

UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY
REGION I ESD

BIOMONITORING PROTOCOLS
JULY 1, 1990

ATTACHMENT A

Example Chain of Custody Documentation

(July 1, 1990)

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Printed on Recycled Paper

ATTACHMENT A

Example Chain of Custody Documentation*

Chain of Custody Documentation provides vital sample information and establishes a clear paper trail for tracking the sample from the moment of collection to the final reporting of analytical results. This subsequently upholds the integrity and quality of the reported biomonitoring results.

Chain of Custody Tape to seal the container: The tape has a sample number which corresponds to a Field Data Card (see below), and provides collection date and time, inspector's/sampler's signature, name and title information. If the tape is broken prior to the onset of analysis, this may indicate tampering and decrease sample integrity.

Sample Tag: The tag remains attached to the sample container and provides the following: source of sample; date and time of collection; preservation technique; analyses to be performed, a sample number which corresponds to the Field Data Card (see below); sampling crew identification and sample type (composite vs. grab/chlorinated vs. prechlorinated). The tag should be water resistant to improve longevity and may also be used to note special remarks about the sample.

Field Data Card: This card basically replicates the information contained on the sample tag and also records other environmental observations and analytical field results made at the time of collection. Additionally, the card is marked with a pre-printed sample number which is used to trace back to the sample tag and the actual sample in the laboratory's possession. The card is kept with the project coordinator or individual who ultimately receives the analytical report.

Chain of Custody Record: This form is completed by the sampling crew and is submitted along with the tagged samples to the analytical laboratory, after the sampler signs off and dates the "Relinquished" line and a laboratory representative signs off and dates the "Received" line. The Record should be duplicated so that the receiving laboratory and the project coordinator/sampler each have a copy of the signed document.

*These forms are provided as examples only.

SAMPLE A

Field Data Card

SOURCE Effluent WWTP #1 PERMIT NO. (optional)
 CITY Boston, MA COLLECTOR John Smith
 WEATHER CLEAR RAIN SNOW (CIRCLE ONE)
 AIR TEMP (°C) < 0° 0°-10° 11°-20° > 21°
 SAMPLE LOCATION SUPPLY WATER WASTEWATER RAW TREATED
 (Circle One) Other _____

LAB CODE NU 55560
 PROJECT #
 STATION # 1210001
 SAMPLE TYPE GRAB 24 C.V.C

COLLECTION DATE
 Y Y M M D D Y Y M M D D
7 10 02 02 7 10 02 03
 START END
 COLLECTION TIME
0750 0850
 START END
 SAMPLE TEMP (°C) 06
 pH (SU) 08.51
 TOT Cl₂ (mg/l) 0.0
 SETTLEABLE SOLIDS (ml/l)
conductivity 120.0 0.0
 FLOW METER CM
 TOTALIZER
 START 7/4/93
 END 967542
 MULTIPLIER X1

PARAMETERS (CHECK APPROPRIATE)

Bacti	<input type="checkbox"/>	NH ₃	<input type="checkbox"/>	Phenol	<input type="checkbox"/>	Tot	<input type="checkbox"/>
BOD	<input type="checkbox"/>	NO ₂ -N	<input type="checkbox"/>	COD	<input type="checkbox"/>	Cd	<input type="checkbox"/>
TSS	<input checked="" type="checkbox"/>	TKN	<input type="checkbox"/>	PCB	<input type="checkbox"/>	Cu	<input type="checkbox"/>
Turb	<input type="checkbox"/>	T P	<input type="checkbox"/>	X Ray	<input type="checkbox"/>	Cr (T)	<input type="checkbox"/>
Organics	<input type="checkbox"/>	O&G	<input type="checkbox"/>	Other <u>Chronic</u>	<input type="checkbox"/>	Cr (+6)	<input type="checkbox"/>
VOA's	<input type="checkbox"/>	CN	<input type="checkbox"/>	Other <u>Toxicity</u>	<input type="checkbox"/>	Fe	<input type="checkbox"/>

FLOW MEASUREMENT
 None ☐
 Magmeter ☐
 Venturi ☐
 Parshall ☒
 V Notch ☐
 Rectangular ☐
 Other ☐

SIZE
100
 (inches) (Degrees)

METALS
 Diss ☐
 Ni ☐
 Pb ☐
 Sn ☐
 Zn ☐
 Other ☐

(Note preserved with ice to 4°C) Unpreserved sample

Chain of Custody Tape

Official Sample Tape	Sample No. <u>55800</u>	Date <u>7/2/90</u>
<u>Effluent Chronic Toxicity</u>	Signature <u>John Smith</u>	
	Print Name and Title <u>John Smith, Inspector</u>	

Sample Tag

Sample Tag (Front)	
NAME OF CLIENT AND ADDRESS <u>Environmental Sampling, Inc</u> <u>70 Main Street</u> <u>Boston, MA</u>	DATE RECEIVED <u>7/2/93</u> TIME <u>1:00pm - 2:00pm</u> BY <u>KINPBI</u>
SOURCE OF SAMPLE <u>Discharge #1 from</u> <u>WWTP (Chlorinated)</u>	LABORATORY <u>55181010</u> PROJECT <u>766</u> ANALYST <u>406</u> REMARKS <u>See Back</u>
COLLECTOR'S NAME (PRINT, LAST NAME) <u>John Smith,</u>	

(July 1, 1990)

(Back)

REMARKS <u>Composite</u> <u>Sample to be Tested for</u> <ul style="list-style-type: none"> • Chronic Toxicity of Chlorinated Eff. • TSS • Total Solids • Alkalinity <u>(dechlorinate prior to testing for toxicity)</u>
--

SAMPLE B

SOURCE Effluent WWTWP001 PERMIT NO 901001
 CITY Boston, MA COLLECTOR John Smith
 WEATHER CLEAR, RAIN, SNOW (CIRCLE ONE)
 AIR TEMP (°C) < 0° 0° 10° 11° 20° > 21°
 SAMPLE LOCATION SUPPLY WATER, WASTEWATER, RAW, TREATED
 (Circle One) Other _____

LAB CODE NY 55561
 PROJECT #
 STATION # WWTWP001
 SAMPLE TYPE GRAB, 24 H, C, V, C

COLLECTION DATE
 Y Y M M D D Y Y M M D D
9 0 0 2 0 3 9 0 0 2 0 3

PARAMETERS (CHECK APPROPRIATE) (Chlorinated sample)
 Bacti ☐ NH3 ☒ Phenol ☐
 BOD ☐ NO2-3 ☐ COD ☐
 TSS ☐ TKN ☐ PCB ☐
 Turb ☐ TP ☐ X-Ray ☐
 Organics ☐ O&G ☐ Other Total Organic Carbon
 VOA's ☐ CN ☐
 FLOW MEASUREMENT
 None ☐ SIZE 2.0
 Magmeter ☐ inches Degrees
 Venturi ☐
 Parshall ☒
 V-Notch ☐
 Rectangular ☐
 Other _____

METALS
 Tot Diss
 Cd ☐
 Cu ☐
 Cr (T) ☐
 Cr (+6) ☐
 Fe ☐
 Hg ☐
 Mn ☐
 Ni ☐
 Pb ☐
 Sn ☐
 Zn ☐
 Other _____

START _____ END _____
 COLLECTION TIME
 START 0850 END 0850
 SAMPLE TEMP (°C) 06
 pH (SU) 08.5
 TOT Cl₂ (mg/l) 0.07
 SETTLEABLE SOLIDS (ml/l)
120.0 0.000
 FLOW METER
 TOTALIZER
 START 714.633
 END 767.548
 MULTIPLIER 1

(Note: preserved with ice & H₂SO₄) Unpreserved sample

Official Sample Tape	Sample No. <u>55801</u>	Date <u>2/2/90</u>
<u>Effluent (Chlorinated) 001</u>	Signature <u>John Smith</u>	
	Print Name and Title <u>John Smith, Inspector</u>	

Sample Tag (Front)	
NAME OF UNIT AND ADDRESS <u>Environmental Samples, Inc.</u> <u>70 Main Street</u> <u>Boston, MA</u>	DATE OF ANALYSIS <u>90/2/2-3</u>
SOURCE OF SAMPLE <u>Discharge eff from</u> <u>WWT (Chlorinated)</u>	ANALYST <u>John Smith</u>
ANALYST'S FIRST, INITIAL, LAST NAME <u>John Smith</u>	REMARKS <u>see Back</u>

(Back)

REMARKS

Composite
Sample to be tested for:

- Ammonia
- Total organic carbon

(July 1, 1990)

SAMPLE C

SOURCE Effluent WWTP 001 PERMIT NO optional
 CITY Boston, MA COLLECTOR John Smith
 WEATHER: CLEAR RAIN SNOW (CIRCLE ONE)
 AIR TEMP (°C): < 0° 0°-10° 11°-20° > 21°
 SAMPLE LOCATION SUPPLY WATER WASTEWATER RAW, TREATED
 (Circle One) Other _____

PARAMETERS (CHECK APPROPRIATE) Total METALS

Bacti ☐ NH3 ☐ Phenol ☐
 BOD ☐ NO2+3 ☐ COD ☐
 TSS ☐ TKN ☐ PCB ☐
 Turb ☐ T P ☐ X-Ray ☐
 Organics ☐ O&G ☐ Other Hardness
 VOA's ☐ CN ☐

FLOW MEASUREMENT

None ☐
 Magmeter ☐
 Venturi ☐
 Parshall ☒
 V-Notch ☐
 Rectangular ☐
 Other _____

SIZE

20
 Inches/Degrees

Tot. Diss.
 Cd ☐
 Cu ☐
 Cr (T) ☐
 Cr (+6) ☐
 Fe ☐
 Hg ☐
 Mn ☐
 Ni ☐
 Pb ☐
 Sn ☐
 Zn ☐
 Other Ag

