RICHARD J. NEVES

wade et al. 1990

TENNESSEE WILDLIFE RESOURCES AGENCY

ELLINGTON AGRICULTURAL CENTER
P. O. BOX 40747
NASHVILLE, TENNESSEE 37204

MEMORANDUM

TO: Kentucky Lake Task Force Members

FROM: David McKinney ADM
Tennessee Wildlife Resources Agency

DATE: September 20, 1990

SUBJECT: Juvenile Mussel Bioassay Test Results

Please find attached materials related to whole effluent bioassay testing for Inland Paper's main process discharge and the ash pond discharge from the Tennessee Valley Authority New Johnsonville steam-electric facility, both of which discharge to the lower Tennessee River in Humphreys County.

Both effluents were tested utilizing (1) short-term nine-day static renewal with juvenile mussels, Anodonta imbecilis, as test organisms and (2) chronic seven-day static renewal with Ceriodaphnia dubia as test organisms. The results of these tests are summarized as follows:

Inland Container

LC_{50} (acute) mussel test 5.2% effluent
NOEC* (acute) mussel test 1.0% effluent
LC_{50} (chronic) Ceriodaphnia test 9.6% effluent
NOEC (chronic) Ceriodaphnia test 3.125% effluent

*The calculated chronic NOEC for Inland Container is 0.516% effluent for non-persistent waste and 0.052% effluent for persistent waste.

New Johnsonville Ash Pond

LC_{50} (acute) mussel test >100% effluent
NOEC (acute) mussel test >100% effluent
LC_{50} (chronic) Ceriodaphnia test >100% effluent
NOEC (chronic) Ceriodaphnia test >100% effluent

The enclosed results are from our most recent industrial Ceriodaphnia. Our approach has been to convert the nine-day acute juvenile test results to a calculated non-persistent chronic value (x 0.10) for comparison of the relative sensitivity of juvenile mussels and Ceriodaphnia. Using this method, our comparative tests to date indicate that juvenile mussels are more sensitive than Ceriodaphnia to Tennessee River Pulp and Paper whole effluent by a factor of no less than 25x; juvenile mussels are more sensitive than Ceriodaphnia to Inland Paper whole effluent by a factor of no less than 19x; and juvenile mussels and Ceriodaphnia both exhibit 100% survival in whole effluent from the ash pond at the New Johnsonville Steam Plant.

Recent discussions with EPA staff involved in toxicity testing and use of bioassay to evaluate whole effluent toxicity suggest that a more appropriate method of comparison for juvenile mussel and Ceriodaphnia results is the comparison of the no observable effect concentration (NOEC) for Ceriodaphnia with a calculated NOEC from the nine-day juvenile mussel test (see attachment). Utilizing this methodology, juvenile mussels are more sensitive to Tennessee River Pulp and Paper whole effluent by a factor of 28.4x; juvenile mussels are more sensitive to Inland Container whole effluent by a factor of 25.9x; juvenile mussels and Ceriodaphnia both exhibit 100% survival in whole effluent from the New Johnsonville Ash Pond.

We are continuing our efforts to have EPA adopt the mussel test for all NPDES permits for discharge of industrial wastewater to stream segments having mussel resources. We are also continuing with our joint project with Water Pollution Control and TVA to perform comparative bioassay on all industrial point source discharges to Kentucky Lake. We would appreciate any comments and recommendations on this work.

If we may further assist, please advise.

ADM:mjc
Attachments

David McKinney

COMPARATIVE SENSITIVITY OF JUVENILE MUSSELS (ANODONTA IMBECILLIS) AND CERIODAPHNIA TO PAPER COMPANY EFFLUENTS

INLAND CONTAINER TOXICITY

	Ceriodaphnia*	Anodonta imbecillis*
96-h Mortality in 100% Effluent	100%	100%
End-of test mortality in 100% Effluent	100%	100%
	(% Effluent)	(% Effluent)
96-hour LC50:	9.6	5.2 (dead only) 2.8 (dead and stressed)
9-day LC50: (mussels)		2.2 (dead) Test Endpoint
Chronic NOEC (Survival): (non-persistent waste)	6.25 Test Endpoint	0.22**
Chronic NOEC (Survival): (persistent waste)	0.63	0.02
Relative sensitivity:	6.25/0.22 = 28.4 (0.625/0.022 = 28.4)	

TENNESSEE RIVER PULP AND PAPER

	Ceriodaphnia*	Anodonta imbecillis*
96-h Mortality in 100% Effluent	0%	38% (dead only) 73% (dead and stressed)
End-of test mortality in 100% Effluent	0%	100%
	(% Effluent)	(% Effluent
96-hour LC50:	>100	>100 (dead only) 78.9 (dead and stressed)
9-day LC50: (mussels)		38.63 (dead) Test Endpoint
Chronic NOEC (Survival): (non-persistent waste)	>100 Test Endpoint	3.86**
Chronic NOEC (Survival): (persistent waste)	>10	0.386
Relative sensitivity:	>100/3.86 = >25.9 (>10/0.386 = >25.9)	

*Ceriodaphnia: 7-day chronic test; A. imbecillis: 9-day short-term (acute) test.
**Calculated (9-day LC50/10); 10 = Acute Chronic Ratio used to convert an acute LC50 to a chronic NOEC.

COMPARATIVE EVALUATION

OF

WHOLE EFFLUENT TOXICITY UTILIZING JUVENILE MUSSELS AND *CERIODAPHNIA*

FOR

INLAND CONTAINER AND NEW JOHNSONVILLE STEAM ELECTRIC FACILITY

MAY 1990

DON C. WADE
AUBREY D. MCKINNEY
JANET POSEY

JUVENILE FRESHWATER MUSSELS AS A LABORATORY TEST SPECIES FOR EVALUATING ENVIRONMENTAL TOXICITY

SUMMARY OF METHOD:

Thousands of juvenile mussels are made available through *in vitro* mass culture techniques of Isom and Hudson(1982) for solid phase toxicity testing. Six to ten day old mussels are exposed by chemical spiking of reference sediments or by using site collected sediments, for a period of nine days, to evaluate "safe" chemical concentrations or natural sediments supportive of aquatic life. Pollutants present in sediment pore water or sorbed to sediment particles are available through the feeding habits of the physiologically active young mussels. Toxicity endpoints of stress and mortality are readily obtained through direct observation. Exposed organisms are fed daily by adding indigenous algal concentrates. Sediments and/or spiked toxicants may be renewed daily to maximize exposure and to facilitate daily endpoint observations.

RECOMMENDED TEST PROTOCOL:

TEST SPECIES	: *Anodonta imbecilis* (freshwater mussel, Unionidae)
TEST TYPE	: Solid phase, static renewal
TEMPERATURE	: 24 ± 1° C
PHOTOPERIOD	: Dark
TEST CHAMBER VOLUME	: 250 ml
RENEWAL OF TEST SOLUTIONS	: Daily
AGE OF TEST ORGANISMS	: 6-10 days old*
NUMBER MUSSELS PER REPLICATE	: 15
REPLICATES PER CONCENTRATION	: 3
FEEDING REGIME	: Daily - Concentrated indigenous algae bloomed in dilution water. Also fresh silt (filtered through 100 mesh nitex to facilitate visual observations)
AERATION	: None
DILUTION WATER	: 5 µm bag filtered receiving water or synthetic water
TEST DURATION	: 9 days
EFFECT MEASURED	: Stress - impaired movement Mortality - absence of ciliary action or empty shells

*Younger mussels can be used; however, by 6 days the activity of young mussels has increased to a level which insures inclusion of viable individuals into the test.

ADVANTAGES OF USING JUVENILE MUSSELS:

1. The juvenile mussel is the first life stage having independent existence and fully exposed to toxicity within the aquatic environment.

2. Thousands of juveniles can be produced *in vitro* from a single gravid adult, providing sufficient mussels for conducting large scale toxicity testing.

3. Larval mussels (glochidia) are available for transformation into juveniles through a large segment of the year (i.e., during spring, summer, and autumn).

4. Mussels utilize both silt and materials filtered from the water in their diet. They are, therefore, ideal test organisms for evaluating toxicity in the total aquatic environment.

5. Juvenile mussels are shelled and can withstand rinsing, pipetting, and washing from one container to another, resulting in high survival in controls and low mortality in test treatments due to handling.

6. Juvenile mussels remain very active (mobile) during the entire test and normally do not close their shells except for brief periods. Even when closed, internal structures (heart, foot, cilia, digestive system) and movements are plainly visible, facilitating observations of condition (living active, living stressed, or dead).

RESULTS SUMMARY:

Test	Observation	Test Type
1. Manganese toxicity	LC50 = 36.2 mg Mn/L (Oxygenated)	9-day static, daily renewal
	LC50 = 19.6 mg Mn/L (Daily pulsed low DO - N_2 stripped to 0.5 mg/L)	9-day static, daily renewal
2. Aquathol K toxicity (herbicide)	Not toxic at 2X field application rate	48-h static, non-renewal*
3. 2,4-D toxicity (herbicide)	Not toxic at 2X field application rate	48-h static, non-renewal*
4. BTI toxicity (mosquito larvicide)	Not toxic at 2X field application rate	9-day static, daily renewal
5. Paper mill complex effluent	LC50 = 38.6% effluent	9-day static, daily renewal
6. Container Co. complex effluent	LC50 = 5.2% effluent	96-h static, daily renewal
	NOEC = 1% effluent	9-day static, daily renewal
7. Fossil plant ashpond complex effluent	NOEC = 100% effluent	9-day static, daily renewal
8. Coal mine effluent (wetland treated)	NOEC = 100% and 70% effluents	9-day static, daily renewal
9. Receiving water and sediment pore water: coal mine effluents	NOEC = 100% samples	9-day static, daily renewal

*Test period shortened to simulate natural field decay rate of herbicide.

CONCLUSIONS:

Results from several years of testing show that in vitro-transformed juvenile mussels exhibit: (1) long-term survivability under laboratory culture conditions, (2) resistance to damage incurred in following test protocols, (3) low mortality in controls during short-term (9-day)

testing, and (4) sensitivities greater than Ceriodaphnia for some toxicants. These factors indicate that juvenile mussels are promising test organisms for a wide range of ecotoxicological studies involving benthic fauna. These studies are also implicated where freshwater mussels occur, or where mussel populations have declined, or where endangered species exist with regard to certifying use of pesticides.

OTHER RELATED STUDIES:

1. 90-day continuous flow-through evaluation of manganese toxicity to juvenile mussels with a 30-day post exposure period for monitoring recovery - Concluded January 1990.

Preliminary Results:

98% survival in controls over 120 day test and recovery period.

Size (total shell length) impaired with increasing Mn concentrations during 90-day exposure (Control mean = 5.1 mm; 32 mg Mn/L treatment mean = 1.8 mm).

Growth recovery indicated during 30-day post exposure period (Control growth during recovery = 6%; Growth of 32 mg Mn/L treatment during recovery = 106%).

Preliminary Conclusions:

The highest concentration of manganese (32 mg Mn/L) was not fatal to juvenile mussels over a 90-day exposure; however, higher concentrations of manganese (especially the 20 and 32 mg Mn/L treatments) retarded shell growth. After 30 days of recovery (all mussels returned to ambient control water), the greatest increase in size (recovery) occurred in the 32 mg Mn/L treatment which had demonstrated the greatest growth retardation.

2. Toxicity of sediment interstitial waters (collected downstream of cotton spraying and in areas near industrial outfalls with mussels and where mussels have died) - Scheduled for late August 1990.

ABD-0156K

3 replicates / Treatment

Toxicity Results, 9-Day Exposure of Juvenile Anodonta imbecillis to Industrial Effluents, Wetland-Treated Coal Mine Seeps, and Receiving Water and Sediment Interstitial Water (Pore Water) for Combined Coal Mine Drainage, May 16-25, 1990.

Treatment*	Alive	Dead	% Mortality	Comments
1. Control (TR)	46	0	0	
2. JOF 100%	43	0	0	
3. JOF 50%	44	0	0	
4. IC 100%	0	45	100	< 24-h
5. IC 50%	0	45	100	< 24-h
6. HR(S) 70%	44	2	4	
7. HR(S) 50%	44	2	4	
8. HR(S) 35%	41	0	0	
9. IMP(1) 100%	45	0	0	
10. C. Bay 100%	45	0	0	
11. Control (PW)	41	0	0	
12. C. Bay (PW) 100%	45	0	0	
13. JOF 25%	40	3	7	
14. IC 5%	0	44	100	Day 6**
15. IC 1%	44	0	0	

*Control (TR) = Filtered Tennessee River water, Aquatic Research Lab.
JOF = Johnsonville Fossil Plant ashpond effluent, Kentucky Reservoir.
IC = Inland Container effluent, Kentucky Reservoir.
HR(S) = Hard Rock South mine seep, Fabius (100% effluent contains approx. 12 mg Mn/L).
IMP(1) = Impoundment No. 1, Fabius.
C. Bay = Receiving water, Coon Bay, Guntersville Reservoir.
Control (PW) = Control pore water, city reservoir, Haleyville, Al.
C.Bay (PW) = Pore water extracted from receiving stream, Coon Bay, Guntersville Reservoir.

**Day 2 - All slow, retarded movement.
Day 3 - 41% stressed or dead.
Day 4 - 91% stressed or dead.
Day 5 - 100% stressed or dead.
Day 6 - All dead.

INLAND CONTAINER — 188 mussels in Test

Dead Mussels Only	Dead and Stressed Mussels
96-h LC1 = 2.137% effluent	96-h EC1 = 0.979% effluent
LC50 = 5.161% effluent	EC50 = 2.750% effluent
LC99 = 12.467% effluent	EC99 = 7.719% effluent

9-day (short-term) NOEC = 1% effluent
9-day (short-term) LOEC = 5% effluent

Chronic (calculated) NOEC = 0.516% effluent (non-persistent waste)
= 0.052% effluent (persistent waste)

ABD-0166K

ABSTRACT

WPCA CONFERENCE
Chattanooga, TN
June 1990

SHORT-TERM TOXICITY TESTING OF JUVENILE FRESHWATER MUSSELS AS A SUPPLEMENTAL MEASURE TO INSURE ADEQUACY OF NATIONAL TOXICS CONTROL POLICY

Aubrey D. McKinney, Tennessee Wildlife Resources Agency, Nashville, Tennessee
Donald C. Wade, Tennessee Valley Authority, Muscle Shoals, Alabama

National toxics control policy promulgated by the U.S. Environmental Protection Agency (EPA) and implemented through the NPDES permits program recommends an integrated strategy for controlling discharge of toxic substances in complex effluents to less than toxic amounts. This strategy uses a whole effluent toxicity-based assessment procedure, in addition to the more traditional pollutant-specific assessment, and recommends conducting toxicity tests on a minimum of three species, initially, to insure that results adequately protect aquatic biota exposed in the natural environment. Recommended test species are normally those contained in acute and short-term chronic test procedures published by EPA.

