1-<0.1)
TR	-	25.0 (24.0-25.9)	7.0 (6.7-7.3)	-	7.8 (7.7-8.0)	7.9 (7.8-8.0)	-	8.2 (8.2-8.3)	179 (178-181)	-	59 (56-61)	68.4 (66.7-70.1)	-

PLARC501-1914

*mg/L as $CaCO_3$