*Unpreserved sample

LAB CODE NO 55802
 PROJECT # ☐
 STATION # 1200007
 SAMPLE TYPE: GRAB 24 hr C.V.C

COLLECTION DATE
 Y Y M M D D Y Y M M D D
9 12 02 02 9 12 02 02
 START END

COLLECTION TIME
0850 0850
 START END

SAMPLE TEMP (°C) 22
 pH (SU) 08.5
 TOT Cl₂ (mg/l) 0.0

SETTLABLE SOLIDS (ml/l)
0.0 0.0

FLOW METER
 TOTALIZER
 START 71468
 END 76754
 MULTIPLIER 1

Note: (preserved with HNO₃)

Official Sample Tape	Sample No. <u>55802</u>	Date <u>12-3/90</u>
<u>Effluent (Chlorinated) 001</u>	Signature <u>John Smith</u>	
	Print Name and Title <u>John Smith, Inspector</u>	

Sample Tag (FRONT)	
NAME OF UNIT AND ADDRESS <u>Environmental Sampling, Inc.</u> <u>70 Main Street</u> <u>Boston, MA</u>	DATE/TIME <u>9/2/2-3</u> <u>8:50 AM - 8:55 AM</u>
SOURCE OF SAMPLE <u>Discharge 001 from</u> <u>WWTP (Chlorinated)</u>	PERMIT NO. <u>001</u>
SAMPLING CREW (FIRST, INITIAL, LAST NAME) <u>John Smith</u>	ANALYST <u>See Back</u>

(Back)
REMARKS <u>Composite</u> <u>Sample to be tested for:</u> <ul style="list-style-type: none"> • Hardness • Metals (Cd, Cu, Cr, Fe, Hg, Pb, Zn, Al, Ag)

(July 1, 1990)

SAMPLE D

SOURCE Effluent WWTP #01 PERMIT NO optional
 CITY Boston, MA COLLECTOR John Smith
 WEATHER CLEAR RAIN, SNOW (CIRCLE ONE)
 AIR TEMP (°C) < 0° 0° 10° 11° 20° > 21°
 SAMPLE LOCATION SUPPLY WATER, WASTEWATER RAW, TREATED
 (Circle One) Other _____

PARAMETERS (CHECK APPROPRIATE)

Bacti	<input type="checkbox"/>	NH3	<input type="checkbox"/>	Phenol	<input type="checkbox"/>
BOD	<input type="checkbox"/>	NO2 - 3	<input type="checkbox"/>	COD	<input type="checkbox"/>
TSS	<input type="checkbox"/>	TKN	<input type="checkbox"/>	PCB	<input type="checkbox"/>
Turb	<input type="checkbox"/>	T P	<input type="checkbox"/>	X Ray	<input type="checkbox"/>
Organics	<input type="checkbox"/>	O & G	<input type="checkbox"/>	Other	_____
VOA s	<input type="checkbox"/>	CN	<input checked="" type="checkbox"/>		

FLOW MEASUREMENT

None ☐
 Magmeter ☐
 Venturi ☐
 Parshall ☐
 V-Notch ☐
 Rectangular ☐
 Other _____

SIZE ☐ ☐ ☐
 Inches/Degrees

NO sulfide present

METALS

Tol	<input type="checkbox"/>	Diss	<input type="checkbox"/>
Cd	<input type="checkbox"/>		
Cu	<input type="checkbox"/>		
Cr (T)	<input type="checkbox"/>		
Cr (+6)	<input type="checkbox"/>		
Fe	<input type="checkbox"/>		
Hg	<input type="checkbox"/>		
Mn	<input type="checkbox"/>		
Ni	<input type="checkbox"/>		
Pb	<input type="checkbox"/>		
Sn	<input type="checkbox"/>		
Zn	<input type="checkbox"/>		
Other	_____		

LAB CODE Nº 55503
 PROJECT #
 STATION # NEW0101
 SAMPLE TYPE GRAB T _____ V _____
 COLLECTION DATE
 Y Y M M D D Y Y M M D D
9 0 0 2 1 0 3
 START END
 COLLECTION TIME
0930
 START END
 SAMPLE TEMP (°C) 06
 pH (SU) 08.7
 TOT Cl₂ (mg/l) 001
 SETTLEABLE SOLIDS (m/l) 0000
 COND = 1200 $\mu\text{mhos/cm}$
 FLOWMETER
 TOTALIZER
 START 74.637
 END 967.542
 MULTIPLIER X1

NOTE: preserved with ice & NaOH

*Unpreserved sample

Effluent Sample Type	Sample No <u>55503</u>	Date <u>2/3/80</u>
<u>Effluent (Chlorinated) #01</u>	Signature <u>John Smith</u>	
	Print Name and Title <u>John Smith, Inspector</u>	

Sample Tag (Front)

NAME OF UNIT AND ADDRESS <u>Environmental Samples, Inc.</u> <u>70 Main Street</u> <u>Boston, MA</u>	DATE-TIME-TEMP <u>90/2/3</u> <u>9:30 am</u> <u>NEW01</u>
SOURCE OF SAMPLE <u>Discharge #01 from</u> <u>WWTP (chlorinated)</u>	<u>55503</u>
SAMPLING CODE (FIRST, INITIAL, LAST NAME) <u>John Smith</u>	<u>Ice + NaOH</u> <u>100 mL</u> <u>700 Gyde</u>

(Back)

(grab sample)

Tested sample with lead acetate paper prior to pH adjustment. —
 No sulfide present.
 (See 40 CFR Part 136 Table II)

SAMPLE E

SOURCE Effluent WWTP #01 PERMIT NO. 010001
 CITY Boston, MA COLLECTOR John Smith
 WEATHER CLEAR RAIN SNOW (CIRCLE ONE)
 AIR TEMP (°C) < 0° 0° 10° 11° 20° > 21°
 SAMPLE LOCATION SUPPLY WATER WASTEWATER RAW, TREATED
 (Circle One) Other _____

PARAMETERS (CHECK APPROPRIATE)

Bacti	<input type="checkbox"/>	NH3	<input type="checkbox"/>	Phenol	<input checked="" type="checkbox"/>	Tol	<input type="checkbox"/>
BOD	<input type="checkbox"/>	NO2 - J	<input type="checkbox"/>	COD	<input type="checkbox"/>	Cd	<input type="checkbox"/>
TSS	<input type="checkbox"/>	TKN	<input type="checkbox"/>	PCB	<input type="checkbox"/>	Cu	<input type="checkbox"/>
Turb	<input type="checkbox"/>	T P	<input type="checkbox"/>	X Ray	<input type="checkbox"/>	Cr (T)	<input type="checkbox"/>
Organics	<input type="checkbox"/>	O & G	<input type="checkbox"/>	Other	_____	Cr (+6)	<input type="checkbox"/>
VOA's	<input type="checkbox"/>	CN	<input type="checkbox"/>			Fe	<input type="checkbox"/>

FLOW MEASUREMENT

None ☐ SIZE ☐ ☐
 Magmeter ☐
 Venturi ☐
 Parshall ☐
 V Notch ☐
 Rectangular ☐
 Other _____ Inches/Degrees

METALS

Tol ☐ Diss ☐
 Cd ☐
 Cu ☐
 Cr (T) ☐
 Cr (+6) ☐
 Fe ☐
 Mg ☐
 Mn ☐
 Ni ☐
 Pb ☐
 Sn ☐
 Zn ☐
 Other _____

*Unpreserved sample

LAB CODE NY 55864
 PROJECT #
 STATION # 111111
 SAMPLE TYPE GRAB T V
 COLLECTION DATE
 Y Y M M D D Y Y M M D D
9 0 0 0 0 0 9 0 0 0 0 0
 START END
 COLLECTION TIME
0930
 START END
 SAMPLE TEMP (°C) 26
 pH (SU) 0.7
 TOT Cl₂ (mg/l) 0.7
 SETTLEABLE SOLIDS (ml/l)
0.0 0.0
 FLOW METER
 TOTALIZER
 START 714.63
 END 962.54
 MULTIPLIER X1

note: preserved with ice & H₂SO₄

Official Sample Tape	Sample No. <u>55864</u>	Date <u>2/3/90</u>
<u>Effluent (Chlorinated) #01</u>	Signature <u>John Smith</u>	
	Print Name and Title <u>John Smith, Inspector</u>	

Sample Tag (Front)

NAME OF UNIT AND ADDRESS <u>Environmental Samples, Inc.</u> <u>70 Main Street</u> <u>Boston, MA</u>	DATE/TIME/TEMP <u>90/02/3</u> <u>7:37am</u> <u>60°F</u>
SOURCE OF SAMPLE <u>Discharge #01 from</u> <u>WWTP</u> <u>(chlorinated)</u>	ANALYST <u>John Smith</u>
SAMPLING CREW (NAME, INITIAL, LAST NAME) <u>John Smith</u>	ANALYSIS <u>402 + 4ml</u> <u>Total phenols</u>

(Back)

(grab sample)

40 CFR Part 136, Table II -
Specifies that
phenolic samples must be collected
in a glass container

EXAMPLE CHAIN OF CUSTODY RECORD FOR
SAMPLES A-E

PROJ NO		PROJECT NAME		NO OF CONTAINERS		REMARKS	
A.O.K.1		Effluent Sampling @ DDI					
SAMPLERS (Signature)							
John Smith							
STA NO	DATE	TIME	CODE	STATION LOCATION			
KIN001	2/3/90	930am	X	Discharge #1 - chlorinated	1		Sample No. 55800
KIN001	"	"	X	"	1		" 55801
KIN001	"	"	X	"	1		" 55802
KIN001	2/3/90	930am	X	"	1		" 55803
KIN001	2/3/90	937am	X	"	1		" 55804
<p>Note: This document should be carbonboxed for multiple copies</p>							
Relinquished by (Signature)		Date / Time		Received by (Signature)		Date / Time	
John Smith		2/3/90 1:15pm					
Relinquished by (Signature)		Date / Time		Received by (Signature)		Date / Time	
Relinquished by (Signature)		Date / Time		Received for Laboratory by (Signature)		Date / Time	
				Betty Shedd		2/3/90 1:15pm	
						Remarks	

(July 1, 1990)

ATTACHMENT B

**Daphnid (Ceriodaphnia dubia) Survival and Reproduction Test
and
Fathead Minnow (Pimephales promelas) Growth and Survival Test**

(July 1, 1990)

ATTACHMENT B

Chronic Toxicity Test Procedure and Protocol:

- o Daphnid (Ceriodaphnia dubia) chronic (and modified acute) static renewal survival and reproduction test.
- o Fathead Minnow (Pimephales promelas) chronic (and modified acute) static renewal larval growth and survival test.