Data are presented to suggest that testing of species contained in the EPA procedures may not adequately protect freshwater mussels for some complex effluents. These data summarize toxicity test results for juvenile (6-8 days old) freshwater mussels exposed to a metal, three pesticides, and a pulp and paper mill whole effluent sample. Test protocols are also described for conducting the freshwater mussel test. Testing of freshwater juvenile mussels, in addition to EPA test species, is strongly recommended where viable mussel beds and/or endangered mussel species occur near discharge points.

REPORT OF RESULTS
CHRONIC TOXICITY EVALUATIONS
OF DISCHARGES FROM INLAND PAPER
AND
TVA-NEW JOHNSONVILLE PLANT

MAY 16-23, 1990

Prepared for

TENNESSEE WILDLIFE RESOURCES AGENCY

NASHVILLE, TENNESSEE

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LIST OF TABLES

I. GENERAL INFORMATION

Facility: **Tennessee Wildlife Resources Agency**
Address: **P.O. Box 40747**
Contractor: **EMPE, Inc.**
Primary Contact: **Jo Ellen Flanagan**
Toxicity Testing Requirements or Requested: **Chronic Toxicity Evaluations**

II. SOURCE OF EFFLUENT AND DILUTION WATER

A. Effluent Samples

1. Sampling Point: **Inland Paper Mill**

Collection Date(s)	Time	Sample Type
5/15/90	NA	Grab

Pretreatment: **Due to low dissolved oxygen reading, daily aeration was necessary as provided for in EPA 600/4-89/001 test method 1000.0, paragraph 11.4.**
Physical/Chemical Data: **See Table 1**

2. Sampling Point: **TVA - New Johnsonville Plant**

Collection Date(s)	Time	Sample Type
5/15/90	NA	Grab

Pretreatment: **None**
Physical/Chemical Data: **See Table 1**

B. Dilution Water

Type: **20% La Croix in deionized water**
Pretreatment: **Aerated a minimum of 24 hours prior to use**
Physical/Chemical Data: **See Table 1**

III. TEST METHODS

A. Toxicity Test Method Utilized: **Cladoceran Survival and Reproduction Test, EPA/600/4-89/001, Method 1002.0**
Date of Initiation: **5/16/90**
Date of Termination: **5/23/90**
Deviations From Reference Method and Reason(s): **None**
Type and Volume of Test Chamber: **Disposable plastic 30 ml cups**
Volume of Solution Used per Chamber: **Approximately 20 ml**
Test Concentrations Evaluated: **0% (control) 3.125% to 100% (See Appendix for bench sheets)**
Number of Organisms per Test Chamber: **One (1)**
Number of Replicate Test Chambers per Treatment Group: **Ten (10)**

Acclimation of Test Organisms: **Cultured in control water**
Test Temperature: $25^{0} \pm 2^{0}C$

IV. TEST ORGANISMS

A. Taxonomic Name: *Ceriodaphnia dubia*
Age at Test Initiation: **Neonates (less than 24 hours old)**
Source: **Originated from EPA stock, Environmental Monitoring and Support Laboratories (EMSL), Cincinnati, Ohio. Neonates selected from EMPE individual monocultures established prior to test initiation**

V. QUALITY ASSURANCE

Standard Toxicant Utilized and Source: **Cadmium chloride - Sigma Chemical Company**

Date of Most Recent Test(s): **May 16, 1990**

Dilution Water Utilized in Test: **20% La Croix in deionized water**
Results: **Acceptable range for the test species. See attached bench sheets for results and reference toxicant information in the Appendix of this report.**

VI. RESULTS

Test results are analyzed utilizing Dunnett's procedure and/or Probit Analysis. The computer program which provided the statistical analysis of this data was made available through the EPA Environmental Monitoring and Support Laboratories, Cincinnati, Ohio.

Chemical/Physical Data - Range and Mean: .....**Table 1**

Ceriodaphnia dubia Survival and Reproduction Data:.....**Table 2**

Ceriodaphnia dubia Statistical Designations:.....**Table 3**

VII. SUMMARY

Chronic toxicity evaluations were conducted on whole effluent from the Inland Paper Mill and TVA's New Johnsonville Plant at the request of the Tennessee Wildlife Resources Agency (TWRA). *Ceriodaphnia dubia* served as the test species due to its reputation for being the most sensitive of test species to toxicants.

Within 24 hours of exposure to Inland's 25%, 50% and 75% effluent concentrations, acute toxicity was observed with the test species exhibiting total mortality at the aforementioned concentrations. After 48 hours of exposure to the same discharge, the 12.5% effluent concentration hosted only 20% survival. Therefore, the Inland 96-hour LC_{50} is statistically designated as 9.6% effluent. Whole effluent from the New Johnsonville TVA plant demonstrated no acute toxicity at any test concentration within 96 hours of exposure. Therefore, the New Johnsonville 96-hour LC_{50} is theoretically greater than 100% effluent concentration.

Upon termination of the seven (7) day chronic evaluations, organism survival and either reproduction or growth rates are examined and compared to a control group. Survival rates for *C. dubia* in the Inland discharge indicated a significant difference when compared to the control group for effluent concentrations above 6.25%. Diminished reproductive rates observed during the test period resulted in an NOEC value for *C. dubia* at 3.125% effluent. The New Johnsonville discharge exhibited no chronic affect in either survival or reproductive rates throughout the test period. Therefore the NOEC value for TVA's New Johnsonville plant lies at 100% effluent.

In summary, the Inland Paper Mill effluent was found to have a 96-hour LC_{50} value of 9.6% effluent and a 7-day NOEC value of 3.125% effluent concentration for *C. dubia*. Discharge from the New Johnsonville TVA Plant exhibited no acute or chronic toxicity and thus a 96-hour and 7-day NOEC value of 100% or greater is designated.

TABLE 1

CHEMICAL PHYSICAL DATA-RANGE AND MEAN
CHRONIC BIOASSAYS
MAY 16-23, 1990
TENNESSEE WILDLIFE RESOURCES AGENCY

Test Medium	D.O. (mg/l)	pH (su)	Alk. (mg/l)	Hdns. (mg/l)	Conductivity (umhos/cm)
Inland Pater	8.1* (7.8-8.8)	8.06 (7.6-8.3)	608.27 (513-649.8)	**	2208.57 (2180-2250)
New Johnsonville	8.51 (7.9-9.0)	7.54 (7.3-7.9)	100.16 (85.5-119.7)	136.80 (119.7-153.9)	259.71 (255-272)
Dilution Water	8.07 (7.8-8.6)	7.58 (7.3-7.9)	102.6 (85.5-119.7)	80.61 (68.4-102.6)	110 (110)

*Data reflects D.O. post-aeration values. Pre-aeration range = 1.0 to 3.2 and mean 2.03.

**Due to dark color of effluent, it was not possible to get a hardness reading with the titration method employed by the EMPE laboratory.

TABLE 2

INLAND PAPER MILL
SURVIVAL AND REPRODUCTION DATA
CHRONIC *Ceriodaphnia dubia*
MAY 16-23, 1990
TENNESSEE WILDLIFE RESOURCES AGENCY

Concentration (%)	Survival % for 7 Days	Mean Offspring Per Female
0 (control)	90	18.1
3.125	100	21.5
6.25	90	8.7
12.5	10	0
25	0	0
50	0	0
75	0	0

TABLE 3

INLAND PAPER MILL
STATISTICAL DESIGNATIONS FOR *Ceriodaphnia dubia* EVALUATIONS
MAY 16-23, 1990
TENNESSEE WILDLIFE RESOURCES AGENCY

Sample	LC_{50} 96-Hour (%)	NOEC 7 Days (%)
Discharge	9.6	3.125
Dilution Water	NT	100

NT = Not Toxic - LC_{50} value cannot be computed with one concentration variable.

TABLE 4

TVA - NEW JOHNSONVILLE PLANT
SURVIVAL AND REPRODUCTION DATA
CHRONIC-*Ceriodaphnia dubia*
MAY 16-23, 1990
TENNESSEE WILDLIFE RESOURCES AGENCY

Concentration (%)	Survival (%) for 7 Days	Mean offspring Per Female
0 (control)	90	18.1
6.25	100	21.6
12.5	100	22.1
25	100	19.9
50	100	15.9
100	100	22.1

TABLE 5

TVA - NEW JOHNSONVILLE PLANT
STATISTICAL DESIGNATIONS FOR *Ceriodaphnia dubia* EVALUATIONS
MAY 16-23, 1990
TENNESSEE WILDLIFE RESOURCES AGENCY

Sample	LC_{50} 96-Hour (%)	NOEC 7 Days (%)
Discharge	>100	100
Dilution Water	NT	100

NT = Not Toxic-LC_{50} value cannot be computed with one concentration variable.

APPENDIX

CHRONIC CERIODAPHNIA EFFLUENT TOXICITY TEST DATA SHEET
EMPE, INC. CONSULTING ENGINEERS

Industry: TWRA
NPDES No.:
Test Organism: Ceriodaphnia dubia
Life Stage: neonates
Type Bioassay: Chronic   Begin 5/16/90   End
Dilution Water Source: 20% LaCroix
Comments: Sample A = Inland Paper Mill

Effl. Conc.	Day No.	A	B	C	D	E	F	G	H	I	J		Total Live Young	No. Live Adults	Most by any Adult
(1) Control	3	0	0	0	0	0	0	0	X:2	0	0				
	4	0	0	4	2	3	0	2	|	0	3				
	5	8	0	8	1	4	0	1	|	0	7				
	6	3	7	0	16	0	11	4	|	1	0				
	7	12	11	12	15	15	14	10	↓	0	13				
(2) 3.125%	3	0	0	0	0	0	0	0	0		0				
	4	3	1	3	0	5	6	5	5	4	3				
	5	6	0	10	4	0	0	0	0	5	0				
	6	0	14	0	0	4		11	15	10					
	7	15	2	9	6	17	13	0	14	14	0				
(3) 6.25%	3	0	0	0	0	0	0	0	0	0	0				
	4	1	2	3	4	1	2	0	4	1	0				
	5	4	0	0	0	1	0	0	0	0	4				
	6	0	7	7	6	5	0	1	4	0	1				
	7	1	4	5	0	0	8	8	6	10	X(1)				
(4) 12.5%	3	X:1	X:2	X:2	X:2	X:2	X:2	X:2	X:2	0	0				
	4	|	|	|	|	|			|	0	0				
	5	|	|	|	|	|	|	|	|	0	0				
	6	|	|	|	|	|	|	|	|	0	0				
	7	|	|	|	|	|	|	|	|	X	0				
(5) 25%	3	X:1	X:1	X:1	X:1	X:1	X:1	X:1	X:1	X:1	X:1	X:1			
	4														
	5		-												
	6														
	7														
(6) 50%	3	X:1	X:1	X:1	X:1	X:1				X					
	4														
	5														
	6														
	7														

0 = 1-2
1 = 9/4
2 - [illegible] - 1 - [illegible]
3 = 1/4
4 - [illegible]

6 = [illegible]
7 - 8B

CHRONIC CERIODAPHNIA EFFLUENT TOXICITY TEST DATA SHEET
EMPE, INC. CONSULTING ENGINEERS

Industry: TWRA
NPDES No.:
Test Organism:
Life Stage:
Type Bioassay: Begin End
Dilution Water Source:
Comments: Sample A

Effl. Conc.	Day No.	Replicate A	B	C	D	E	F	G	H	I	J		Total Live Young	No. Live Adults	Most by any Adult
(7) ~~Controls~~ 75%	3														
	4														
	5														
	6														
	7														
	3														
	4														
	5														
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EMPE, INC.
PRELIMINARY DATA FORM

Client TWRA Room Temp ~25°

Sample Identification Sample A

Type Bioassay for Sample Use Chronic Tested By [illegible]

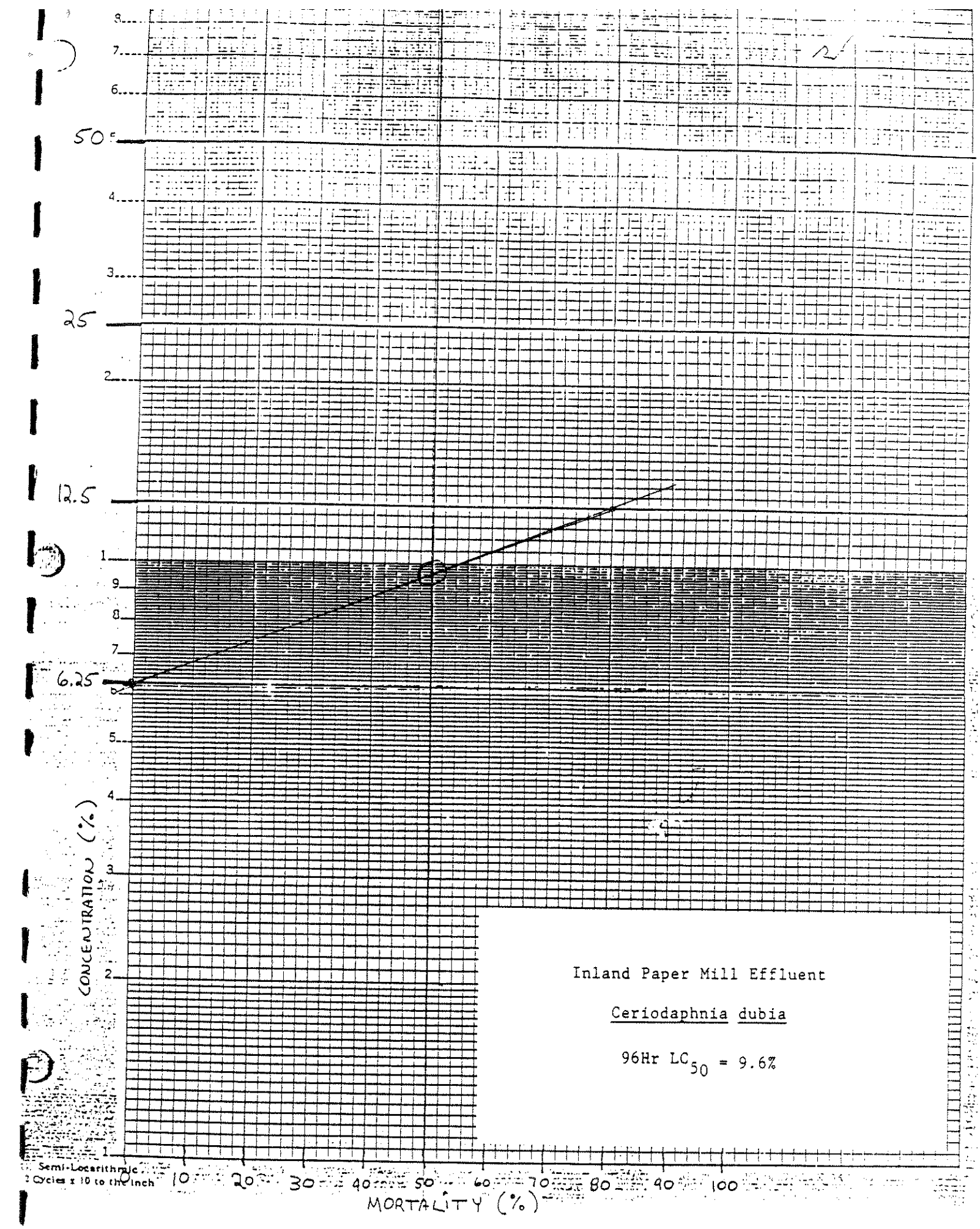
Comments [illegible]

100% Concentration:

	Zero Hr.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	5-16	5-17	5-18	5-19	5-20		5-22	
Dissolved Oxygen	10 mins 1.0 → 8.2	15 mins 1.2 → 7.8	15 mins 1.8 → 7.8	1.4 → 8.2	2.8 → 7.9	[illegible] 7.5	3.2 → 8.0	
pH	8.3	8.1	8.2	7.9	8.3	8.0	7.6	
Conductivity	2220	2180	2180	2200	2230	2200	2250	
Alkalinity	649.8	649.8	598.5	513.9 159.9	649.8	632.7	[illegible]	
Total Hardness	Not	Possible	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Date Collected	5-15	5-15	5-15	5-15	5-15			
Date Tested	5-16	5-17	5-18	5-19	5-20	5-21	5-22	
	JW	JW	JW	JW		JW	JW	

Control H_2O:

	Zero Hr.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Dissolved Oxygen	8.0	8.6	8.0	8.1	8.0	7.8	8.0	
pH	7.7	7.4	7.5	7.5	7.8	7.9	7.3	
Conductivity	113	110	110	110	110	110	110	
Alkalinity	122.6	119.7	85.5	102.6	102.6	102.6	102.6	
Total Hardness	[illegible]	102.6	68.4	85.5	68.4	85.5	[illegible]	


50
25
12.5
6.25
CONCENTRATION (%)
MORTALITY (%)
Inland Paper Mill Effluent
Ceriodaphnia dubia
96Hr LC50 = 9.6%
Semi-Logarithmic
2 Cycles x 10 to the Inch

TWRA Inland Paper C.dubia 7-Day Survival

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	.9000	.3162	35.1
2	10	1.0000	.0000	.0
3	10	.9000	.3162	35.1
4*	10	.1000	.3162	316.2
5*	10	.0000	.0000	.0
6*	10	.0000	.0000	.0
7*	10	.0000	.0000	.0

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -.217568
This difference corresponds to -24.17 percent of control

Between groups sum of squares = 14.285714 with 6 degrees of freedom.

Error mean square = .042857 with 63 degrees of freedom.

****
*
* Warning - the test for equality of variances *
* could not be computed as 1 or more of the *
* variances is zero. *
*
****

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	18.1000	9.8031	54.2
2	10	21.5000	10.5856	49.2
3*	10	8.7000	6.0562	69.6
4*	10	.0000	.0000	.0
5*	10	.0000	.0000	.0
6*	10	.0000	.0000	.0
7*	10	.0000	.0000	.0

*) the mean for this group is significantly less than
the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -6.215400
This difference corresponds to -34.34 percent of control

Between groups sum of squares = 5322.800000 with 6 degrees of freedom.

Error mean square = 34.976190 with 63 degrees of freedom.

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**************************************************
*                                                *
* Warning - the test for equality of variances   *
* could not be computed as 1 or more of the      *
* variances is zero.                             *
*                                                *
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```

CHRONIC CERIODAPHNIA EFFLUENT TOXICITY TEST DATA SHEET
EMPE, INC. CONSULTING ENGINEERS

Industry: TVRA
NPDES No.:
Test Organism: C. dubia
Life Stage: neonates
Type Bioassay: Chronic Begin 5/16/90 End
Dilution Water Source: 20% LaCroix
Comments: Sample B = TVA Plant in New Johnsonville

Effl. Conc.		Day No.	Replicate A	B	C	D	E	F	G	H	I	J	Total Live Young	No. Live Adults	Most by any Adult
Control	(1)	3	See Sample A Bench Sheet												
		4													
		5													
		6													
		7													
6.25	(2)	3													
		4	1	0	3	0	0	0	2	0	0	0			
		5	0	0	0	0	0	0	0	9	0	0			
		6	0	11	10	8	10	1	11	1	12	9			
		7	11	14	16	15	8	13	14	16	14	7			
12.5	(3)	3													
		4	3	0	0	1	0	2	0	0	0	0			
		5	0	0	0	0	0	0	0	0	0	0			
		6	0	7	8	9	10	8	9	8	13	10			
		7	9	12	16	13	15	11	13	14	14	16			
25	(4)	3													
		4	3	0	1	2	1	0	3	3	0	0			
		5	0	0	0	0	0	1	0	0	0	0			
		6	4	8	10	10	9	9	8	8	8	6			
		7	17	13	0	13	0	17	14	0	16	15			
50	(5)	3													
		4	0	0	0	0	3	3	0	0	2	0			
		5	0	0	0	0	0	1	0	0	0	0			
		6	9	9	8	9	0	7	7	3	3	0			
		7	15	14	12	7	8	0	13	1	12	13			
100	(6)	3													
		4	4	3	-	0	0	1	0	1	2	1			
		5	9	10	0	0	1	0	0	0	0	0			
		6	0	0	13	11	12	5	9	12	14	11			
		7	10	8	12	11	11	11	10	10	0	9			

5 - 4/13
6 - 4/13
7 - 4/13

EMPE, INC.
PRELIMINARY DATA FORM

Client TVRA Room Temp ~25°

Sample Identification Sample B

Type Bioassay for Sample Use CHRONIC Tested By LS + JSt

Comments

100% Concentration:

	Zero Hr. 5-16	Day 1 5/17	Day 2 5/18	Day 3 5/19	Day 4 5/20	Day 5 5/21	Day 6 5/22	Day 7
Dissolved Oxygen	8.9	9.0	8.4	8.6	8.6	8.2	7.9	
pH	7.3	7.4	7.5	7.4	7.8	7.9	7.5	
Conductivity	261	255	260	255	272	255	260	
Alkalinity	102.6	85.15	102.6	102.	102.6	119.7	[illegible]	
Total Hardness	136.8	153.9	136.8	119.	119.7	153.9	136.8	
Date Collected	5-15	5-15	5-15	5-15	5-15	5-15	5-15	
Date Tested	5-16	5/17	5-18	5/19	5-20	5-21	[illegible]	
	JN	JN	JB	JSt		JW	JW	

Control H_2O:

	Zero Hr. 5-16	Day 1 5/17	Day 2 5/18	Day 3 5/19	Day 4 5/20	Day 5 5/21	Day 6 5/22	Day 7
Dissolved Oxygen	8.0	8.6	8.0	8.1	8.0	7.8	8.0	
pH	7.7	7.4	7.5	7.5	7.8	7.9	7.3	
Conductivity	110	110	110	110	110	110	110	
Alkalinity	102.6	119.7	85.5	102.6	102.6	102.6	102.6	
Total Hardness	85.5	102.6	68.4	85.5	68.4	85.5	51.3	

/RA New Johnsonville C.dubia 7-Day Survival

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
= control	10	.9000	.3162	35.1
2	10	1.0000	.0000	.0
3	10	1.0000	.0000	.0
4	10	1.0000	.0000	.0
5	10	1.0000	.0000	.0
6	10	1.0000	.0000	.0

the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

inumum detectable difference for Dunnett's test = -.132791
iis difference corresponds to -14.75 percent of control

etween groups sum of squares = .083333 with 5 degrees of freedom.

rror mean square = .016667 with 54 degrees of freedom.

****
*
Warning - the test for equality of variances *
could not be computed as 1 or more of the *
variances is zero. *
*
****

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	18.1000	9.8031	54.2
2	10	21.6000	6.0590	28.1
3	10	22.1000	4.2804	19.4
4	10	19.9000	6.6240	33.3
5	10	15.9000	6.2263	39.2
6	10	22.1000	4.2544	19.3

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -6.664417
This difference corresponds to -36.82 percent of control

Between groups sum of squares = 317.950000 with 5 degrees of freedom.

Error mean square = 41.979630 with 54 degrees of freedom.

Bartlett's test p-value for equality of variances = .120

THE USE OF JUVENILE FRESHWATER MUSSELS AS A LABORATORY TEST SPECIES FOR EVALUATING ENVIRONMENTAL TOXICITY

Donald C. Wade, TVA Aquatic Research Lab, Decatur, Al; Robert G. Hudson, Dept. of Biology, Presbyterian College, Clinton, SC; Aubrey D. McKinney, TWRA, Nashville, Tn.

Hi Neil — Material for the SETAC Poster Session. I may not use all of these and will have additional color photographs — Call me if you want to make changes.

Thank you —
Don Wade

ABSTRACT

In response to discovery of manganese concentrations in Tennessee River sediment elutriates which were toxic to Ceriodaphnia dubia/affinis, short-term laboratory studies were conducted to determine toxicity of manganese to juvenile freshwater mussels (Anodonta imbecilis) and evaluate potential for manganese inhibition on reservoir mussel recruitment during drought (low flow/low dissolved oxygen) conditions. Following in vitro transformation of mussel glochidia into juveniles, toxicity testing protocols were developed and tests were performed on 8-day old juveniles. EC50 and LC50 values were determined for manganese ($MnSO_4$) under both oxygenated and low oxygen (N_2 stripped) conditions. Additional toxicity screening tests of two aquatic herbicides (2,4-D and Aquathol K), a paper mill effluent, and a mosquito larvicide (BTI) were conducted. Results are applicable for evaluating use of juvenile mussels as a benthic test organism for a wide range of ecotoxological studies.

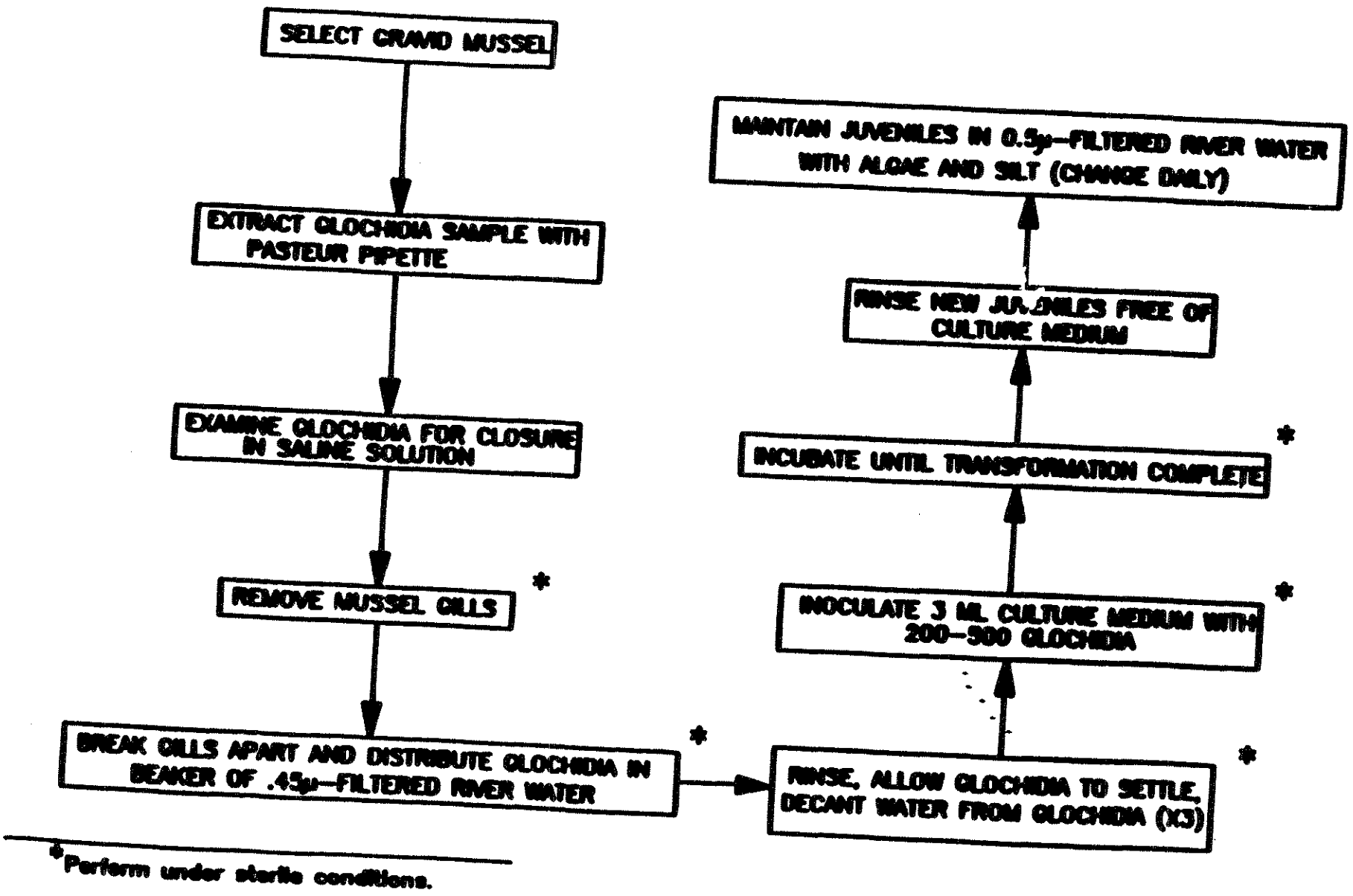
INTRODUCTION

Reports of dying mussels and diseased, blemished catfish captured in commercial haravests from Kentucky Lake (Tennessee River) led to toxicity investigations of sediment elutriates collected throughout the 295 kilometer-long reservoir. Discovery of toxicity to Ceriodaphnia and fathead minnows in deep, oxygen-poor habitats during extreme drought conditions, identification of manganese as the probable toxic agent, and examination of anthropologic activities on the reservoir led to short-term toxicity studies using juvenile (6-8 days old) freshwater mussels. A large number of juveniles were produced for testing, using the transformation procedures of Isom and Hudson (1982). Described below are methods, protocols, and results of studies conducted to examine toxic responses to: manganese (under both oxygenated and low dissolved oxygen conditions), three pesticides used to control mosquitoes and aquatic plants on the reservoir, and a paper mill effluent.