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee must collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Chronic and acute toxicity data shall be reported as outlined in Section IX. The chronic fathead minnow and daphnid tests can be used to calculate the acute LC50 at the 48 hour exposure interval where both an acute (LC50) and a chronic (C-NOEC) test is specified in the permit.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

A chronic toxicity test sampling event is defined as three discharge (composite or grab) samples collected over the seven-day test period (see Section IV).

III. METHODS

Methods to follow are those recommended by EPA in:

Weber, C.I., et al, 1989. Short Term Methods For Estimating The Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Second Edition. Office of Research and Development, Cincinnati, OH, EPA/600/4-89/001.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

For each sampling event, three discharge samples shall be collected over a 7-day exposure period. An initial sample (day 0) is used to start the test. The additional two samples are collected for use at the start of day 3 and 5. Renewal of test concentrations is conducted daily with the most recently collected discharge sample. The initial (day 0) sample will be analyzed chemically. Day 3 and 5 samples will be held until test completion. If either the day 3 or 5 renewal sample is of

sufficient potency to cause lethality to 50 percent or more test organisms in any of the dilutions for either species, then a chemical analysis shall be performed on the appropriate sample(s) as well.

Aliquots shall be split from the samples, containerized and preserved (as per 40 CFR Part 136) for chemical and physical analyses. The remaining samples shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual chlorine (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I) EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for chronic toxicity analysis shall be collected at a point upstream of the discharge free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a standard dilution water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate surface water or standard dilution water of known quality with a hardness, pH, conductivity, alkalinity, and total suspended solids similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information see Section 7, page 24 of EPA/600/4-89/001.

VI. REGION I RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR THE DAPHNID (Ceriodaphnia dubia) SURVIVAL AND REPRODUCTION TEST¹

1. Test type:	Static, renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Photoperiod:	16 hr. light, 8 hr. dark
5. Test chamber size:	30 ml
6. Test solution volume:	15 ml
7. Renewal of test solutions:	Daily using most recently collected sample.
8. Age of test organisms:	Less than 24 hr.; and all released within an 8 hr. period of each other.
9. Number of neonates per test chamber:	1
10. Number of replicate test chambers per treatment:	10
11. Number of neonates per test concentration:	10
12. Feeding regime:	Feed 0.1 ml each of YTC and algal suspension per exposure chamber daily.
13. Aeration:	None
14. Dilution water: ²	Receiving water, other surface water, moderately hard synthetic water (prepared using either Millipore Milli-Q [®] or equivalent deionized water and reagent grade chemicals) or deionized water combined with mineral water.

15. Effluent concentrations:³ 5 concentrations and a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
16. Dilution factor: 0.5
17. Test duration: Until 60% of control females have three broods (may require 7 days).
18. End points: Survival and reproduction
19. Test acceptability: 80% or greater survival and an average of 15 or more young/female in the control solutions. At least 60% of surviving females in controls must have produced third brood.
20. Sampling requirements: For on-site tests, samples are collected daily and used within 24 hr. of the time they are removed from the sampling device. For off-site tests, a minimum of three samples are collected (i.e. days 0, 3, 5) and used for renewal (see Sec IV). Off-site tests samples must be first used within 48 hours of collection.
21. Sample volume required: Minimum 2 liters/day.

Footnotes:

1. Adapted from EPA/600/4-89/001.
2. Standard dilution water must have hardness requirements to generally reflect characteristics of the receiving water.
3. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. REGION I RECOMMENDED EFFLUENT TEST CONDITIONS FOR THE
FATHEAD MINNOW (Pimephales promelas) LARVAL SURVIVAL
AND GROWTH TEST

1. Test type:	Static, renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Photoperiod:	16 hr. light, 8 hr. dark
5. Test chamber size:	250 - 1000 ml
6. Test solution volume:	Minimum 200 ml/replicate
7. Renewal of test concentrations:	Daily using most recently collected sample.
8. Age of test organisms:	Newly hatched larvae less than 24 hr. old
9. No. larvae/test chamber and control:	15 (minimum of 10)
10. No. of replicate chambers/concentration:	4 (minimum of 3)
11. No. of larvae/concentration:	60 (minimum of 30)
12. Feeding regime:	Feed 0.1 ml newly hatched brine shrimp nauplii twice daily, 6 hrs. between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient larvae are added to provide an excess.
13. Cleaning:	Siphon daily, immediately before test solution renewal.
14. Aeration:	None, unless DO concentrations falls below 40% saturation. Rate should be less than 100 bubbles/min.

15. Dilution water: ²	Receiving water, other surface water, moderately hard synthetic water (prepared using either Millipore Milli-Q ^R or equivalent deionized and reagent grade chemicals) or deionized water combined with mineral water.
16. Effluent concentrations: ³	5 and a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
17. Dilution factor:	0.5
18. Test duration:	7 days
19. End points:	Survival and growth (weight)
20. Test acceptability:	80% or greater survival in controls: average dry weight of controls equals or exceeds 0.25 mg
21. Sampling requirements:	For on-site tests, samples are collected daily and used within 24 hours of the time they are removed from the sampling device. For off-site tests, a minimum of three samples are collected (i.e. days 0, 3, 5) and used for renewal (see Sec.IV). Off-site tests samples must be first used within 48 hours of collection.
22. Sample volume required:	Minimum 2.5 liters/day.

Footnotes:

1. Adapted from EPA/600/4-89/001.
2. Standard dilution water must have hardness requirements to generally reflect characteristics of the receiving water.
3. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VIII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>	<u>Effluent</u>	<u>Diluent</u>	<u>Minimum Detection Limit (mg/l)</u>
Hardness ^{*1}	x	x	0.5
Alkalinity	x	x	2.0
pH	x	x	--
Specific Conductance	x	x	--
Total Solids and Suspended Solids	x	x	--
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5
Total Residual Chlorine (TRC) ^{*2}	x	x	0.02

Total Metals

Cd	x		0.005
Cr, Ni	x		0.05
Pb, Cu	x	x	0.005
Zn, Al	x	x	0.02
Mg, Ca	x	x	0.05

In addition, the following chemical analyses shall be performed as part of each daily renewal procedure on each dilution and the controls.

<u>Parameter</u>	<u>Beginning of 24-hr Exposure Period</u>	<u>End of 24-hr Exposure Period</u>
Dissolved Oxygen	x	x
Temperature	x	
pH	x	
Specific Conductance	x ^{.3}	
Alkalinity	x ^{.3}	
Hardness	x ^{.3}	

Superscripts:

^{*1} Method 314 A (hardness by calculation) from APHA (1985) Standard Methods for the Examination of Water and Wastewater. 16th Edition.

^{*2} Total Residual Chlorine
Methods: either of the following methods from the 16th Edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses:

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titrimetric Method).

^{*3} These are performed on the 100% effluent and control samples only.

IX. TOXICITY TEST REPORT ELEMENTS

A report of results will include the following:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

Chronic

- Daily survival of test organisms in the controls and all replicates in each dilution. Survival data should be analyzed by Fisher's Exact Test prior to analysis of reproduction data.
- Young per female for all replicates in each dilution for Ceriodaphnia and weight for minnow larvae.
- Chronic test data shall undergo hypothesis testing to determine if the distribution of results is normal using the Shapiro-Wilks test. The variance must also be tested for homogeneity using Bartlett's Test. Then the endpoint estimates, NOEC and LOEC must be determined using Dunnett's Procedure, Bonferroni's T-Test, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test. The choice of test depends on the number of replicates and whether the variance is homogeneous or not. See EPA/600/4-87/028 for details. (Any printouts and graphical displays must be submitted, along with the name of the program, the date, and the author(s). When data is analyzed by hand, worksheets should be included.)
- C-NOEC: Chronic-No Observed Effect Concentration.
- LOEC: Lowest Observed Effect Concentration.
- MATC: Maximum Allowable Toxicant Concentration.

Acute (These data points are to be obtained 48-hrs into the chronic test.)

- Survival for each concentration and replication at time 24, and 48 hours; and
- LC50 and 95% confidence limits using one of the following methods in order of preference, Probit, Trimmed Spearman Karber, Moving Average Angle, or Graphical method; printout or copy of these calculations.

The Probit, Trimmed Spearman Karber, and Moving Average Angle methods of analyses can only be used when mortality of some of the test organisms are observed in at least two of the (% effluent) concentrations tested (i.e. partial mortality). If a test results in a 100% survival and 100% mortality in adjacent treatments ("all or nothing" effect), a LC50 may be estimated using the graphical method.

Chronic and Acute

- All chemical/physical data generated. (Include detection limits.)
- Raw data and bench sheets. (See sample data sheets, pp. 22-29.)
- Provide a description of dechlorination procedures (as applicable).
- Any other observations or test conditions affecting test outcome.

X. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by Part I of the permit.

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EXAMPLE

Data form for the Ceriodaphnia survival and reproduction test.
Daily record.

(The chart on the right was reduced to save space).

Discharger: _____

Location: _____

Date Sample Collected: _____

Analyst: _____

Test Dates: _____

Template No.: _____

Dilution Water: _____

Test Chambers (glass/plastic): _____

Food: _____

Test Temp: _____

Test Organisms (age): _____

Comments: _____

/ = Test organism alive
x = Test organism dead
0 = Number of live young
(-0) = Number of dead young
M = Lost or missing
y = Male

	0	20	30	40	50	60
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
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16						
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Example
Data form for the Ceriodaphnia survival and reproduction test.
Summary of data

Discharger: _____ Analyst: _____
Location: _____ Test Start-Date/Time: _____
Date Sample Collected: _____ Test Stop -Date/Time: _____

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	Total													

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	Total													

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	Total													

Example

Data form for the Ceriodaphnia survival and reproduction test.
Summary of data (Continued)

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	Total													

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	Total													

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
	1													
	2													
	3													
	4													
	5													
	6													
	7													
	8													
	Total													

Example

Data form for the fathead minnow larval survival and growth test. Routine chemical and physical determinations.