ADVANTAGES OF USING JUVENILE MUSSELS IN TOXICITY TESTING

- ☐ The juvenile mussel is the first life stage having independent existence and being fully exposed to toxicity within the aquatic environment.
- ☐ Thousands of juveniles can be produced *in vitro* from a single gravid adult, providing sufficient mussels for conducting large-scale toxicity testing.
- ☐ Larval mussels (glochidia) are available for transformation into juveniles through most of the year (i.e., *Anodonta imbecilis*).
- ☐ Mussels utilize both silt and materials filtered from the water in their diet. They are, therefore, ideal test organisms for evaluating toxicity in the total aquatic environment.
- ☐ Juvenile mussels are shelled and can withstand rinsing, pipetting, and washing from one container to another, resulting in low mortality in controls.
- ☐ Juvenile mussels remain very active during the entire test and normally do not close their shells except for brief periods. Even when closed, internal structures and movements are plainly visible, facilitating observations of condition (living, stressed, dead).

TRANSFORMATION OF FRESHWATER MUSSEL GLOCHIDIA INTO JUVENILES IN VITRO PROCEDURE



*Perform under sterile conditions.

COMPONENTS OF FRESHWATER MUSSEL ARTIFICIAL CULTURE MEDIUM*

COMPOUND	CONCENTRATION mg/L
Nonessential Amino Acids	
L – alanine	8.9
L – asparagine	13.2
L – aspartic acid	13.3
Glycine	7.5
L – glutamic acid	14.7
L – proline	11.5
L – serine	10.5
Essential Amino Acids	
L – arginine	105
L – cystine	24
L – histidine	31
L – isoleucine	52
L – leucine	52
L – lysine	58
L – methionine	15
L – phenylalaniine	32
L – threonine	48
L – tryptophane	10
L – tyrosine	36
L – valine	46
Inorganic Salts	
$CaCl_2$	120
$MgCl_2 \cdot 6H_2O$	1000
NaCl	1530
KCl	99
$NaHCO_3$	2200

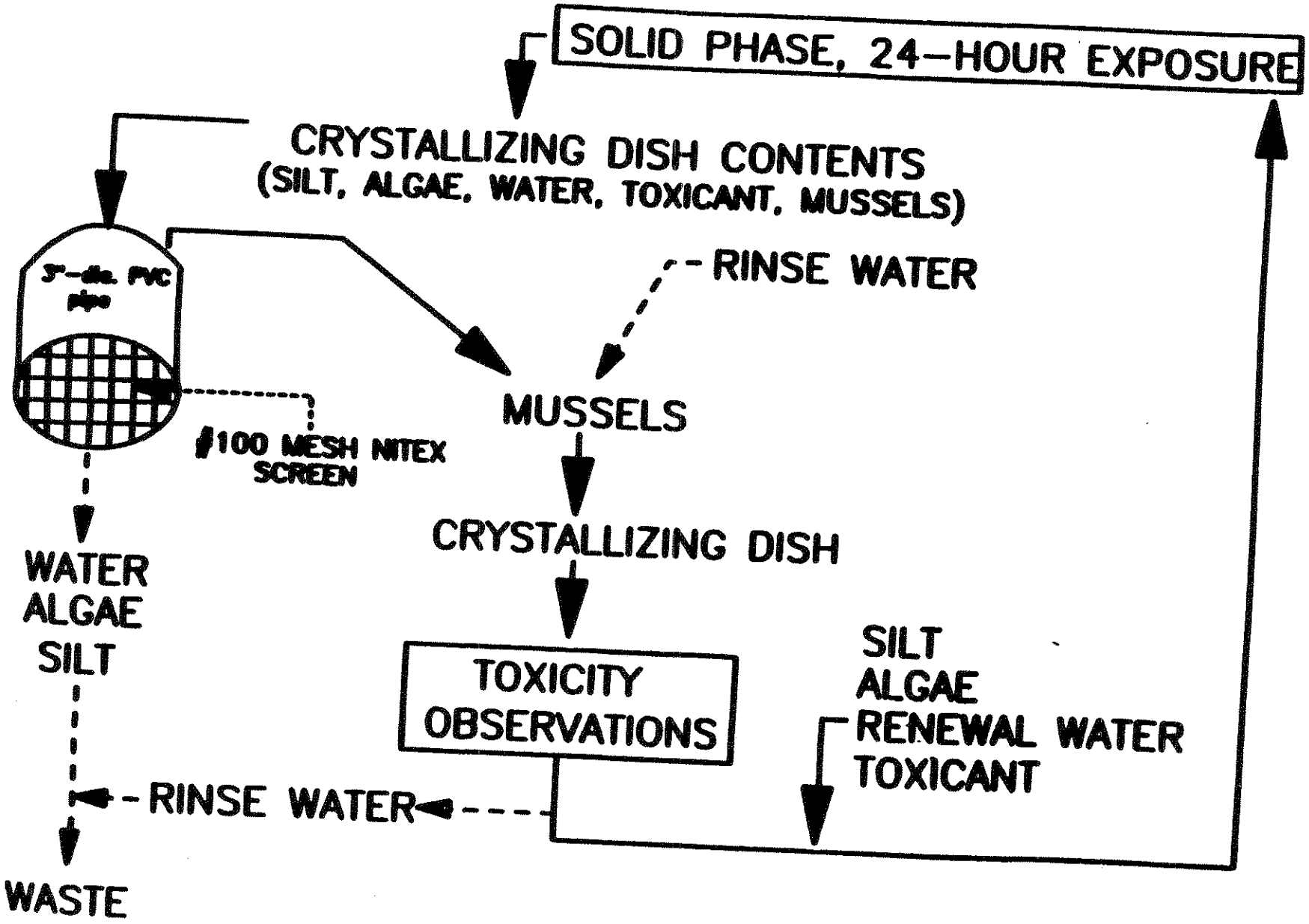
COMPOUND	CONCENTRATION mg/L
Vitamins	
Choline chlorine	1.0
Folic acid	1.0
Inositol	2.0
Nicotinamide	1.0
Calcium pantothenate	1.0
Pyridoxal	1.0
Riboflavin	0.1
Thiamine	1.0
Antibiotics & Antimycotics	(μg/L)
Antibiotic	
Carbenicillin	100
Gentamicin sulfate	100
Rifampin	100
Antimycotic	
Amphotericin B	5
Other Compounds	(mg/L)
Glucose	1000
Phenol red (optional)	10

* Isom and Hudson (1982)

RECOMMENDED CONDITIONS FOR SHORT-TERM TOXICITY TESTING ON FRESHWATER JUVENILE MUSSELS (ANODONTA IMBECILIS)

1. TEST TYPE:	Solid phase, static renewal
2. TEMPERATURE:	24 $\pm^{\circ}$1 C
3. PHOTOPERIOD:	Dark
4. TEST CHAMBER VOLUME:	250 mL
5. RENEWAL OF TEST SOLUTIONS:	Daily
6. AGE OF TEST ORGANISMS:	6–10 Days old
7. NO. MUSSELS PER REPLICATE:	15
8. REPLICATES PER CONCENTRATION:	2
9. FEEDING REGIME:	Daily – dilution water seeded with indigenous algae and bloomed, plus 800 mg silt/L (filtered through #100-mesh nitex screen)
10. AERATION:	None
11. DILUTION WATER:	5 µm-Filtered receiving water or synthetic water
12. TEST DURATION:	9 Days
13. EFFECT MEASURED:	Stress – impaired movement Mortality – absence of ciliary action or empty shells

JUVENILE MUSSEL TOXICITY TESTING
DAILY RENEWAL PROCEDURE



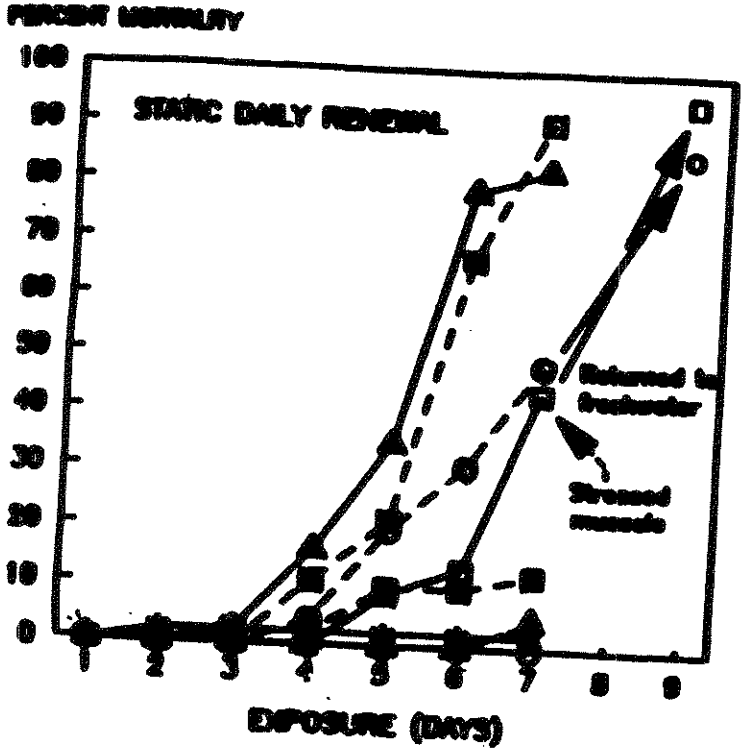
TOXICITY STUDIES CONDUCTED BY TVA
KENTUCKY RESERVOIR

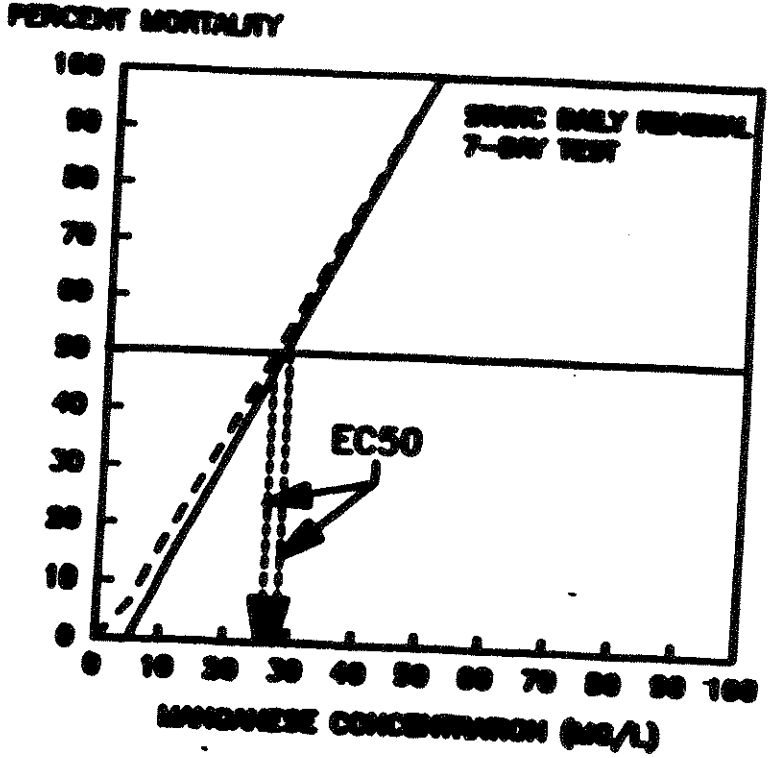
STUDY	OBSERVATIONS	TEST TYPE
1. Sediments, reservoir wide (1987)	Toxicity in deep, oxygen-poor areas	Chronic, Ceriodaphnia & fathead minnows
2. Sediments, selected locations (1987)	Less toxicity with improved DO	Chronic, Ceriodaphnia & fathead minnows
3. Toxicity characterization (1987)	Toxic agent = manganese	Acute, Ceriodaphnia (EPA, Duluth)
4. Manganese toxicity,* screening (1988)	Toxic between 5 & 50 mg/L	Short-term (7 days), juvenile mussels
5. Manganese toxicity, definitive (1989)	LC50 = 36.2 mg/L (oxygenated) LC50 = 19.6 mg/L (low DO)	Short-term (9 days), juvenile mussels
6. 2,4-D toxicity,* screening (1988)	Not toxic ● 2X applied conc.	Acute (48-h), juvenile mussels
7. Aquathol K toxicity,* screening (1988)	Not toxic ● 2X applied conc.	Acute (48-h), juvenile mussels
8. BTI toxicity (1989)	Not toxic ● 2X applied conc.	Short-term (9 days), juvenile mussels
9. Paper mill whole effluent toxicity (1989)	LC50 = 38.6%, not toxic ● 100:1 dil.	Short-term (9 days), juvenile mussels
10. Manganese toxicity, subchronic (1989)	Testing underway	90-day, flow-through, juvenile mussels

*First toxicity studies to test juvenile freshwater mussels.