Discharger: _____ Test Dates: _____
Location: _____ Analyst: _____

Control:	Day							Remarks
	1	2	3	4	5	6	7	
Temp.								
D.O. Initial								
Final								
pH Initial								
Final								
Alkalinity								
Hardness								
Conductivity								
Chlorine								

Conc:	Day							Remarks
	1	2	3	4	5	6	7	
Temp.								
D.O. Initial								
Final								
pH Initial								
Final								
Alkalinity								
Hardness								
Conductivity								
Chlorine								

Conc:	Day							Remarks
	1	2	3	4	5	6	7	
Temp								
D.O. Initial								
Final								
pH Initial								
Final								
Alkalinity								
Hardness								
Conductivity								
Chlorine								

Example

Data form for the fathead minnow larval survival and growth test. Routine chemical and physical determinations.
(Continued).

Discharger: _____ Test Dates: _____
Location: _____ Analyst: _____

	Day							
Conc:	1	2	3	4	5	6	7	Remarks
Temp.								
D.O. Initial								
Final								
pH Initial								
Final								
Alkalinity								
Hardness								
Conductivity								
Chlorine								

	Day							
Conc:	1	2	3	4	5	6	7	Remarks
Temp.								
D.O. Initial								
Final								
pH Initial								
Final								
Alkalinity								
Hardness								
Conductivity								
Chlorine								

	Day							
Conc:	1	2	3	4	5	6	7	Remarks
Temp.								
D.O. Initial								
Final								
pH Initial								
Final								
Alkalinity								
Hardness								
Conductivity								
Chlorine								

Example

Survival data for fathead minnow larval survival and growth test.

Discharger: _____ Test Dates: _____
 Location: _____ Analyst: _____

		No. Survivors							
Conc:	Rep. No.	Day							
		1	2	3	4	5	6	7	Remarks
Control									
Conc:									
Conc:									
Conc:									
Conc:									
Conc:									
Conc:									

Comments:

(July 1, 1990)

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EXAMPLE

. Weight data for fathead minnow larval survival and growth test

Discharge: _____ Test Date(s): _____ Drying Temperature (°C): _____
Location: _____ Weighing Date: _____ Drying Time (h): _____
Analyst: _____

Conc :	Rep. No.	A Wgt. of boat (mg)	B Dry wgt: foil and larvae (mg)	B-A Total dry wgt of larvae (mg)	C No. of larvae	(B-A)/C Mean dry wgt of larvae (mg)	Remarks
Control							
Conc :							
Conc :							
Conc :							
Conc :							
Conc :							

Adapted from Hughes, et al., 1987.

EXAMPLE

Summary data for fathead minnow larval survival
and growth test.¹

Discharger: _____ Test Dates: _____
Location: _____ Analyst: _____

Treatment	Control					
No. live larvae						
Survival (%)						
Mean dry wgt of larvae (mg) + SD						
Temperature range (°C)						
Dissolved oxygen range (mg/L)						
Hardness						
Conductivity						

Comments:

ATTACHMENT C

Daphnid (Ceriodaphnia dubia and Daphnia pulex)
and
Fathead Minnow (Pimephales promelas) 48 Hour Acute Tests

ATTACHMENT C

Acute Toxicity Test Procedure and Protocol:

- ° Daphnids (Ceriodaphnia dubia and Daphnia pulex) definitive 48 hour acute test.
- ° Fathead Minnow (Pimephales promelas) definitive 48 hour acute test.

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee must collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Acute toxicity test data shall be reported as outlined in Section IX.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

An acute toxicity test sampling event is defined as a single discharge (composite or grab) sample.

III. METHODS

Methods should follow those recommended by EPA in:

Peltier, W., and Weber, C.I., 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Third Edition. Office of Research and Development, Cincinnati, OH. EPA/600/4-85/013.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

A discharge sample shall be collected. Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for chemical and physical analyses required. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual chlorine (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I) EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for acute toxicity analysis shall be collected at a point upstream of the discharge free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a standard dilution water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate standard dilution water of known quality with a hardness, pH, conductivity, alkalinity, and total suspended solids similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information see Section 6, page 22 of EPA/600/4-85/013.

VI. REGION I RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR THE DAPHNIDS (Ceriodaphnia dubia and Daphnia pulex) 48 HOUR ACUTE TESTS¹

1. Test type	Static, non-renewal
2. Temperature (°C)	25 ± 1°C
3. Light quality	Ambient laboratory illumination
4. Photoperiod	16 hour light, 8 hour dark
5. Test chamber size	Minimum 30 ml
6. Test solution volume	Minimum 25 ml
7. Age of test organisms	1-24 hours (neonates)
8. No. daphnids per test chamber	5
9. No. of replicate test chambers per treatment	4
10. Total no. daphnids per test concentration	20
11. Feeding regime	None
12. Aeration	None
13. Dilution water ²	Receiving water, other surface water, moderately hard synthetic water (prepared using either Millipore Milli-Q ^R or equivalent deionized water and reagent grade chemicals) or deionized water combined with mineral water.
14. Dilution factor	0.5
15. Number of dilutions ³	5 plus a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
16. Effect measured	Mortality - no movement of body or appendages on gentle prodding

- | | |
|----------------------------|---|
| 17. Test acceptability | 90% or greater survival of test organisms in control solution |
| 18. Sampling requirements | For on-site tests, samples must be used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must first be used within 48 hours of collection. |
| 19. Sample volume required | Minimum 2 liters |
-

Footnotes:

1. Adapted from EPA/600/4-85/013.
2. Standard prepared dilution water must have hardness requirements to generally reflect the characteristics of the receiving water.
3. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. REGION I RECOMMENDED TEST CONDITIONS FOR THE FATHEAD MINNOW
(Pimephales promelas) 48 HOUR ACUTE TEST¹

1. Test Type:	Static, non-renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Photoperiod:	16 hr light, 8 hr dark
5. Size of test vessels:	250-1000 ml
6. Volume of test solution:	Minimum 200ml/replicate
7. Age of fish:	1-14 days
8. No. of fish per chamber:	10 (not to exceed loading limits)
9. No. of replicate test vessels per treatment:	2
10. Total no. organisms per concentration:	20
11. Feeding regime:	None
12. Aeration:	None, unless DO concentration falls below 40% of saturation, at which time gentle single-bubble aeration should be started at a rate of less than 100 bubbles/min. (Routine DO check recommended.)
13. Dilution Water: ²	Receiving water, other surface water, moderately hard synthetic water (prepared using either Millipore Milli-Q ^R or equivalent deionized and reagent grade chemicals) or deionized water combined with mineral water.
14. Dilution factor:	0.5

15. Number of dilutions:³ 5 plus a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
16. Effect measured: Mortality-no movement on gentle prodding
17. Test acceptability: 90% or greater survival of test organisms in control solution
18. Sampling requirements: For on-site tests, samples must be used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must be first used within 48 hours of collection.
19. Sample volume required: Minimum 4 liters
-

Footnotes:

1. Adapted from EPA/600/4-85/013.
2. Standard dilution water must have hardness requirements to generally reflect characteristics of the receiving water.
3. When receiving water is used for dilution, an additional control made up of standard dilution water (0% effluent) is required.

VIII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>	<u>Effluent</u>	<u>Diluent</u>	<u>Minimum Detection Limit (mg/L)</u>
Hardness ^{*1}	x	x	0.5
Alkalinity	x	x	2.0
pH	x	x	---
Specific Conductance	x	x	---
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5
Total Residual Chlorine (TRC) ^{*2}	x	x	0.02

Total Metals

Cd	x		0.005
Cr, Ni	x		0.05
Pb, Cu	x	x	0.005
Zn, Al	x	x	0.02
Mg, Ca	x	x	0.05

Superscripts:

^{*1} Method 314A (hardness by calculation) from APHA (1985) Standard Methods for the Examination of Water and Wastewater. 16th Edition.

^{*2} Total Residual Chlorine

Methods: either of the following methods the 16th edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses.

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titrimetric Method).

IX. TOXICITY TEST REPORT

The following must be reported:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

- Survival for each concentration and replication at time 24, and 48 hours.
- LC50 and 95% confidence limits shall be calculated using one of the following methods in order of preference Probit, Trimmed Spearman Karber, Moving Average Angle, or the graphical method. All printouts (along with the name of the program, the date, and the author(s)) and graphical displays must be submitted. When data is analyzed by hand, worksheets should be submitted.

The Probit, Trimmed Spearman Karber, and Moving Average Angle methods of analyses can only be used when mortality of some of the test organisms are observed in at least two of the (% effluent) concentrations tested (i.e. partial mortality). If a test results in a 100% survival and 100% mortality in adjacent treatments ("all or nothing" effect), a LC50 may be estimated using the graphical method.

- All chemical/physical data generated (include detection limits).
- Raw data and bench sheets. (See sample data sheets, pp. 41-42.)
- Describe method of dechlorination where applicable.
- Any observations and test conditions which affected the outcome of testing.

X. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by of Part I of the NPDES permit.

1. EXPOSURE CHAMBER

Total vessel capacity: _____ ml
Test solution volume: _____ "
Water depth: constant - _____ cm
 cyclic - _____ to _____ cm.

3. AERATION

None: _____
Slow: _____ (ml/min)
Moderate: _____ "
Vigorous: _____ "
Beginning _____ (hour)

2. FEEDING SCHEDULE

Not fed: _____
Fed daily: _____
Fed irregularly (describe): _____

4. SCREENED ANIMAL ENCLOSURES

Not used: _____
Used: _____ (cm) Diameter

5. Condition of surviving organisms at end of test: _____

6. Comments: _____

Example
Check list on back of effluent toxicity data sheet.

ATTACHMENT D

**Sea Urchin (Arbacia punctulata) Fertilization Test
and
Algal (Champia parvula) Reproduction Test**

ATTACHMENT D

Chronic Toxicity Test Procedure and Protocol:

- ° Sea Urchin (Arbacia punctulata) 1 hour fertilization test.
- ° Algal (Champia parvula) 2 day exposure, 5 to 7 day recovery, reproduction test.

I. INTRODUCTION

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee must collect discharge samples and perform toxicity tests that are required by Part I of the NPDES permit. Chronic toxicity data shall be reported as outlined in Section IX.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

A sampling event is defined as a single discharge (composite or grab) sample.

III. METHODS

Methods to follow are those recommended by EPA in:

Weber, C.I., et al, 1988. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters To Marine and Estuarine Organisms, Office of Research and Development, Cincinnati, OH. EPA/600/4-87/028.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

A discharge sample shall be collected. Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual oxidants (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I) EPA/600/3-88/034, Section 8.7 provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for toxicity analysis shall be collected at a point away from the discharge which is free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a standard dilution water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate surface water or standard dilution water of known quality with a conductivity, salinity, total suspended solids, and pH similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information, see Section 7, page 19 of EPA/600/4-87/028.

Dilution water used for marine chronic toxicity shall be of sufficient quality to meet minimum acceptability of test results. (See Sections VI and VII.)

For Arbacia punctulata, an uncontaminated source of natural seawater, deionized water mixed with hypersaline brine, or artificial sea salts with a salinity of 30 o/oo \pm 2 o/oo must be used.

For Champia parvula, an uncontaminated source of natural seawater (30 o/oo S) or a combination of 50 percent 30 o/oo salinity natural seawater and 50 percent 30 o/oo salinity artificial seawater must be used.

VI. REGION I RECOMMENDED TEST CONDITIONS FOR THE SEA URCHIN
(Arbacia punctulata) FERTILIZATION TEST¹

1. Test type	Static, non-renewal
2. Salinity	30 o/oo \pm 2 o/oo
3. Temperature	20 \pm 1°C
4. Light quality	Ambient laboratory light during test preparation
5. Light intensity	10-20 uE/m ² /s, or 50-100 ft-c (Ambient Laboratory Levels)
6. Test vessel size	Disposable (glass) liquid scintillation vials (20 ml capacity), not pre-cleaned
7. Test solution volume	5 ml
8. Number of sea urchins	Pooled sperm from four males and pooled eggs from four females are used per test
9. Number of egg and sperm cells per chamber	About 2000 eggs and 5,000,000 sperm cells per vial
10. Number of replicate chambers per treatment	4 (minimum of 3)
11. Dilution water	Uncontaminated source of natural seawater; deionized water mixed with hypersaline brine or artificial sea salts
12. Dilution factor	0.5
13. Test duration	1 hour and 20 minutes
14. Effects measured	Fertilization of sea urchin eggs
15. Number of treatments per test ²	5 and a control. An additional dilution at the permitted effluent concentration (% effluent) is required.

16. Acceptability of test results

Recommended sperm:egg ratio must result in fertilization of minimum of 70% of the eggs in the control chambers.

17. Sampling requirements

For on-site tests, samples are to be used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must be first used within 48 hours of collection.

18. Sample volume required

Minimum 2 liters.

Footnotes:

1. Adapted from EPA/600/4-87/028.
2. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. REGION I RECOMMENDED TEST CONDITIONS FOR THE ALGAL (Champia parvula) SEXUAL REPRODUCTION TEST¹

1. Test Type	Static, non-renewal
2. Salinity	30 o/oo \pm 2 o/oo
3. Temperature	22 - 24°C
4. Photoperiod	16 hr. light, 8 hr. dark
5. Light intensity	100 uE/m ₂ /s (500 ft-c)
6. Light source	Cool-white fluorescent lights
7. Test chamber	200 ml polystyrene cups, or 250 ml Erlenmeyer flasks
8. Test solution volume	100 ml
9. Dilution water	30 o/oo salinity natural seawater, or a combination of 50% 30 o/oo salinity natural seawater and 50% 30 o/oo salinity artificial seawater.
10. Dilution factor	0.5
11. Number of dilutions ²	5 and a control. An additional dilution at the permitted effluent concentration (% effluent) is required.
12. Number of replicate chambers per treatment	4 (minimum of 3)
13. Number of organisms per test chamber	5 female branch tips and 1 male plant
14. Test duration	2-day exposure to effluent, followed by 5- to 7-day recovery period in control medium for cystocarp develop- ment.
15. Effects measured	Reduction in cystocarp produc- tion compared to controls

16. Acceptability of test

Control mortality must not exceed 20%.

Plant fragmentation in the controls or lower exposure concentrations should be minimal.

Control plants must average 10 or more cystocarps.

17. Sampling requirements

For on-site tests, samples must be used within 24 hours of the time they are removed from the sampling device.

Off-site test samples must be used within 48 hours of collection.

18. Sample volume

Minimum of 2 liters.

Footnotes:

1. Adapted from EPA/600/4-87/028.

2. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VIII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>			Minimum
	<u>Effluent</u>	<u>Diluent</u>	<u>Detection</u> <u>Limit(mg/L)</u>
pH	x	x	---
Specific Conductance	x	x	---
Salinity	x	x	PPT(o/oo)
Total Residual Oxidants ¹	x	x	0.02
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5

Total Metals

Cd	x	0.01
Cr, Ni	x	0.05
Pb, Zn, Cu	x	0.01
Al	x	0.02

Superscripts:

¹ Total Residual Oxidants

Methods: either of the following methods from the 16th Edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses:

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titrimetric Method).

IX. TOXICITY TEST REPORT ELEMENTS

A report of results will include the following:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

- Daily survival of test organisms in the controls and all replicates in each dilution if applicable.
- Chronic test data shall undergo hypothesis testing to determine if the distribution of results is normal using the Shapiro-Wilks test. The variance must also be tested for homogeneity using Bartlett's Test. Then the endpoint estimates, NOEC and LOEC must be determined using Dunnett's Procedure, Bonferroni's T-Test, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test. The choice of test depends on the number of replicates and whether the variance is homogeneous or not. See EPA/600/4-87/028 for details. (All printouts and graphical displays must be submitted, along with the name of the program, the date, and the author(s). When data is analyzed by hand, the worksheets should be submitted.)
- C-NOEC: Chronic-No Observed Effect Concentration.
- LOEC: Lowest Observed Effect Concentration.
- MATC: Maximum Allowable Toxicant Concentration.
- All chemical/physical data generated (include detection limits).
- Raw data and bench sheets. (See sample data sheets, pp. 53-57.)
- Any test conditions and observations affecting test outcome.

X. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by Part I of the permit.

Example

Data sheet for Champia parvula sexual reproduction test.
Receiving water summary sheet.

SITE _____

COLLECTION DATE _____

TEST DATE _____

LOCATION	INITIAL SALINITY	FINAL SALINITY	SOURCE OF SALTS FOR SALINITY ADJUSTMENT*

*e. natural seawater, GP2 brine, GP2 salts, etc
(include some indication of amount)

COMMENTS

Example
Data sheet for Champia parvula sexual reproduction test.
Cystocarp data sheet.

COLLECTION DATE _____ RECOVERY BEGAN (date) _____

EXPOSURE BEGAN (date) _____ COUNTED (date) _____

EFFLUENT OR TOXICANT _____

TREATMENTS (% EFFLUENT, μ G/L, or REC. WATER SITES)

REPLICATES	CONTROL						
------------	---------	--	--	--	--	--	--

A 1							
2							
3							
4							
MEAN							

B 1							
2							
3							
4							
MEAN							

C 1							
2							
3							
4							
MEAN							

OVERALL MEAN							
-----------------	--	--	--	--	--	--	--

Temperature _____

Salinity _____

Light _____

Source of Dilution Water _____

From Thursby and Steele, 1987.

Example

Data sheet for (1) fertilization test using Arbacia punctulata.

TEST DATE: _____

SAMPLE: _____

COMPLEX EFFLUENT SAMPLE: _____

COLLECTION DATE: _____

SALINITY/ADJUSTMENT: _____

PH/ADJUSTMENT REQUIRED: _____

PHYSICAL CHARACTERISTICS: _____

STORAGE: _____

COMMENTS: _____

SINGLE COMPOUND: _____

SOLVENT (CONC): _____

TEST CONCENTRATIONS: _____

DILUTION WATER: _____

CONTROL WATER: _____

TEST TEMPERATURE: _____

TEST SALINITY: _____

COMMENTS: _____

Example

Data sheet (2) for fertilization test using Arbacia punctulata

TEST DATE: _____

SAMPLE: _____

SPERM DILUTIONS:

HEMACYTOMETER COUNT, E: _____ $\times 10^4$ = SPM SOLUTION E = _____

SPERM CONCENTRATIONS: SOLUTION E $\times 40$ = SOLUTION A = _____ SPM
SOLUTION E $\times 20$ = SOLUTION B = _____ SPM
SOLUTION E $\times 5$ = SOLUTION C = _____ SPM

SOLUTION SELECTED FOR TEST (_____ = 5×10^7 SPM):

DILUTION: $\text{SPM} / (5 \times 10^7) = \frac{\text{_____}}{\text{_____}} \text{ DF}$
 $[(\text{DF}) \times 5] - 5 = \text{_____} + \text{SW, mL}$

FINAL SPERM COUNTS = _____

EGG DILUTIONS:

ORIGINAL EGG STOCK CONCENTRATION = $10 \times (\text{INITIAL EGG COUNT})$ = _____
VOLUME OF SW TO ADD TO DILUTE EGG STOCK TO 2000/mL: _____
(EGG COUNT) - 200 = _____
CONTROL WATER TO ADD EGG STOCK, mL = _____
FINAL EGG COUNT = _____

TEST TIMES:

SPERM COLLECTED: _____

EGGS COLLECTED: _____

SPERM ADDED: _____

EGGS ADDED: _____

FIXATIVE ADDED: _____

SAMPLES READ: _____

Data sheet (3) for fertilization test using *Arbacia punctulata*.