SCREENING OF MANGANESE TOXICITY TO FRESHWATER MUSSELS (8-DAY OLD ANODONTA IMBECILIS)

OXYGENATED AND PULSED LOW DO* CONDITIONS

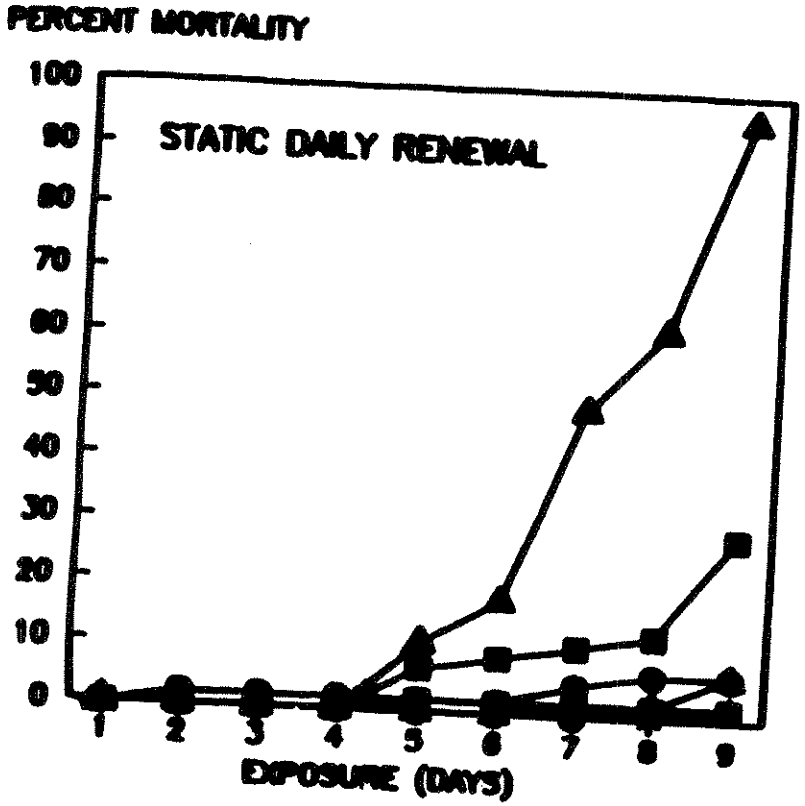




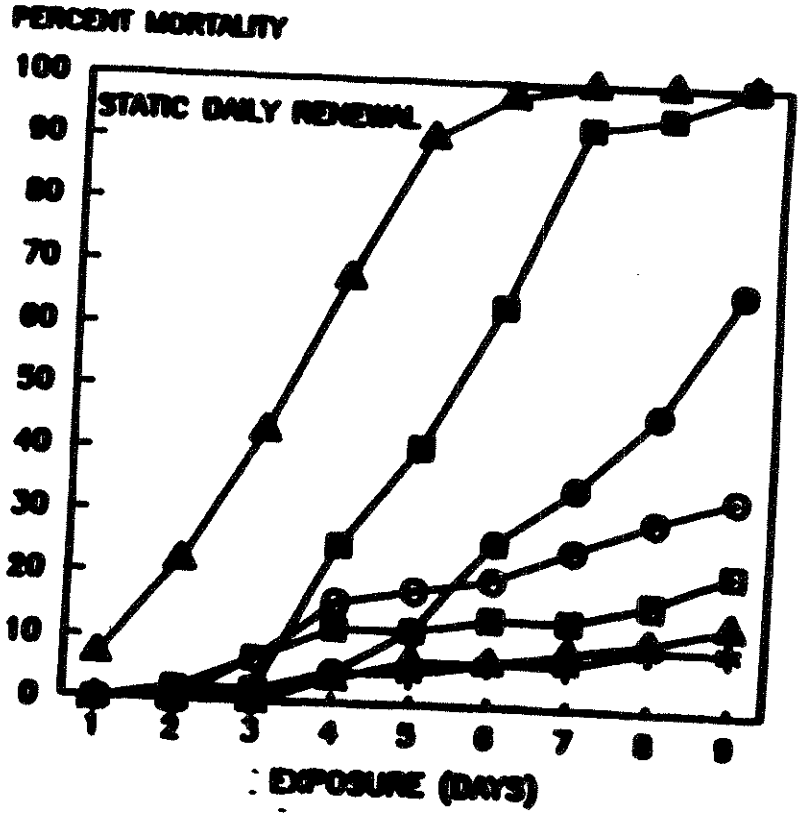
* Pulsed to ≤ 0.5 mg/L

(8-DAY OLD ANODONTA IMBECILIS)

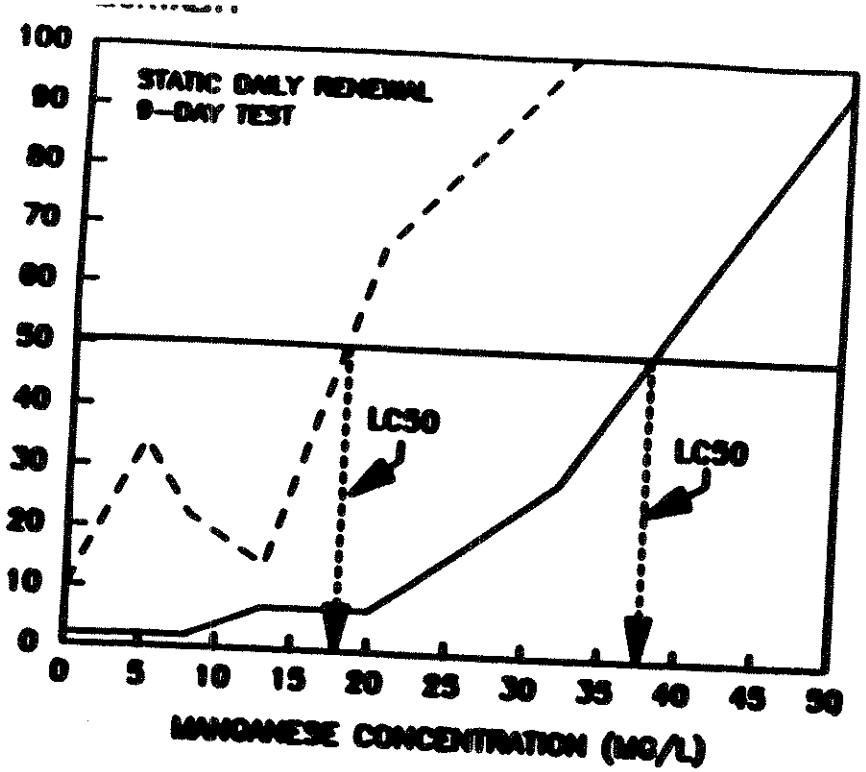
OXYGENATED CONDITIONS (DO > 7 MG/L)



PULSED LOW DO CONDITIONS (DO ≤ 0.5 MG/L)



CONTROL
Mn 5 MG/L
Mn 8 MG/L
Mn 13 MG/L
Mn 20 MG/L
Mn 32 MG/L
Mn 50 MG/L



PROBIT ANALYSIS

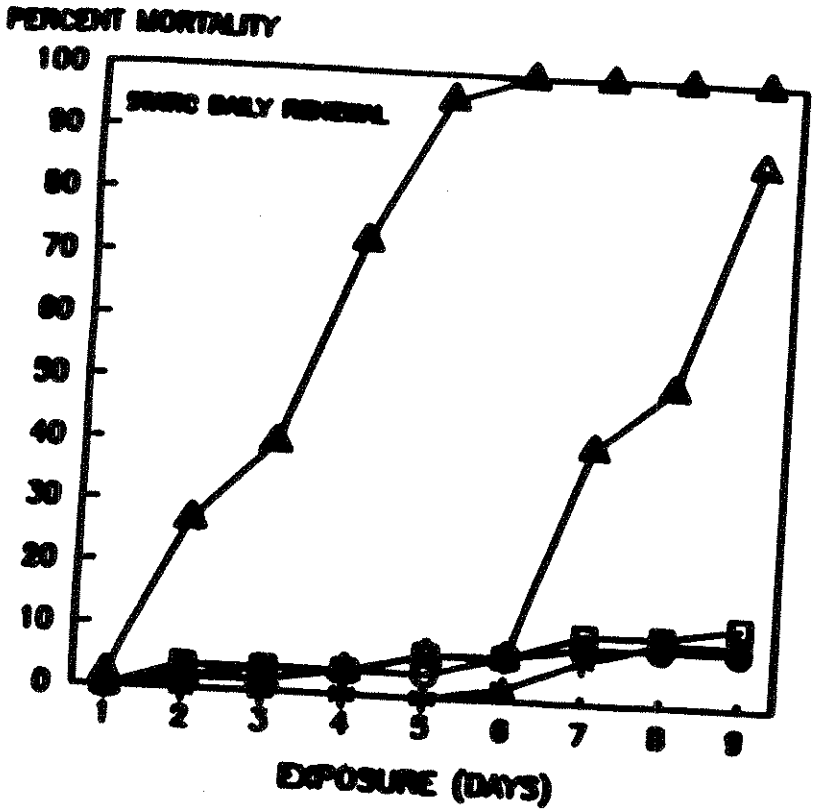
Oxygenated

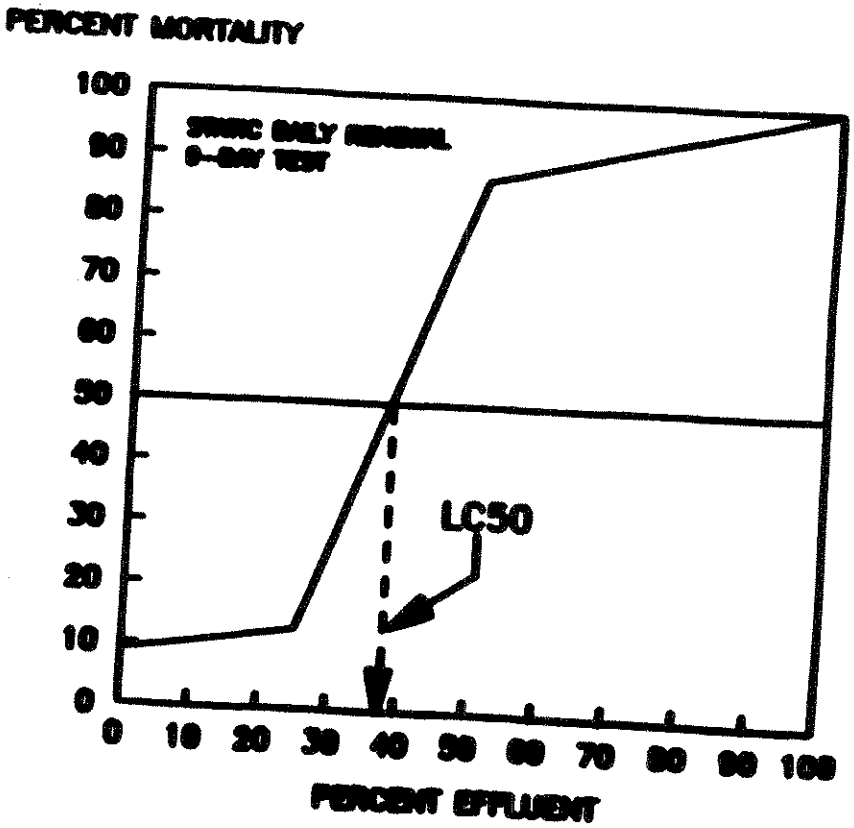
Point	Concentration mg/L	95% Confidence Limits Lower	Upper
LC1	23.07	17.99	28.31
LC50	36.19	33.56	38.88
LC99	56.77	50.11	71.76

Pulsed low DO

Point	Concentration mg/L	95% Confidence Limits Lower	Upper
LC1	15.06	13.13	16.24
LC50	19.55	18.77	20.42
LC99	24.49	23.27	25.86

(6-DAY OLD ANODONTA IMBECILIS)





PROBIT ANALYSIS

Point	Concentration %	95% Confidence Limits Lower	Upper
LC1	20.41	18.62	22.50
LC50	38.63	34.58	43.46
LC99	73.10	64.19	83.94

TOXICITY OF PAPER MILL EFFLUENT TO 6-DAY OLD FRESHWATER MUSSELS (*ANODONTA IMBECILIS*) AND *CERIODAPHNIA DUBIA*

SHORT-TERM/* ACUTE TESTS

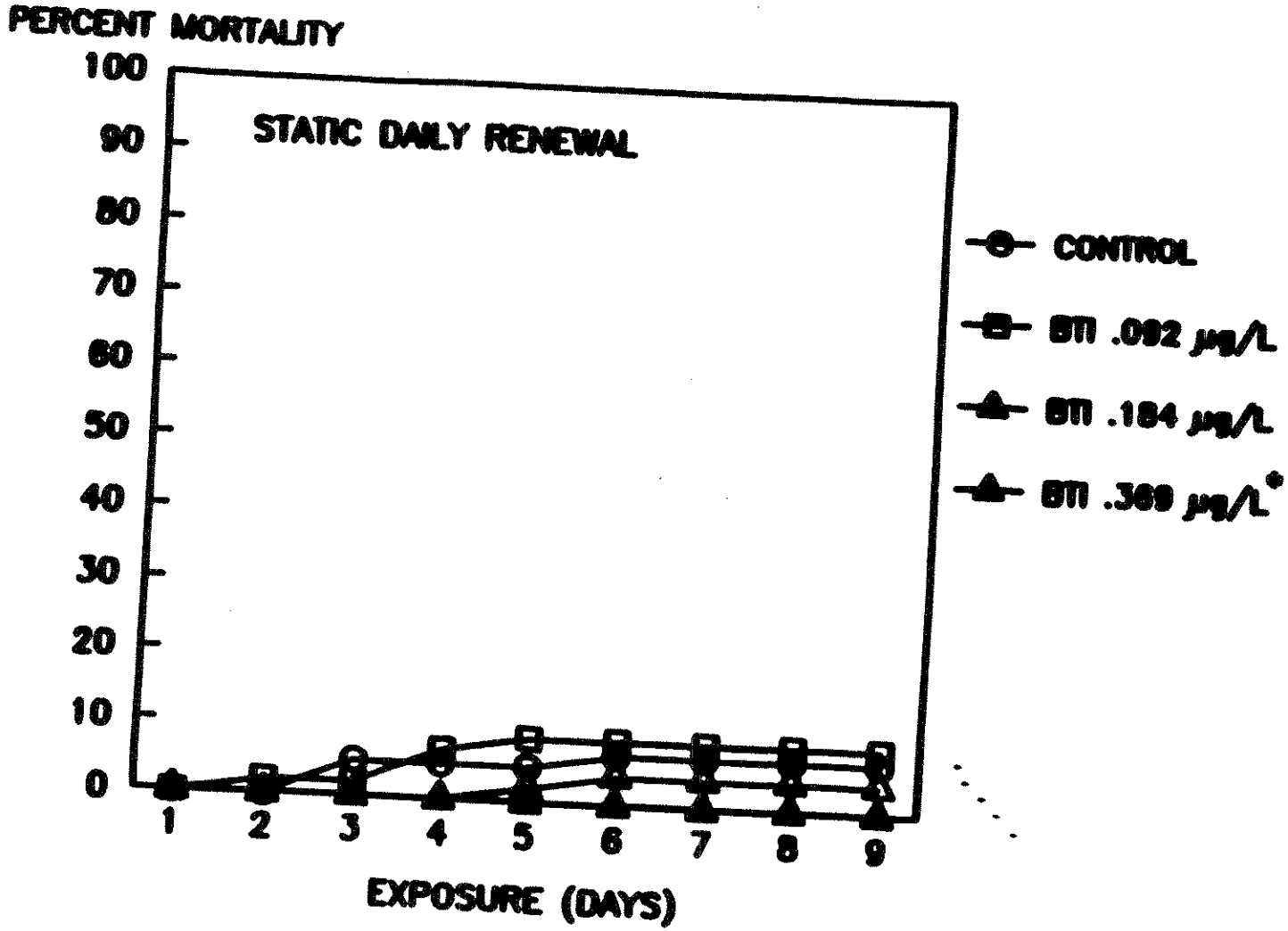
	TEST TYPE	EXPOSURE	LC50
Anodonta imbecilis	Static, daily renewal	9 days	38.6%
Ceriodaphnia dubia	Static, daily renewal	96-h	>100%

CHRONIC TESTS*

	TEST TYPE	EXPOSURE	LOEC	NOEC
Anodonta imbecilis	NT**	NT**	NT**	NT**
Ceriodaphnia dubia	Static, daily renewal	7 days	50%	25%

* Split samples: *Anodonta imbecilis* - TVA; *Ceriodaphnia* - EMPE, Nashville
**NT = Not tested.

BTI TOXICITY TO 6-DAY OLD JUVENILE MUSSELS (ANODONTA IMBECILIS)



*Two times field application rate.

SUMMARY OF TOXICITY DATA FOR JUVENILE FRESHWATER MUSSELS (ANODONTA IMBECILIS)

TOXICANT*	LC1	LC50	LC99
Manganese (as $MnSO_4$)			
Oxygenated	23.1 mg/L	36.2 mg/L	56.8 mg/L
Pulsed low DO	15.7 mg/L	19.6 mg/L	24.4 mg/L
Paper mill effluent	20.4 %	38.6 %	73.1 %
Herbicide, 2,4-D	>4.6 mg/L**	>4.6 mg/L**	>4.6 mg/L**
Herbicide, Aquathol K	>2.8 mg/L**	>2.8 mg/L**	>2.8 mg/L**
Larvicide, BTI	>0.45 L/acre ft.**	>0.45 L/acre ft.**	>0.45 L/acre ft.**

* Exposure: Herbicides, 48-h static; Other, static, daily renewal, 9 days.
** Two times the concentration applied at the reservoir's surface.

CONCLUSIONS

Toxicity testing showed that juvenile mussels were not sensitive to the herbicides 2,4–D and Aquathol K and the larvicide BTI at two times the concentrations applied to Kentucky Reservoir. The LC50 concentration for manganese, 19.6 mg/L tested under low dissolved oxygen conditions, was sufficiently high to warrent concern over recruitment to reservoir mussel populations during drought/low flow periods. Paper mill effluent was toxic to juvenile mussels, but not at the 100:1 minimum dilution permitted for discharging to the reservoir's headwaters. Juvenile mussels were more sensitive than Ceriodaphnia to the paper mill effluent in split–sample tests conducted by two toxicity laboratories.

Results from two years of testing show that in vitro–transformed juvenile mussels exhibit: 1) long–term survivability under laboratory culture conditions, 2) resistance to damage incurred in following test protocols, 3) low mortality in controls during short–term and extended testing, and 4) sensitivities in the range of Ceriodaphnia for some toxicants. These factors indicate that juvenile mussels are promising test organisms for a wide range of ecotoxicological studies.

TOXICITY OF TAPP EFFLUENT TO JUVENILE MUSSELS

KENTUCKY RESERVOIR

1989

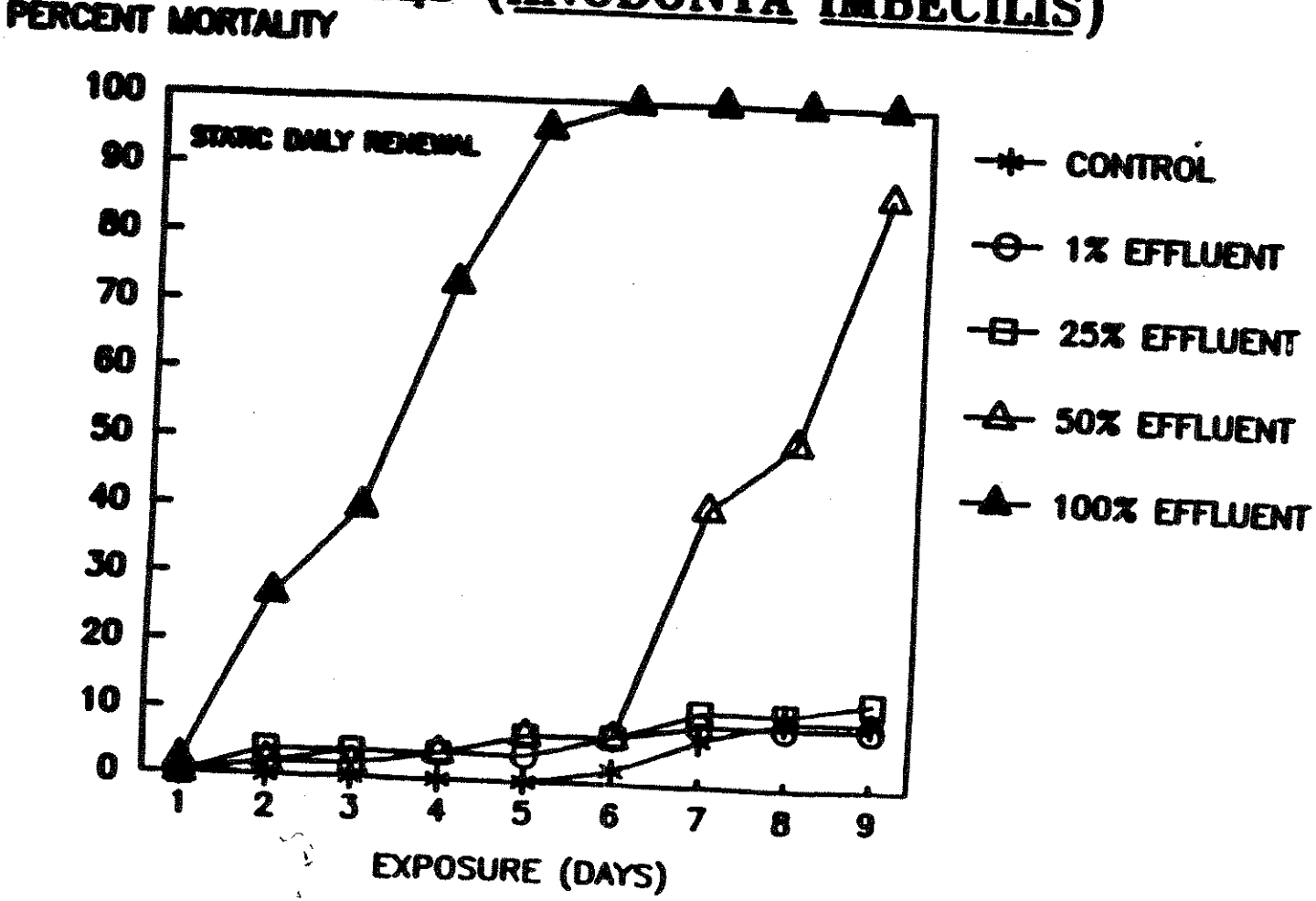
TVA/TWRA

Table 1. PERCENT SURVIVAL OF 6-DAY OLD ANCODONTA IMBECILIS DURING 9 DAYS EXPOSURE* TO TRPP EFFLUENT

Treatment	Rep	Test Exposure (Days)								
		1	2	3	4	5	6	7	8	9
TRPP CONTROL	1	100	100	100	100	100	100	93	93	93
	2	100	100	100	100	100	100	100	92	92
	3	100	100	100	100	100	93	87	87	87
	1-3	100	100	100	100	100	98	93	90	90
% Mortality:	1-3	0	0	0	0	0	2	7	10	10
TRPP 1%	1	100	100	100	100	100	100	93	93	93
	2	100	93	93	93	93	93	93	93	93
	3	100	100	93	93	93	87	87	87	87
	1-3	100	98	96	96	96	93	91	91	91
% Mortality:	1-3	0	2	4	4	4	7	9	9	9
TRPP 25%	1	100	100	100	100	93	93	87	87	87
	2	100	93	93	93	93	93	87	87	80
	3	100	93	93	93	93	93	93	93	93
	1-3	100	96	96	96	93	93	89	89	87
% Mortality:	1-3	0	4	4	4	7	7	11	11	13
TRPP 50%	1	100	100	100	93	93	93	67	40	7
	2	100	93	93	93	93	93	47	47	13
	3	100	100	100	100	93	93	60	60	20
	1-3	100	98	98	96	93	93	59	49	13
% Mortality:	1-3	0	2	2	4	7	7	41	51	87
TRPP 100%	1	93	67	60	40	7	0	0	0	0
	2	100	57	53	7	0	0	0	0	0
	3	100	93	67	33	7	0	0	0	0
	1-3	98	73	60	27	4	0	0	0	0
% Mortality:	1-3	2	27	40	73	96	100	100	100	100

*Static daily renewal test.

TRPP WHOLE EFFLUENT TOXICITY TO FRESHWATER MUSSELS (ANODONTA IMBECILIS)



TOXICITY* OF TRPP EFFLUENT TO 6-DAY OLD JUVENILE MUSSELS (ANODONTA IMBECILIS)

POINT	CONCENTRATION (PERCENT)	95% CONFIDENCE LIMITS	
		LOWER	UPPER
LC 1	20.4101	18.624	22.500
LC 50	38.6270	34.576	43.459
LC 99	73.1024	64.193	83.944

* DATA BASED ON 9 DAYS, STATIC, DAILY RENEWAL EXPOSURE

ALLOWABLE TOXICITY BASED ON 100:1 DILUTION OF TRPP EFFLUENT

ALLOWABLE ACUTE TOXICITY	TEST RESULTS
$\leq$ CMC* x Dilution Factor $\leq$ 0.3 Acute Toxicity Units (TU_a) x 100 $\leq$ 30 TU_a or $LC_{50} \geq$ 3.3%	2.6 TU_a – Within Limit or LC_{50} = 38.6% – Within Limit
ALLOWABLE CHRONIC TOXICITY	**(ESTIMATED)** **
$\leq$ CCC* x Dilution Factor $\leq$ 1.0 Chronic Toxicity Units (TU_c) x 100 $\leq$ 100 TU_c or NOEC $\geq$ 1.0%	25.9 TU_c – Within Limit or NOEC = 3.86% – Within Limit

*CMC = Criteria Maximum Concentration
CCC = Criteria Continuous Concentration

**Based on Acute/Chronic Ratio = 10

TOXICITY OF BTI (VECTOBAC) TO JUVENILE MUSSELS

1989

TVA/TWRA

TOXICITY EVALUATION OF THE MOSQUITO LARVICIDE BTI (VECTOBAC®) USING JUVENILE FRESHWATER MUSSELS (ANODONTA IMBECILIS SAY)

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ACKNOWLEDGEMENTS

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EXECUTIVE SUMMARY

A 9-day static daily renewal toxicity test was conducted evaluat effects of up to twice the field application concentration of the mosquito larvicide BTI (Vectobac®) on 6-day old juvenile mussels (Anodonta imbecilis). Survival was greater than 90 percent in all treatments, with 1(percent of the mussels exposed to two times the field application rate surviving the test. All surviving mussels remained very active and showed n signs of stress. Conclusion is that field application of this pesticide use in accordance with label instructions is not toxic to freshwater mussels.

TOXICITY EVALUATION OF THE MOSQUITO LARVICIDE BTI (VECTOBAC®) USING JUVENILE FRESHWATER MUSSELS (ANODONTA IMBECILIS SAY)

Background

Mussel mortalities and purported general decline of the mussel community in Kentucky Reservoir has led to a broad range of concerns about reservoir water quality and potential problems both within the reservoir and its watershed. Among the concerns expressed has been the use of pesticides by TVA to control problematic aquatic plants and populations of mosquitos.

In 1988 TVA's Aquatic Research Laboratory conducted toxicity evaluations of the two major herbicides used in Kentucky Reservoir, 2,4-D and Aquathol® K. Exposure of 7-day old juvenile mussels (Anodonta imbecilis) to twice the concentrations of active ingredients applied in TVA's aquatic weed control operations to the surface of TVA reservoirs, failed to produce mortality, with 100 percent of the mussels tested surviving a 48-h exposure period (Wade, 1988). Although TVA uses only approved and labeled pesticides supported by a large number of toxicity studies documenting absence of effects to non-target species, TVA was requested by the Tennessee Wildlife Resources Agency to evaluate the toxicity of the mosquito and black fly larvicide BTI (Vectobac®) on freshwater mussels. BTI is a bacterium (Bacillus thuringiensis) which, when ingested by mosquito and black fly larvae, is acted upon by enzymes to release endotoxins which cause gut paralysis and ultimately death of the target pest organisms. Since mussels also filter bacteria from the water as a food source, it seemed appropriate to conduct the toxicity

evaluation, although Pelecypoda (freshwater mussels) is a non-target organism which has been shown to be unaffected by BTI. This test evaluated toxicity of BTI on very young (6-day old) mussels, representing what is likely the sensitive life stage most appropriate for testing.