DATE TESTED: _____

SAMPLE: _____

TOTAL AND UNFERTILIZED EGG COUNT AT END OF TEST:

[illegible]

STATISTICAL ANALYSIS:

ANALYSIS OF VARIANCE: _____

CONTROL: _____

DIFFERENT FROM CONTROL (P): _____

COMMENTS: _____

ATTACHMENT E

**Mysid Shrimp (Mysidopsis bahia)
and
Inland Silverside (Menidia beryllina) Acute Tests**

ATTACHMENT E

Acute Toxicity Test Procedure and Protocol:

- o Mysid Shrimp (Mysidopsis bahia) definitive 48 hour test.
- o Inland Silverside (Menidia beryllina) definitive 48 hour test.

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee shall collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Acute toxicity data shall be reported as outlined in Section IX.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

A sampling event is defined as a single discharge (composite or grab) sample.

III. METHODS

Methods to follow are those recommended by EPA in:

Peltier, W., and Weber, C.I., 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Third Edition. Office of Research and Development, Cincinnati, OH. EPA/600/4-85/013.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

A discharge sample shall be collected. Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual oxidants (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I) EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for acute toxicity analysis shall be collected at a point away from the discharge which is free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a standard dilution water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate surface water or standard dilution water of known quality with a conductivity, salinity, total suspended solids, and pH similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information, see Section 6, page 22 of EPA/600/4-85/013.

VI. REGION I RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR
THE MYSID (Mysidopsis bahia) 48 HOUR TEST¹

1. Test type	Static, non-renewal
2. Salinity	25 ppt \pm 10 percent for all dilutions
3. Temperature ($^{\circ}$ C)	25 $^{\circ}$ C \pm 1 $^{\circ}$ C
4. Light quality	Ambient laboratory illumination
5. Photoperiod	16 hour light, 8 hour dark
6. Test chamber size	250 ml
7. Test solution volume	200 ml
8. Age of test organisms	1-5 days
9. No. Mysids per test chamber	10
10. No. of replicate test chambers per treatment	2
11. Total no. Mysids per test concentration	20
12. Feeding regime	Light feeding (2 drops concentrated brine shrimp nauplii, approx 100 nauplii/mysid) twice daily
13. Aeration ²	None
14. Dilution water	Natural seawater, synthetic salt water, or deionized water mixed with hypersaline brine.
15. Dilution factor	0.5
16. Number of dilutions ³	5 plus a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
17. Effect measured	Mortality - no movement of body or appendages on gentle prodding

18. Test acceptability	90% or greater survival of test organisms in control solution
19. Sampling requirements	For on-site tests, samples are used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must be first used within 48 hours of collection.
20. Sample volume required	Minimum 4 liters

Footnotes:

1. Adapted from EPA/600/4-85/013.
2. If dissolved oxygen falls below 40% saturation, aerate at rate of less than 100 bubbles/min. Routine DO checks are recommended.
3. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. REGION I RECOMMENDED TOXICITY TEST CONDITIONS FOR THE
INLAND SILVERSIDE (Menidia beryllina) 48 HOUR TEST¹

1. Test Type	Static, non-renewal
2. Salinity	25 ppt \pm 2 ppt
3. Temperature	25°C \pm 1°C
4. Light Quality	Ambient laboratory illumination
5. Photoperiod	16 hr light, 8 hr dark
6. Size of test vessel	250-1000 ml
7. Volume of test solution	Minimum 200ml/replicate
8. Age of fish	7-21 days
9. No. fish per chamber	10 (not to exceed loading limits)
10. No. of replicate test vessels per treatment	2
11. Total no. organisms per concentration	20
12. Aeration ²	None
13. Dilution water	Natural seawater, synthetic saltwater, or deionized water mixed with hypersaline brine
14. Dilution factor	0.5
15. Number of dilutions ³	5 plus a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
16. Effect measured	Mortality-no movement on gentle prodding.
17. Test acceptability	90% or greater survival of test organisms in control solution.

18. Sampling requirements

For on-site tests, samples must be used within 24 hours of the time they are removed from the sampling device. Off-site test samples must be used within 48 hours of collection.

19. Sample volume required

Minimum 4 liters

Footnotes:

1. Adapted from EPA/600/4-85/013.
2. If dissolved oxygen falls below 40% saturation, aerate at rate of less than 100 bubbles/min. Routine DO checks recommended.
3. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VIII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>	<u>Effluent</u> <u>Diluent</u>		<u>Minimum</u> <u>Detection</u> <u>Limit (mg/L)</u>
pH	x	x	---
Specific Conductance	x	x	---
Salinity	x	x	PPT(o/oo)
Total Residual Oxidants ¹	x	x	0.02
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5

Total Metals

Cd	x	0.01.
Cr, Ni	x	0.05
Pb, Zn, Cu	x	0.01
Al	x	0.02

Superscript:

¹ Total Residual Oxidants

Methods: either of the following methods from the 16th Edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses:

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titration Method).

IX. TOXICITY TEST REPORT

The following must be reported:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

- Survival for each concentration and replication at time 24, and 48 hours.
- LC50 and 95% confidence limits shall be calculated using one of the following methods in order of preference Probit, Trimmed Spearman Karber, Moving Average Angle, or the graphical method. All printouts (along with the name of the program, the date, and the author(s)) and graphical displays must be submitted. When data is analyzed by hand, worksheets should be submitted.

The Probit, Trimmed Spearman Karber, and Moving Average Angle methods of analyses can only be used when mortality of some of the test organisms are observed in at least two of the (% effluent) concentrations tested (i.e. partial mortality). If a test results in a 100% survival and 100% mortality in adjacent treatments ("all or nothing" effect), a LC50 may be estimated using the graphical method.

- All chemical data/physical generated (include detection limits).
- Raw data and bench sheets.
- Any other observations or test conditions affecting test outcome.

X. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by of Part I of the permit.

ATTACHMENT F

Mysid Shrimp (Mysidopsis bahia) 96 Hour Acute Test

ATTACHMENT F

Acute Toxicity Test Procedure and Protocol:

- o Mysid Shrimp (Mysidopsis bahia) definitive 96 hour test.

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocol described below. The permittee shall collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Acute toxicity data shall be reported as outlined in Section VIII.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

A sampling event is defined as a single discharge (composite or grab) sample.

III. METHODS

Methods to follow are those recommended by EPA in:

Peltier, W., and Weber, C.I., 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Third Edition. Office of Research and Development, Cincinnati, OH. EPA/600/4-85/013.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

A discharge sample shall be collected. Aliquots shall be split from the sample(s), containerized and preserved (as per 40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual oxidants (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I) EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for acute toxicity analysis shall be collected at a point away from the discharge which is free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a laboratory water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate or standard dilution water of known quality with a salinity, pH, conductivity, and total suspended solids similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information see Section 6, page 22 of EPA/600/4-85/013.

VI. REGION I RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR
THE MYSID (Mysidopsis bahia) 96 HOUR TEST¹

1. Test type	Static, renewal ²
2. Salinity	25 ppt \pm 10 percent for all dilutions
3. Temperature ($^{\circ}$ C)	25 $^{\circ}$ C \pm 1 $^{\circ}$ C
4. Light quality	Ambient laboratory illumination
5. Photoperiod	16 hour light, 8 hour dark
6. Test chamber size	250 ml
7. Test solution volume	200 ml
8. Renewal of test solutions	Daily
9. Age of test organisms	1-5 days
10. No. Mysids per test chamber	10
11. No. of replicate test chambers per treatment	2
12. Total no. Mysids per test concentration	20
13. Feeding regime	Light feeding (2 drops concentrated brine shrimp nauplii, approx 100 nauplii/mysid) twice daily
14. Aeration ³	None
15. Dilution water	Natural seawater, synthetic salt water, or deionized water mixed with hypersaline brine.
16. Dilution factor	0.5
17. Number of dilutions ⁴	5 plus a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
18. Effect measured	Mortality - no movement of body or appendages on gentle prodding

19. Test acceptability	90% or greater survival of test organisms in control solution
20. Sampling requirements	For on-site tests, samples are collected daily and used within 24 hours of the time they are removed from the sampling device. For off-site tests, samples must first be used within 48 hours of collection.
21. Sample volume required	Minimum 4 liters

Footnotes:

1. Adapted from EPA/600/4-85/013.
2. If the 96 hour mysid test is being performed concurrently with chronic toxicity test(s), samples shall be collected on days 0 and 3 of the 4-day exposure period. Renewal of test concentrations for toxicity testing shall be conducted with the most recently collected discharge sample. The initial (day 0) sample will be analyzed chemically. Day 3 sample will be held until test completion. If the renewal (day 3) sample is of sufficient potency to cause lethality to 50 percent or more test organisms in any of the dilutions, then a chemical analysis shall be performed on this sample as well.
3. If dissolved oxygen falls below 40% saturation, aerate at rate of less than 100 bubbles/min. Routine DO checks are recommended.
4. When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>	<u>Effluent</u>	<u>Diluent</u>	<u>Minimum Detection Limit(mg/L)</u>
pH	x	x	---
Specific Conductance	x	x	---
Salinity	x	x	PPT(o/oo)
Total Residual Oxidants ¹	x	x	0.02
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5

Total Metals

Cd	x	0.01
Cr, Ni	x	0.05
Pb, Zn, Cu	x	0.01
Al	x	0.02

In addition, the following chemical analyses shall be performed as part of each daily renewal procedure on each dilution and the controls.

<u>Parameter</u>	<u>Beginning of 24-hr Exposure Period</u>	<u>End of 24-hr Exposure Period</u>
Dissolved Oxygen	x	x
Temperature	x	
pH	x	
Specific Conductance	x	

Superscript:

¹ Total Residual Oxidants

Methods: either of the following methods from the 16th Edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses:

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titration Method).

VIII. TOXICITY TEST REPORT

The following must be reported:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

- Survival for each concentration and replication at time 24, 48, 72, and 96 hours.
- LC50 and 95% confidence limits shall be calculated using one of the following methods in order of preference Probit, Trimmed Spearman Karber, Moving Average Angle, or the graphical method. All printouts (along with the name of the program, the date, and the author(s)) and graphical displays must be submitted. When data is analyzed by hand, worksheets should be submitted.

The Probit, Trimmed Spearman Karber, and Moving Average Angle methods of analyses can only be used when mortality of some of the test organisms are observed in at least two of the (% effluent) concentrations tested (i.e. partial mortality). If a test results in a 100% survival and 100% mortality in adjacent treatments ("all or nothing" effect), a LC50 may be estimated using the graphical method.