Selection and Preparation of Test Organisms

The life history of freshwater mussels includes an obligate parasitic larval stage (glochidium) which becomes attached to gill and fin tissues of a host fish. The attached glochidium is soon covered by a layer of gill or fin tissue and remains on the fish host throughout a transformation process which results in a free-living juvenile mussel. The transformed juvenile stage was chosen for testing because it is the first stage fully exposed and completely dependent upon the aquatic environment. The juvenile mussels are metabolically very active in order to meet immediate growth requirements for survival. Unlike adult mussels, juveniles appear unable to "clam up" to resist short-term environmental perturbations, making the juveniles sensitive and ideal for toxicity studies.

In order to provide sufficient juvenile mussels of known age for conducting the toxicity test, glochidia were taken from a gravid female and transformed *in vitro* in artificial culture medium as described by Isom and Hudson (1982), inventors of the technique which was patented and assigned to TVA (1984). *Anodonta imbecillis* was chosen as the test species because its glochidia are available throughout the year (Hudson, et al. 1986) and because of past success in transforming and growing this species under laboratory conditions. This species is also widespread in

the region, preferring impounded waters and streams having muddy or sandy bottoms. Glochidia from a single female produced several thousand viable juveniles, more than enough for conducting the test. Juveniles used in the study were in culture from July 12-19, 1989 (7 days).

Toxicity Test

The toxicity test, initiated July 25, utilized 180 juvenile (6-day old) mussels and was conducted over a 9-day exposure period to concentrations of BTI equal to 1/2, 1, and 2 times the active ingredients applied to the surface of TVA reservoirs for larval mosquito control.

Larvicide Treatment	Concentration
BTI X 1/2	0.92 X 10^{-4} mL BTI/L (113.648 mL/acre foot)
BTI X 1 (field application rate)	1.84 X 10^{-4} mL BTI/L (227.297 mL/acre foot)
BTI X 2	3.68 X 10^{-4} mL BTI/L (454.594 mL/acre foot)

These nominal concentrations were renewed daily by adding full strength insecticide to Tennessee River water which had been collected from Wheeler Reservoir, filtered through a 5 micron bag filter, and placed in 5-gal aquaria inoculated with reservoir plankton (concentrated by centrifugation) to develop a rich plankton (food source) community. The plankton enriched reservoir water was also used as a control in the test. Sediment from the source of the Anodonta imbecillis stock mussels (Haleyville, Alabama, municipal reservoir) was filtered through a 100-mesh nitex screen and added to each treatment at a concentration of

800 mg sediment/liter. Both plankton and sediment are essential for juvenile mussel health and survival (Hudson and Isom, 1984). The sediment/Tennessee River water mixture (control) was analyzed for priority pollutant volatile organics, extractable organics, and metals.

The toxicity test was conducted from July 25-August 3, 1989. Fifteen juvenile mussels were added to each replicate (3/treatment). Test vessels were 250 mL crystallizing dishes filled to the brim with water. Sufficient "dummy" replicates (identical to test medium, but without mussels) were run for each treatment to provide adequate volumes of water for water chemistry analyses which included both initial and final measurements of dissolved oxygen, temperature, pH, alkalinity, hardness, and conductivity at each daily toxicant renewal. All replicates were kept in a dark incubator at 24°C.

After each 24-hr exposure, water and silt were rinsed through a 100-mesh nitex screen which retained the juvenile mussels. Juveniles were immediately rinsed back into the emptied crystallizing dish and enumerated as either live/active, live/stressed, or dead. Following evaluation, renewal water and toxicants were added to each dish.

Results

Criteria used to evaluate stress and differentiate between the living and dead juveniles were:

1. Alive/Active - foot protrudes from shell within a minute of settling to the bottom of the dish; foot locates dish bottom and "flips" shell into an upright position; mussel glides along dish bottom on extended foot.

2. Alive/Stressed - shell gaped open with foot immobile and protruding (hanging) from shell; close observation reveals only slight foot motion and ciliary action; as in active juveniles, algae mass usually rotating within area of stomach.

3. Dead - Shell gaped open with detrital material adhered to soft tissues just within the shell (indicating absence of ciliary movement which sweeps this area clean in live, active and stressed, mussels); and/or decomposition of soft parts (indistinguishable foot, organs, etc., usually accompanied by protozoans within the shell); or empty shells.

Under field conditions, BTI is applied at the surface of the reservoir. Because of dilution and reservoir currents, exposure of benthic (bottom dwelling) organisms is likely at reduced levels from those in this test since treatments of BTI were added directly to the mussels. This acute assay represents a worst-case exposure since the juvenile mussels were exposed to twice the concentration applied to the reservoir's surface in the field and since the toxicant was renewed daily.

Survival during the 9-day exposure is shown in table 1. At the end of nine days survival in the control and all treatments of BTI was ≥ 91 percent. One hundred percent of the mussels in the double field application treatment survived the test. All surviving mussels remained very active throughout the 9 days exposure without signs of stress. The 9-day LC50 for the test was > 100 percent of the 2X concentration tested.

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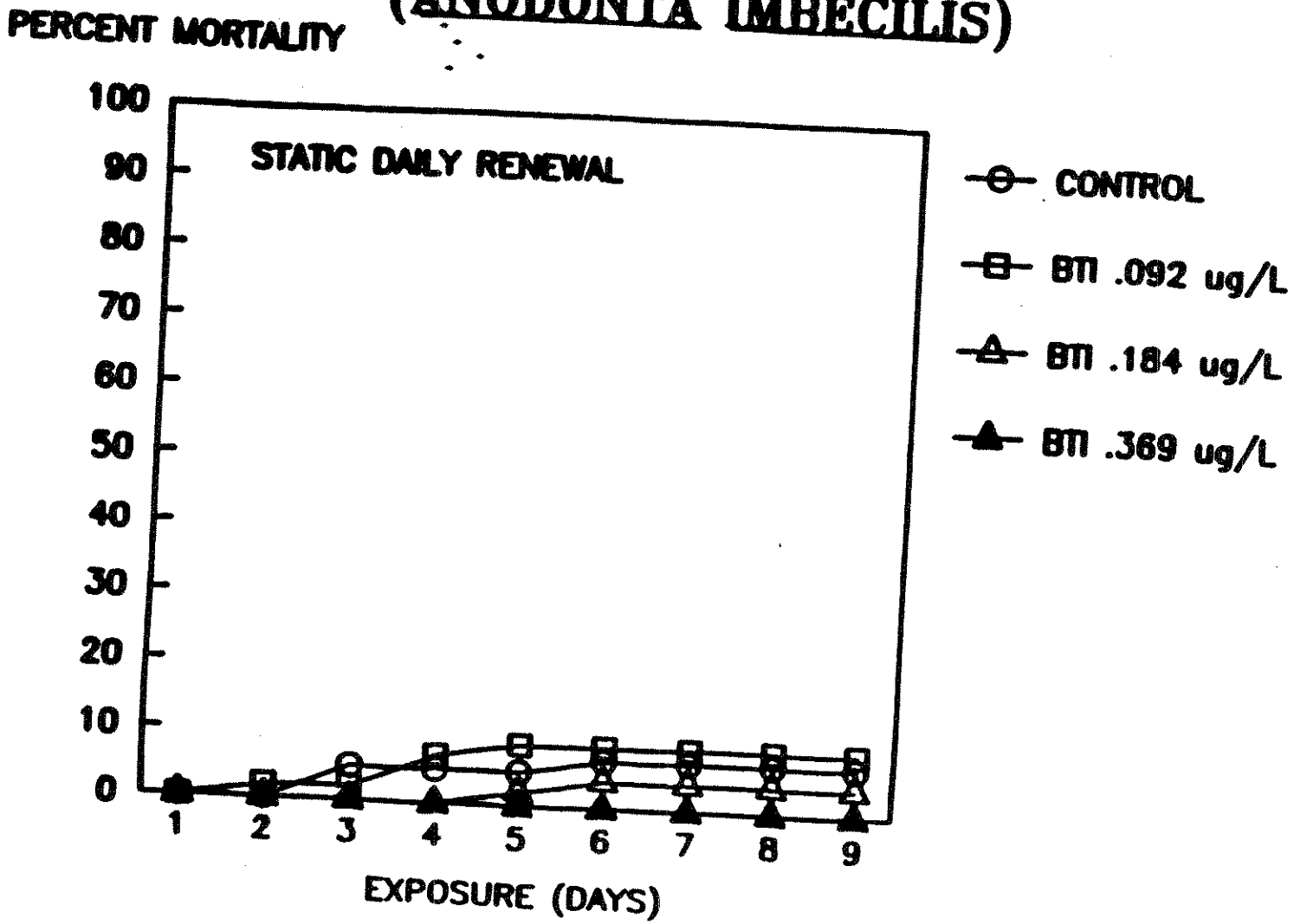
BTI TOXICITY TO 6-DAY OLD JUVENILE MUSSELS (ANODONTA IMBECILIS)



MANGANESE TOXICITY TO JUVENILE MUSSEL (ANODONTA IMBECILIS)

1989

TVA

Table 1. Percent Survival of 6-Day Old *Anodonta Imbecilis* During 9 Days Exposure* to Concentrations of Manganese Under Oxygenated Conditions (DO > 7 mg/L)

Treatment	Rep	Test Exposure (Days)								
		1	2	3	4	5	6	7	8	9
CONTROL	1	100	100	100	100	100	100	100	100	100
	2	100	100	100	100	100	100	100	100	93
	3	100	100	100	100	100	100	100	100	100
	1-3	100	100	100	100	100	100	100	100	98
% Mortality:	1-3	0	0	0	0	0	0	0	0	2
Mn 5 mg/L	1	100	100	100	100	100	100	100	93	93
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100	100
	1-3	100	100	100	100	100	100	100	98	98
% Mortality:	1-3	0	0	0	0	0	0	0	2	2
Mn 8 mg/L	1	100	100	100	100	100	100	100	100	100
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	93	93	93	93	93
	1-3	100	100	100	100	98	98	98	98	98
% Mortality:	1-3	0	0	0	0	2	2	2	2	2
Mn 13 mg/L	1	100	100	100	100	100	100	100	100	93
	2	100	100	100	100	100	100	93	93	94
	3	100	100	100	100	100	100	100	100	93
	1-3	100	100	100	100	100	100	98	98	93
% Mortality:	1-3	0	0	0	0	0	0	2	2	7
Mn 20 mg/L	1	100	100	100	100	100	100	100	100	100
	2	100	93	93	93	93	93	86	86	86
	3	100	100	100	100	100	100	100	94	94
	1-3	100	98	98	98	98	98	95	93	93
% Mortality:	1-3	0	2	2	2	2	2	5	7	7

Treatment	Rep	Test Exposure (Days)								
		1	2	3	4	5	6	7	8	9
Mn 32 mg/L	1	100	100	100	100	100	100	100	100	87
	2	100	100	100	100	87	87	80	73	53
	3	100	100	100	100	93	87	87	87	79
	1-3	100	100	100	100	93	91	89	87	71
% Mortality	1-3	0	0	0	0	7	9	11	13	29
Mn 50 mg/L	1	100	100	100	100	100	100	53	20	0
	2	100	100	100	100	67	47	0	0	0
	3	100	100	100	100	100	100	100	93	13
	1-3	100	100	100	100	89	82	51	38	4
% Mortality	1-3	0	0	0	0	11	18	49	62	96

*Static daily renewal test.

0068K

Table 2. Percent Survival of 6-Day Old *Anodonta Imbecilis* During 9 Days Exposure* to Concentrations of Manganese Under Pulsed Low Dissolved Oxygen Conditions (to ≤ 0.5 mg/L)

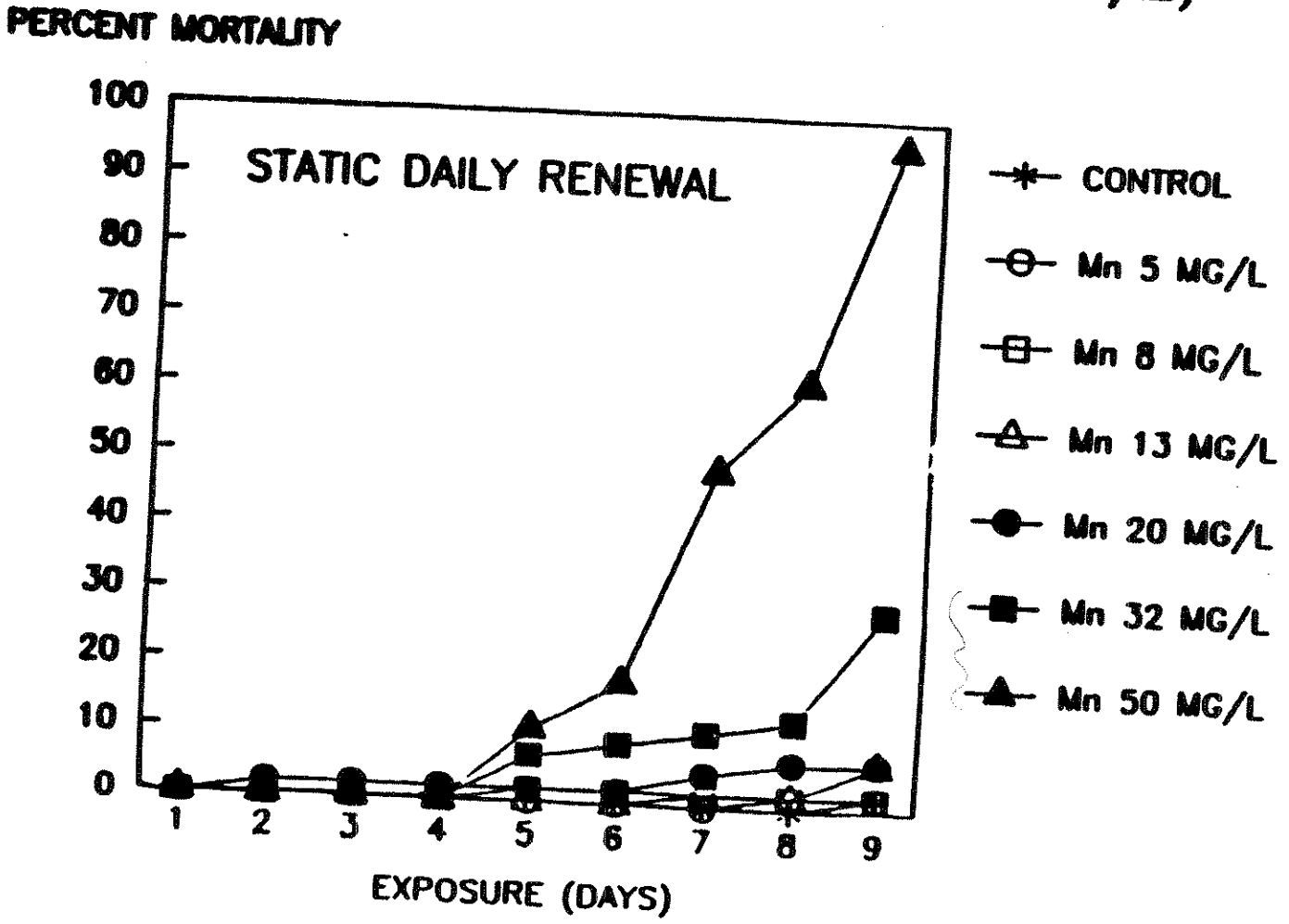
Treatment	Rep	Test Exposure (Days)								
		1	2	3	4	5	6	7	8	9
LOW DO CONTROL	1	100	100	100	93	92	85	85	85	85
	2	100	100	100	100	100	100	100	92	92
	3	100	100	100	93	93	93	93	93	93
	1-3	100	100	100	95	95	93	93	90	90
% Mortality:	1-3	0	0	0	5	5	7	7	10	10
Mn 5 mg/L LOW DO	1	100	100	94	87	87	87	73	6	67
	2	100	100	93	80	80	80	79	71	64
	3	100	100	93	87	80	73	73	73	67
	1-3	100	100	93	84	82	80	75	70	66
% Mortality:	1-3	0	0	7	16	18	20	25	30	34
Mn 8 mg/L LOW DO	1	100	100	100	93	93	93	93	93	79
	2	100	93	87	87	80	73	73	67	67
	3	100	100	92	92	92	92	92	92	92
	1-3	100	98	93	88	88	86	86	83	78
% Mortality:	1-3	0	2	7	12	12	14	14	17	22
Mn 13 mg/L LOW DO	1	100	100	100	100	100	100	100	100	100
	2	100	100	100	93	93	93	93	93	86
	3	100	100	100	93	87	87	80	73	73
	1-3	100	100	100	96	93	93	91	89	86
% Mortality:	1-3	0	0	0	4	7	7	9	11	14
Mn 20 mg/L LOW DO	1	100	100	100	100	100	93	87	53	27
	2	100	100	100	100	80	60	53	47	33
	3	100	100	100	86	85	69	54	47	38
	1-3	100	98	98	95	88	74	65	53	33
% Mortality:	1-3	0	2	2	5	12	26	35	47	67

Treatment	Rep	Test Exposure (Days)								
		1	2	3	4	5	6	7	8	9
Mn 32 mg/L LOW DO	1	100	100	100	100	100	73	21	14	0
	2	100	100	100	100	80	33	0	0	0
	3	100	100	93	27	0	0	0	0	0
	1-3	100	100	98	75	59	36	7	5	0
% Mortality	1-3	0	0	2	25	41	64	93	95	100
Mn 50 mg/L LOW DO	1	87	60	14	0	0	0	0	0	0
	2	100	100	93	67	20	7	0	0	0
	3	93	73	60	27	7	0	0	0	0
	1-3	93	78	57	32	9	2	0	0	0
% Mortality	1-3	7	22	43	68	91	98	100	100	100

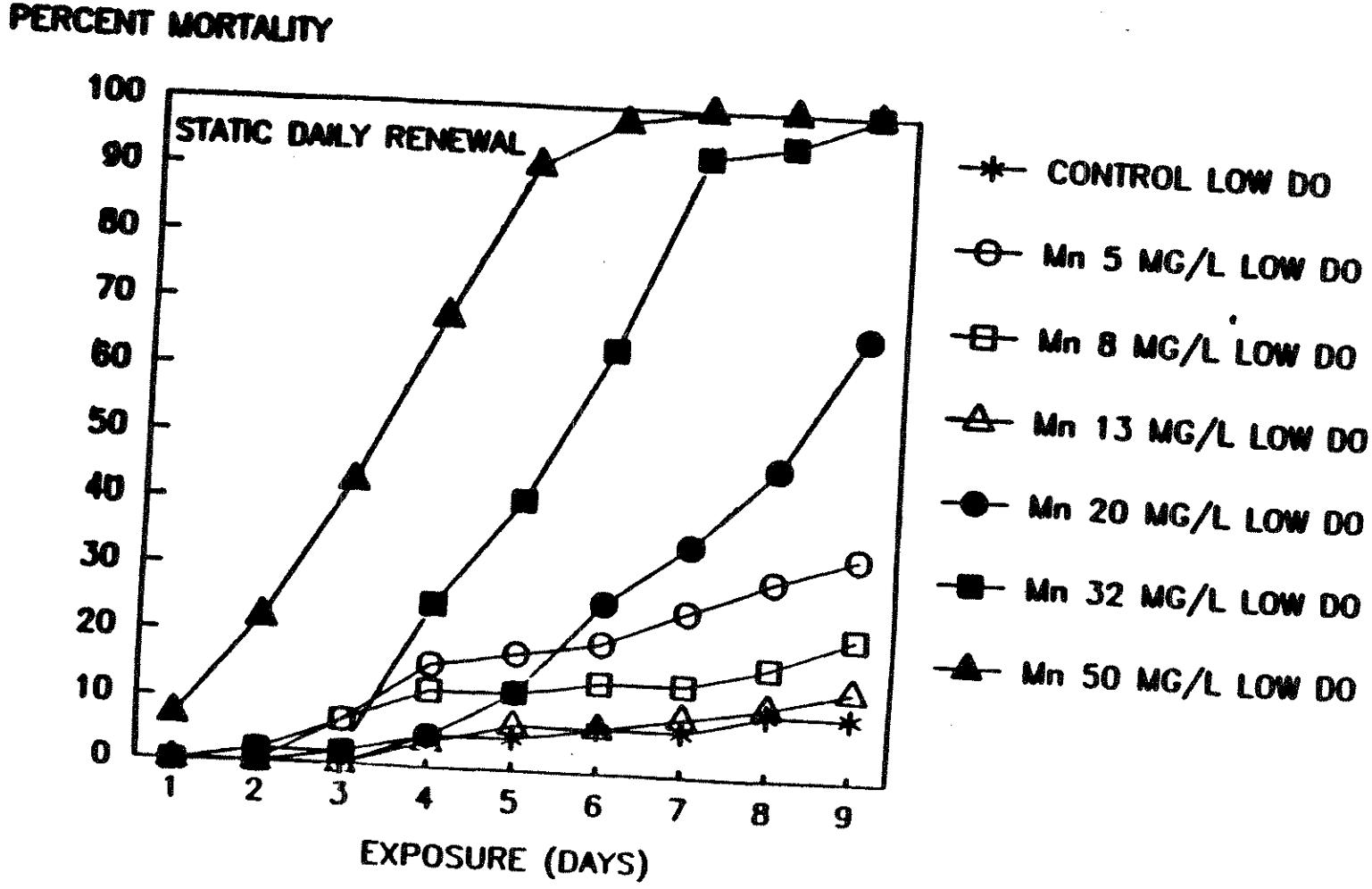
*Static daily renewal test.

0068K

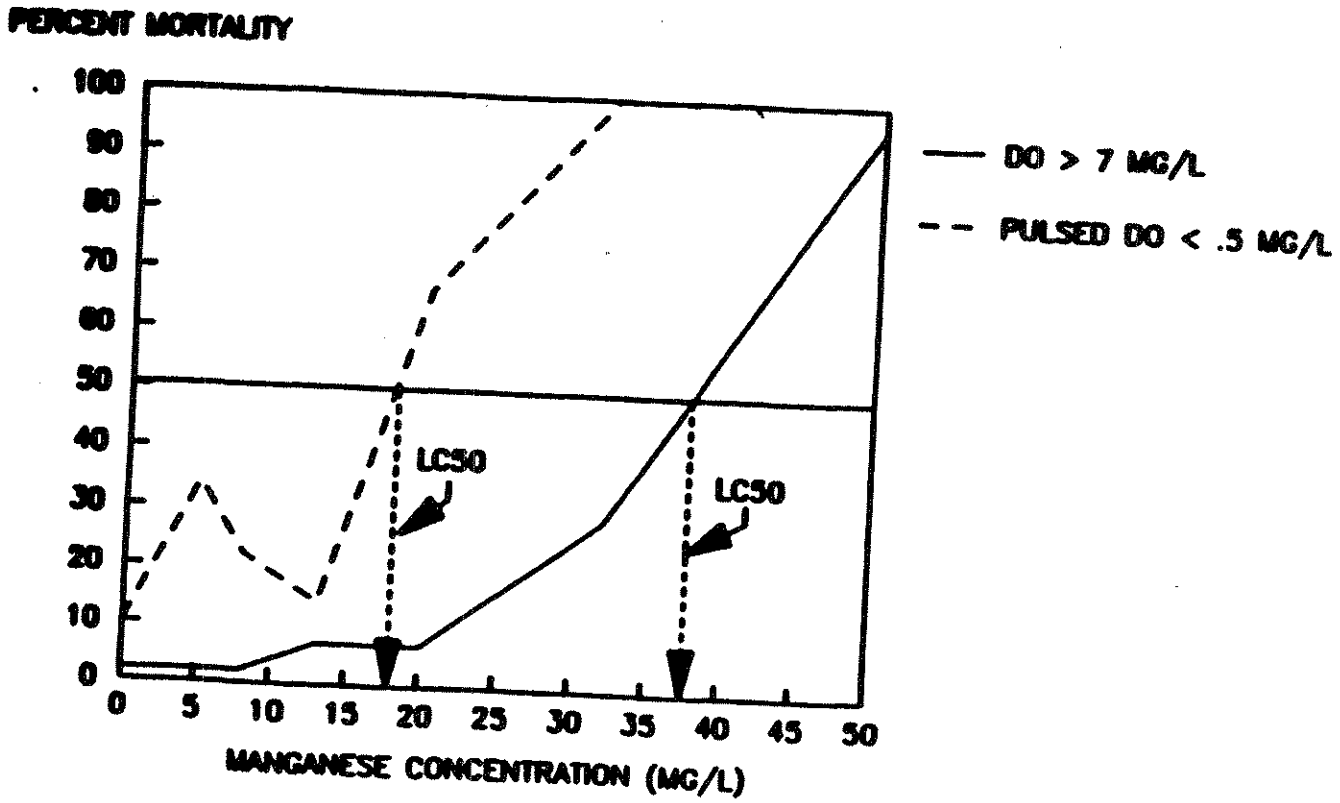
MANGANESE TOXICITY TO FRESHWATER MUSSELS OXYGENATED CONDITIONS (DO > 7 MG/L)



MANGANESE TOXICITY TO FRESHWATER MUSSELS
PULSED LOW DO CONDITIONS



MANGANESE TOXICITY TO JUVENILE MUSSELS
6-DAY OLD ANODONTA IMBECILIS



TOXICITY* OF MANGANESE TO 6-DAY OLD JUVENILE MUSSELS (ANODONTA IMBECILIS)

DISSOLVED OXYGEN > 7 MG/L

POINT	CONCENTRATION (MG/L)	95% CONFIDENCE LIMITS	
		LOWER	UPPER
LC1	23.0685	17.992	26.307
LC50	36.1887	33.556	38.879
LC99	56.7711	50.114	71.756

DISSOLVED OXYGEN PULSED < 0.5 MG/L

POINT	CONCENTRATION (MG/L)	95% CONFIDENCE LIMITS	
		LOWER	UPPER
LC1	15.6614	15.134	16.243
LC50	19.5476	18.765	20.416
LC99	24.3981	23.267	25.661

* BASED ON 9 DAYS, STATIC DAILY RENEWAL

Table 1. Percent Survival of 6-Day Old Anodonta imbecilis During 9 Days Exposure to the Mosquito Larvicide BTI (Vectobac®)

Treatment	Rep	Test Exposure (Days) 1	2	3	4	5	6	7	8	9
BTI CONTROL	1	100	100	93	93	93	93	93	93	93
	2	100	100	100	100	100	100	93	93	93
	3	100	100	93	93	93	87	87	87	87
	1-3	100	100	95	95	95	93	93	93	93
% Mortality:	1-3	0	0	5	5	5	7	7	7	7
BTI X 1/2*	1	100	100	100	100	93	93	93	93	93
	2	100	93	93	80	80	80	80	80	80
	3	100	100	100	100	100	100	100	100	100
	1-3	100	98	98	93	91	91	91	91	91
% Mortality:	1-3	0	2	2	7	9	9	9	9	9
BTI X 1*	1	100	100	100	100	100	100	100	100	100
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	93	87	87	87	87
	1-3	100	100	100	100	98	96	96	96	96
% Mortality:	1-3	0	0	0	0	2	4	4	4	4
BTI X 2*	1	100	100	100	100	100	100	100	100	100
	2	100	100	100	100	100	100	100	100	100
	3	100	100	100	100	100	100	100	100	100
	1-3	100	100	100	100	100	100	100	100	100
% Mortality:	1-3	0	0	0	0	0	0	0	0	0

*Mussels exposed to 1/2, 1, and 2 times the calculated field application rate of 227.2968 mL BTI per acre ft. of water. Daily renewal static test.

0066K