- All chemical/physical data generated (include detection limits).
- Raw data and bench sheets.
- Any observations and test conditions which affected the outcome of testing.

IX. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by of Part I of the permit.

ATTACHMENT G

**Sheepshead Minnow (Cyprinodon variegatus)
Survival and Growth Test**

ATTACHMENT G

Chronic Toxicity Test Procedure and Protocol:

- o **Sheepshead Minnow (Cyprinodon variegatus) growth and survival test.**

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee must collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Chronic toxicity data shall be reported as outlined in Section VIII.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

A chronic toxicity sampling event is defined as three discharge (composite or grab) samples collected over the seven-day period (see Section IV).

III. METHODS

Methods to follow are those recommended by EPA in:

Weber, C.I., et al, 1988. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters To Marine and Estuarine Organisms, Office of Research and Development, Cincinnati, OH. EPA/600/4-87/028.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

For each sampling event, three discharge samples shall be collected over a 7-day exposure period. An initial sample (day 0) is used to start the test. The additional two samples are collected for use at the start of day 3 and 5. Renewal of test concentrations is conducted daily with the most recently collected discharge sample. The initial (day 0) sample will be analyzed chemically. Day 3 and 5 samples will be held until test completion. If either the day 3 or 5 renewal sample is of sufficient potency to cause lethality to 50 percent or more test organisms in any of the dilutions, then a chemical analysis shall be performed on the appropriate sample(s) as well.

Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH,

temperature, and total residual oxidants (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I), EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for toxicity analysis shall be collected at a point away from the discharge which is free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a laboratory water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate or standard dilution water of known quality with a salinity, pH, conductivity, and total suspended solids similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information see Section 7, page 19 of EPA/600/4-87/028.

Dilution water used for marine chronic toxicity shall be of sufficient quality to meet minimum acceptability of test results (see Section VI).

VI. REGION I RECOMMENDED TEST CONDITIONS FOR THE SHEEPSHEAD MINNOW (Cyprinodon variegatus) LARVAL GROWTH AND SURVIVAL TEST¹

1.	Test type	Static, renewal
2.	Salinity	20 o/oo TO 30 o/oo \pm 2 o/oo
3.	Temperature	25 \pm 2°C
4.	Light quality	Ambient laboratory light
5.	Light intensity	10-20 uE/m ² /s, or 50-100 ft-c (Ambient Laboratory Levels)
6.	Photoperiod	14 hr light, 10 hr darkness
7.	Test vessel size	300 - 1000 ml beakers or equivalent
8.	Test solution volume	250 - 750 ml/replicate (loading and DO restrictions must be met)
9.	Renewal of test solutions	Daily using most recently collected sample.
10.	Age of test organisms	Newly hatched larvae (less than 24 hr old
11.	Larvae/test chamber	15 (minimum of 10)
12.	Number of replicate chambers per treatment	4 (minimum of 3)
13.	Source of food	Newly hatched <u>Artemia</u> nauplii less than 24 hr old
14.	Feeding regime	Feed once a day 0.10 g wet wt <u>Artemia</u> nauplii per replicate on days 0-2; feed 0.15 g wet wt <u>Artemia</u> nauplii per replicate on days 3-6
15.	Cleaning	Siphon daily, immediately before test solution renewal
16.	Aeration ²	None
17.	Dilution water	Uncontaminated source of natural seawater; or hypersaline brine; or artificial seawater mixed with deionized water

18. Effluent concentrations ³	5 and a control. An additional effluent concentration (% effluent) is required.
19. Dilution factor	0.5
20. Test duration	7 days
21. Effects measured	Survival and growth (weight)
22. Acceptability of test	The average survival of control larvae is a minimum of 80%, and the average dry wt of unpreserved control larvae is a minimum of 0.6 mg, or the average dry wt of preserved control larvae is a minimum of 0.50 mg.
23. Sampling requirements	For on-site tests, samples are collected daily and used within 24 hours of the time they are removed from the sampling device. For off-site tests, samples must be first used within 48 hours of collection.

Footnotes:

¹ Adapted from EPA/600/4-87/028.

² If DO falls below 60% of saturation, aerate all chambers at a rate of less than 100 bubbles/min. Routine DO checks are recommended.

³ When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>	<u>Effluent</u>	<u>Diluent</u>	<u>Minimum Detection Limit (mg/L)</u>
pH	x	x	---
Specific Conductance	x	x	---
Salinity	x	x	PPT (o/oo)
Total Residual Oxidants ¹	x	x	0.02
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5

Total Metals

Cd	x	0.01
Cr, Ni	x	0.05
Pb, Zn, Cu	x	0.01
Al	x	0.02

In addition, the following chemical analyses shall be performed as part of each daily renewal procedure on each dilution and the controls.

<u>Parameter</u>	<u>Beginning of 24-hr Exposure Period</u>	<u>End of 24-hr Exposure Period</u>
Dissolved Oxygen	x	x
Temperature	x	
pH	x	
Specific Conductance	x	

Superscript:

¹ Total Residual Oxidants

Methods: either of the following methods from the 16th Edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses:

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titrimetric Method).

VIII. TOXICITY TEST REPORT ELEMENTS

A report of results will include the following:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

- Daily survival of test organisms in the controls and all replicates in each dilution if applicable.
- Chronic test data shall undergo hypothesis testing to determine if the distribution of results is normal using the Shapiro-Wilks test. The variance must also be tested for homogeneity using Bartlett's Test. Then the endpoint estimates, NOEC and LOEC must be determined using Dunnett's Procedure, Bonferroni's T-Test, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test. The choice of test depends on the number of replicates and whether the variance is homogeneous or not. See EPA/600/4-87/028 for details. All printouts and graphical displays must be submitted.
- C-NOEC: Chronic No Observed Effect Concentration.
- LOEC: Lowest Observed Effect Concentration.
- MATC: Maximum Allowable Toxicant Concentration.
- All chemical/physical data generated (include detection limits).
- Raw data and bench sheets. (See sample data sheets pp. 85-88.)
- Any other observations and test conditions that may have affected the outcome of the test.

IX. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by Part I of the permit.

Data forms for sheepshead minnow larval survival and growth test.
Daily record of larval survival and test conditions.

Test Dates: _____ Species: _____

Type Effluent: _____ Field _____ Lab _____ Test _____

Effluent Tested: _____

CONCENTRATION:																																
REPLICATE:								REPLICATE:								REPLICATE:								REPLICATE:								
DAYS	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
PLVE LARVAE																																
TEMP (°C)																																
SALINITY (‰)																																
D.O. (mg/l)																																
PLARVAE DRY WT																																

TIME FED						
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COMMENTS:

Adapted from: Hughes, Heber, Schimmel, and Berry, 1987.

EXAMPLE

Test Dates: _____ Species: _____

Type Effluent: _____ Field: _____ Lab: _____ Test: _____

Effluent Tested: _____

CONCENTRATION																																				
REPLICATE									REPLICATE									REPLICATE									REPLICATE									
DAYS	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7				
PLVE LARVAE																																				
TEMP (°C)																																				
SALINITY (°/o)																																				
D O (mg/l)																																				
PLARVAE DRY WT				MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT				MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT					MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT					MEAN WEIGHT / LARVAE (mg) ± S D			

CONCENTRATION:																																				
PLVE LARVAE																																				
TEMP (°C)																																				
SALINITY (°/o)																																				
D O (mg/l)																																				
PLARVAE DRY WT				MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT				MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT					MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT					MEAN WEIGHT / LARVAE (mg) ± S D			

CONCENTRATION.																																				
PLVE LARVAE																																				
TEMP (°C)																																				
SALINITY (°/o)																																				
D O (mg/l)																																				
PLARVAE DRY WT				MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT				MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT					MEAN WEIGHT / LARVAE (mg) ± S D					PLARVAE DRY WT					MEAN WEIGHT / LARVAE (mg) ± S D			

TIME							
FED							

COMMENTS:

(JULY 1, 1990)

Data forms for sheepshead minnow larval survival and growth test. Dry weights of larvae.

[illegible]

Printed on Recycled Paper

Example

Data forms for sheepshead minnow larval survival
and growth test. Summary of test results.

Test Dates _____ Species: _____

Effluent Tested _____

TREATMENT						
# LIVE LARVAE						
SURVIVAL (%)						
MEAN DRY WT / LARVAE (mg) \pm S D						
SIGNIF DIFF FROM CONTROL (o)						
MEAN TEMPERATURE (oC) \pm S D						
MEAN SALINITY 0/00 \pm S D						
AV. DISSOLVED OXYGEN (mg./L) \pm S D						

COMMENTS:

ATTACHMENT H

Chronic (and Modified Acute) Toxicity Test Procedure and Protocol:

- o Inland Silverside (Menidia beryllina) growth and survival test.

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable toxicity tests in accordance with the appropriate test protocols described below. The permittee must collect discharge samples and perform the toxicity tests that are required by Part I of the NPDES permit. Chronic toxicity data shall be reported as outlined in Section VIII.

II. TEST FREQUENCY AND SAMPLING REQUIREMENTS

See Part I of the NPDES permit for sampling location, sample type, test frequency, test species, and test date(s) requirements. Chain of Custody information should be provided for each sample tested.

A chronic toxicity sampling event is defined as three discharge (composite or grab) samples collected over the seven-day period (see Section IV).

III. METHODS

Methods to follow are those recommended by EPA in:

Weber, C.I., et al, 1988. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters To Marine and Estuarine Organisms, Office of Research and Development, Cincinnati, OH. EPA/600/4-87/028.

Any exceptions are stated herein.

IV. SAMPLE COLLECTION

For each sampling event, three discharge samples shall be collected over a 7-day exposure period. An initial sample (day 0) is used to start the test. The additional two samples are collected for use at the start of day 3 and 5. Renewal of test concentrations is conducted daily with the most recently collected discharge sample. The initial (day 0) sample will be analyzed chemically. Day 3 and 5 samples will be held until test completion. If either the day 3 or 5 renewal sample is of sufficient potency to cause lethality to 50 percent or more test organisms in any of the dilutions, then a chemical analysis shall be performed on the appropriate sample(s) as well.

Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if necessary) in the laboratory using sodium thiosulfate for subsequent toxicity testing. Grab samples must be used for pH, temperature, and total residual oxidants (as per 40 CFR Part 122.21).

The Methods for Aquatic Toxicity Identification Evaluations (Phase I), EPA/600/3-88/034, Section 8.7, provides detailed information regarding the use of sodium thiosulfate (i.e. dechlorination).

All samples held overnight shall be refrigerated at 4°C.

V. DILUTION WATER

Dilution water used for toxicity analysis shall be collected at a point away from the discharge which is free from toxicity or other sources of contamination. When using receiving water as the dilution water an additional control (0% effluent), made up from a laboratory water of known quality, will also be run.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate or standard dilution water of known quality with a salinity, pH, conductivity, and total suspended solids similar to that of the receiving water may be substituted AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S). It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. For further information see Section 7, page 19 of EPA/600/4-87/028.

Dilution water used for marine chronic toxicity shall be of sufficient quality to meet minimum acceptability of test results (see Section VI).

VI. REGION I RECOMMENDED TEST CONDITIONS FOR THE INLAND SILVERSIDE (Menidia beryllina) GROWTH AND SURVIVAL TEST¹

1. Test type	Static, renewal
2. Salinity	5 o/oo TO 32 o/oo \pm 2 o/oo
3. Temperature	25 \pm 2°C
4. Light quality	Ambient laboratory light
5. Light intensity	10-20 uE/m ² /s, or 50-100 ft-C (Ambient Laboratory Levels)
6. Photoperiod	16 hr light, 8 hr darkness
7. Test vessel size	300 - 1000 ml beakers or equivalent (glass test chambers should be used)
8. Test solution volume	250 ml minimum (loading and DO restrictions must be met)
9. Renewal of test solutions	Daily using most recently collected sample.
10. Age of test organisms	Seven to eleven days post hatch.
11. Larvae/test chamber	15 (minimum of 10)
12. Number of replicate chambers	4 (minimum of 3) per treatment
13. Source of food	Newly hatched <u>Artemia</u> nauplii less than 24 hr old
14. Feeding regime	Feed once a day 0.10 g wet wt <u>Artemia</u> nauplii per replicate on days 0-2; feed 0.15 g wet wt <u>Artemia</u> nauplii per replicate on days 3-6
15. Cleaning	Siphon daily, immediately before test solution renewal and feeding
16. Aeration ²	None

17. Dilution water	Uncontaminated source of natural seawater; or deionized water mixed with hypersaline brine or equivalent artificial seawater.
18. Effluent concentrations ³	5 and a control. An additional effluent concentration (% effluent) is required.
19. Dilution factor	0.5
20. Test duration	7 days
21. Effects measured	Survival and growth (weight)
22. Acceptability of test	The average survival of control larvae is a minimum of 80%, and the average dry wt of unpreserved control larvae is a minimum of 0.5 mg, or the average dry wt of preserved control larvae is a minimum of 0.43 mg.
23. Sampling requirements	For on-site tests, samples are collected daily and used within 24 hours of the time they are removed from the sampling device. For off-site tests, samples must be first used within 48 hours of collection.
24. Sample Volume Required	Minimum of 5 liters.

Footnotes:

- ¹ Adapted from EPA/600/4-87/028.
- ² If DO falls below 60% of saturation, aerate all chambers at a rate of less than 100 bubbles/min. Routine DO checks are recommended.
- ³ When receiving water is used for dilution an additional control made up of standard dilution water (0% effluent) is required.

VII. CHEMICAL ANALYSIS

The following chemical analyses shall be performed for each sampling event.

<u>Parameter</u>	<u>Effluent</u>	<u>Diluent</u>	<u>Minimum Detection Limit(mg/L)</u>
pH	x	x	---
Specific Conductance	x	x	---
Salinity	x	x	PPT(o/oo)
Total Residual Oxidants ¹	x	x	0.02
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5

Total Metals

Cd	x	0.01
Cr, Ni	x	0.05
Pb, Zn, Cu	x	0.01
Al	x	0.02

In addition, the following chemical analyses shall be performed as part of each daily renewal procedure on each dilution and the controls.

<u>Parameter</u>	<u>Beginning of 24-hr Exposure Period</u>	<u>End of 24-hr Exposure Period</u>
Dissolved Oxygen	x	x
Temperature	x	
pH	x	
Specific Conductance	x	

Superscript:

¹ Total Residual Oxidants

Methods: either of the following methods from the 16th Edition of the APHA (1985) Standard Methods for the Examination of Water and Wastewater must be used for these analyses:

Method 408-C (Amperometric Titration Method)-the preferred method;
Method 408-D (Ferrous Titrimetric Method).

VIII. TOXICITY TEST REPORT ELEMENTS

A report of results will include the following:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended.

Toxicity test data shall include the following:

Chronic

- Daily survival of test organisms in the controls and all replicates in each dilution.
- Chronic test data shall undergo hypothesis testing to determine if the distribution of results is normal using the Shapiro-Wilks test. The variance must also be tested for homogeneity using Bartlett's Test. Then the endpoint estimates, NOEC and LOEC must be determined using Dunnett's Procedure, Bonferroni's T-Test, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni adjustment. The choice of test depends on the number of replicates and whether the variance is homogeneous or not. See EPA/600/4-87/028 for details. All printouts and graphical displays must be submitted.
- C-NOEC: Chronic No Observed Effect Concentration.
- LOEC: Lowest Observed Effect Concentration.
- MATC: Maximum Allowable Toxicant Concentration.
- All chemical/physical data generated (include detection limits).
- Raw data and bench sheets. (See sample data sheets pp. 8-11.)
- Any other observations and test conditions that may have affected the outcome of the test.

Acute

- Survival for each concentration and replication at time 24, and 48 hours.
- LC50 and 95% confidence limits shall be calculated using one of the following methods in order of preference Probit, Trimmed Spearman Karber, Moving Average Angle, or the graphical method. All printouts (along with the name of the program, the date, and the author(s)) and graphical displays must be submitted. When data is analyzed by hand, worksheets should be submitted.

The Probit, Trimmed Spearman Karber, and Moving Average Angle methods of analyses can only be used when mortality of some of the test organisms are observed in at least two of the (% effluent) concentrations tested (i.e. partial mortality). If a test results in a 100% survival and 100% mortality in adjacent treatments ("all or nothing" effect), a LC50 may be estimated using the graphical method.

- All chemical data/physical generated (include detection limits).
- Raw data and bench sheets.
- Any other observations or test conditions affecting test outcome.

IX. REPORTING

Signed copies of the toxicity testing reports shall be submitted as required by Part I of the permit.

Any exceptions are stated herein.

(JULY 1991)

8

EXAMPLE

Data forms for inland silverside larval survival and growth test. Daily record of larval survival and test conditions.

Test Dates: _____ Species _____

Type Effluent: _____ Field _____ Lab _____ Test _____

Effluent Tested: _____

CONCENTRATION:																																			
REPLICATE										REPLICATE								REPLICATE								REPLICATE									
DAYS	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7			
#LIVE LARVAE																																			
TEMP (°C)																																			
SALINITY (‰)																																			
D O (mg/l)																																			
#LARVAE / DRY WT																																			
MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D								

CONCENTRATION:																																			
#LIVE LARVAE																																			
TEMP (°C)																																			
SALINITY (‰)																																			
D O (mg/l)																																			
#LARVAE / DRY WT																																			
MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D								

CONCENTRATION:																																			
#LIVE LARVAE																																			
TEMP (°C)																																			
SALINITY (‰)																																			
D O (mg/l)																																			
#LARVAE / DRY WT																																			
MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D									MEAN WEIGHT / LARVAE (mg) ± S D								

TIME FED							
----------	--	--	--	--	--	--	--

COMMENTS:

EXAMPLE (Continued).

Test Dates: _____ Species: _____

Type Effluent: _____ Field _____ Lab _____ Test _____

Effluent Tested: _____

CONCENTRATION:																																
REPLICATE:								REPLICATE:								REPLICATE:								REPLICATE:								
DAYS	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
#LIVE LARVAE																																
TEMP (°C)																																
SALINITY (‰)																																
D.O. (mg/l)																																
#LARVAE / DRY WT					MEAN WEIGHT / LARVAE (mg) ± S.D.								MEAN WEIGHT / LARVAE (mg) ± S.D.																MEAN WEIGHT / LARVAE (mg) ± S.D.			
CONCENTRATION:																																
#LIVE LARVAE																																
TEMP (°C)																																
SALINITY (‰)																																
D.O. (mg/l)																																
#LARVAE / DRY WT					MEAN WEIGHT / LARVAE (mg) ± S.D.								MEAN WEIGHT / LARVAE (mg) ± S.D.																MEAN WEIGHT / LARVAE (mg) ± S.D.			
CONCENTRATION:																																
#LIVE LARVAE																																
TEMP (°C)																																
SALINITY (‰)																																
D.O. (mg/l)																																
#LARVAE / DRY WT					MEAN WEIGHT / LARVAE (mg) ± S.D.								MEAN WEIGHT / LARVAE (mg) ± S.D.																MEAN WEIGHT / LARVAE (mg) ± S.D.			

TIME							
FED							

COMMENTS:

(July 1991)

EXAMPLE

Data forms for inland silverside larval survival and growth test. Dry weights of larvae.

Test Dates: _____ **Species:** _____

[illegible]

EXAMPLE Data forms for inland silverside larval
survival and growth test. Summary of test
results.

Test Dates: _____ Species: _____

Effluent Tested: _____

TREATMENT						
# LIVE LARVAE						
SURVIVAL (%)						
MEAN DRY WT./ LARVAE (mg) ± S.D.						
SIGNIF. DIFF. FROM CONTROL (o)						
MEAN TEMPERATURE (oC) ± S.D.						
MEAN SALINITY 0/00 ± S.D.						
AV. DISSOLVED OXYGEN (mg./L) ± S.D.						

COMMENTS: