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

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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 harvests 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.

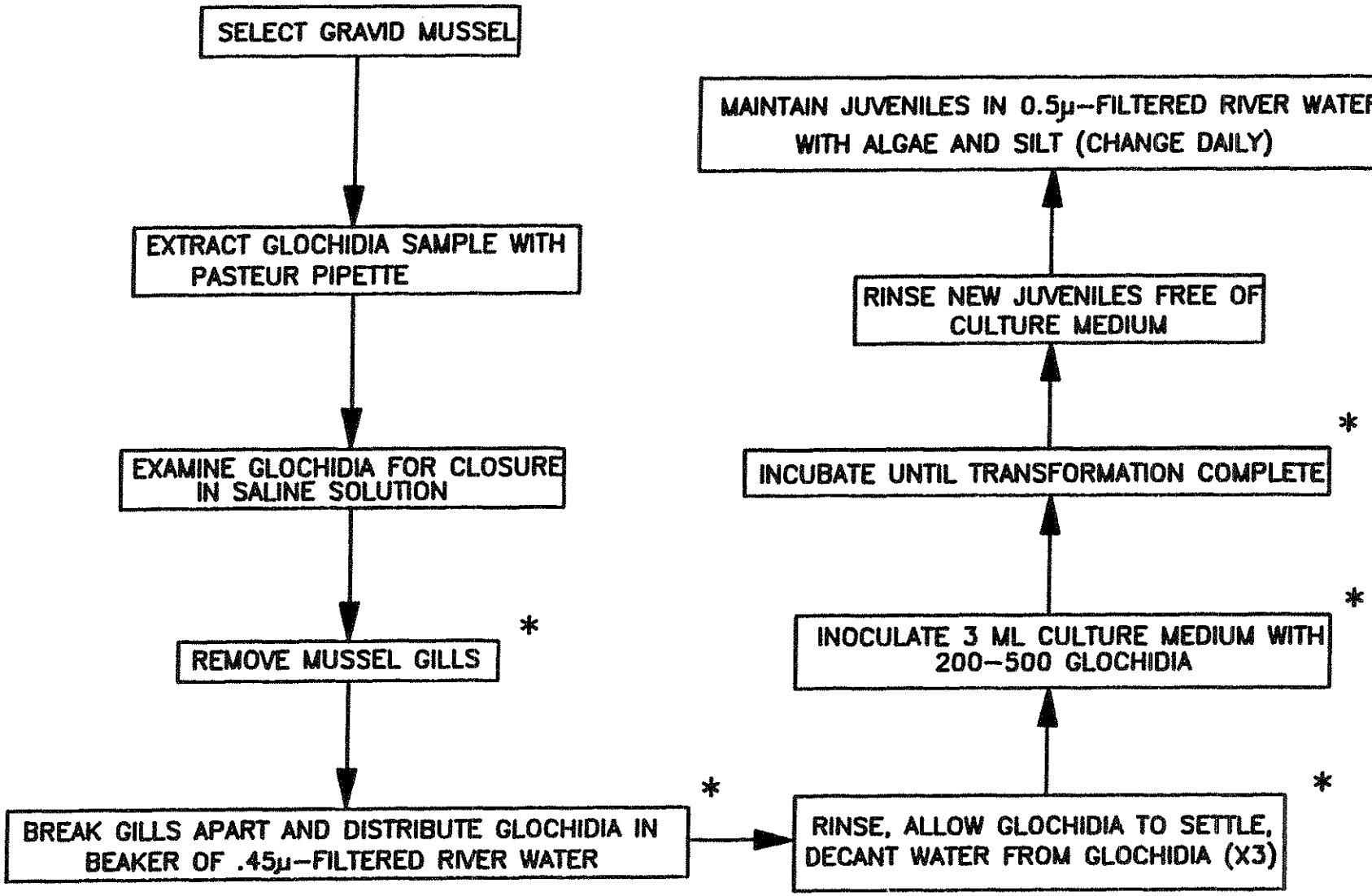
COMPONENTS OF FRESHWATER MUSSEL ARTIFICIAL CULTURE MEDIUM*

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 pantohthenate	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
Natural Compounds	
Plasma (1/3 by volume)	

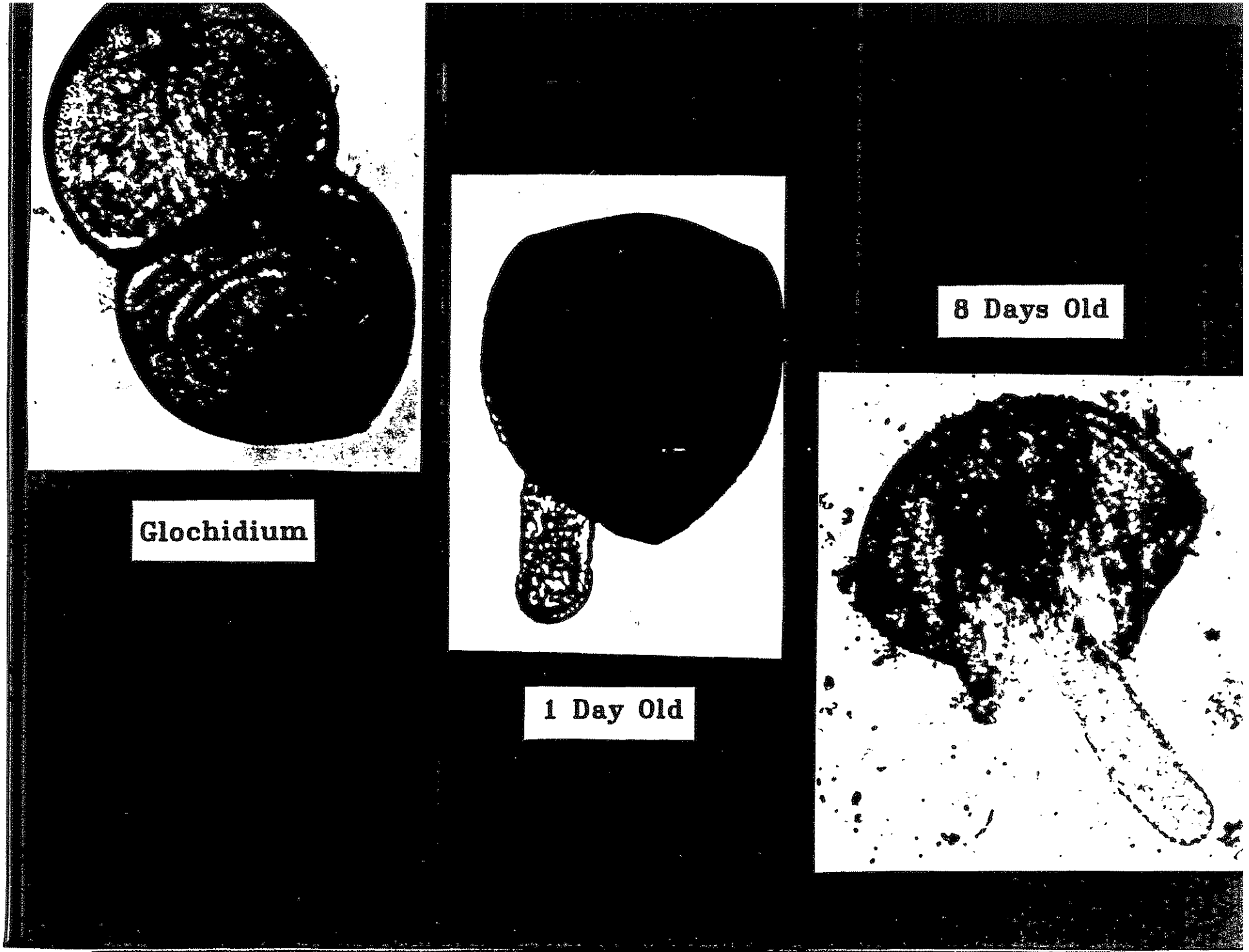
* Isom and Hudson (1982)

TRANSFORMATION OF FRESHWATER MUSSEL GLOCHIDIA INTO JUVENILES
IN VITRO PROCEDURE



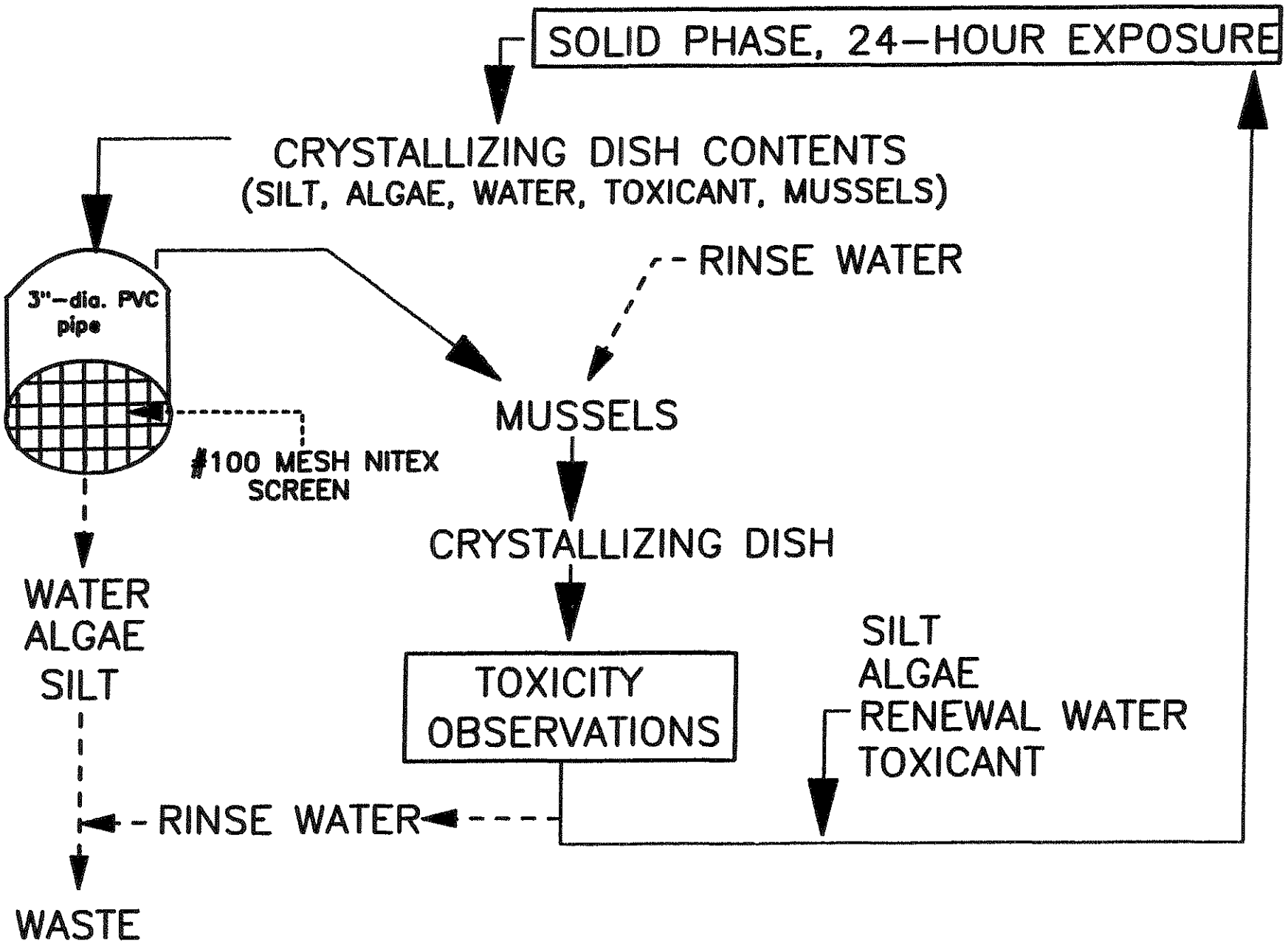
*Perform under sterile conditions.

Freshwater Mussel Glochidia in Culture



Anodonta imbecilis Glochidium and Transformed Juveniles, One and Eight Days Old

JUVENILE MUSSEL TOXICITY TESTING
DAILY RENEWAL PROCEDURE

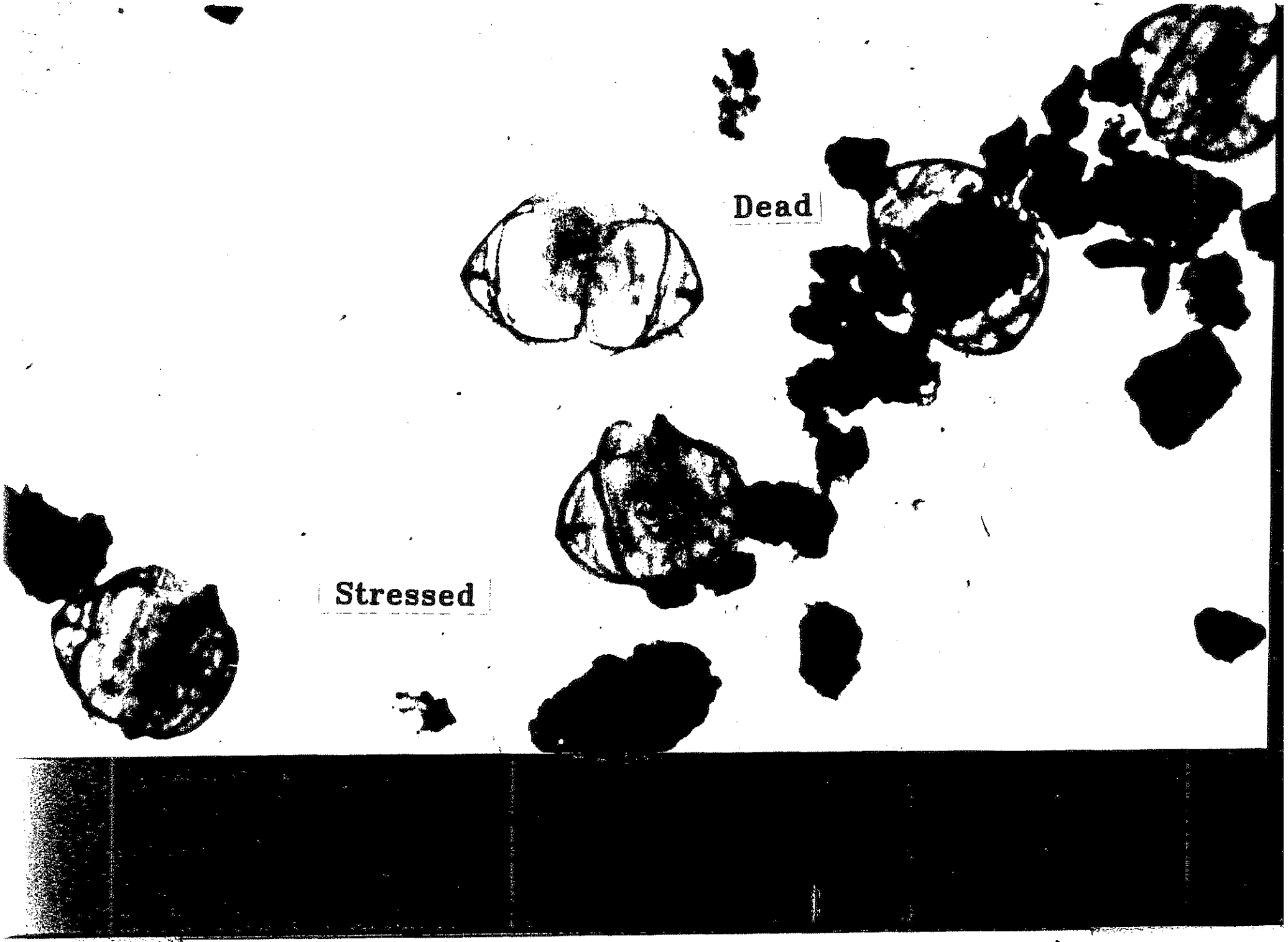


KENTUCKY RESERVOIR TOXICITY STUDIES

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 *in vitro* transformed juvenile freshwater mussels

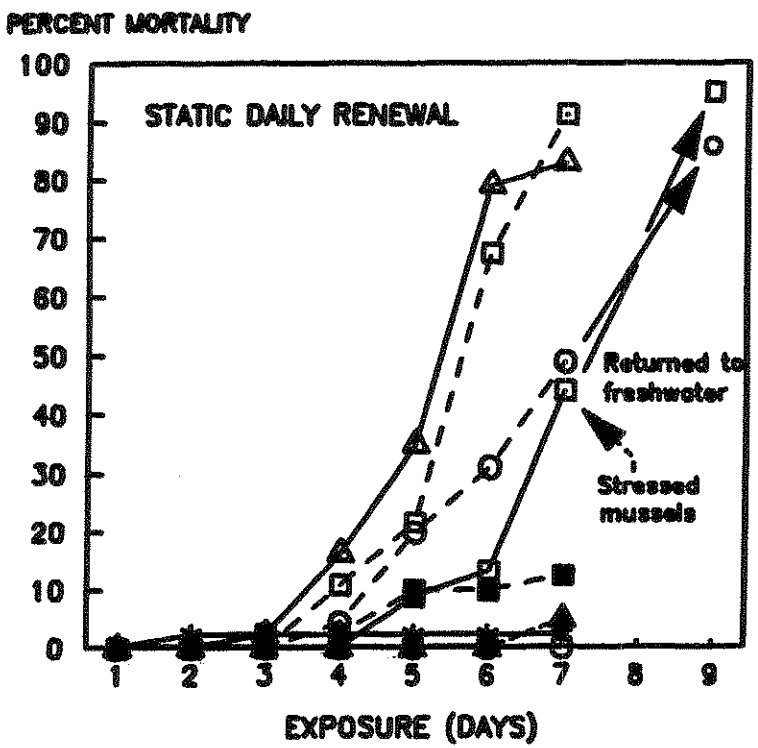
Active, Non-stressed Juvenile Mussels (8 Days Old)

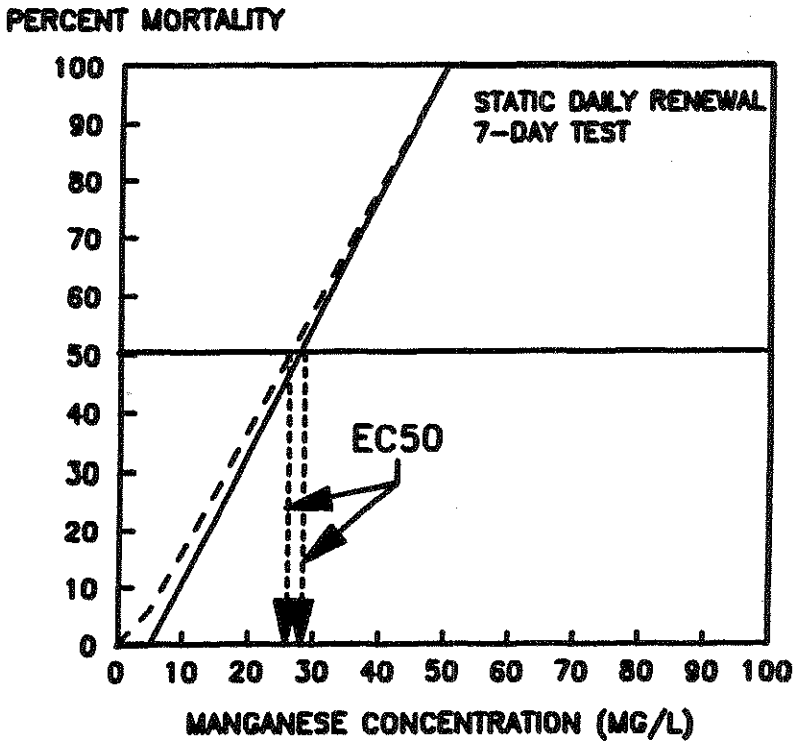


Stressed and Dead Juvenile Mussels (10 Days Old)

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

OXYGENATED AND PULSED LOW DO* CONDITIONS

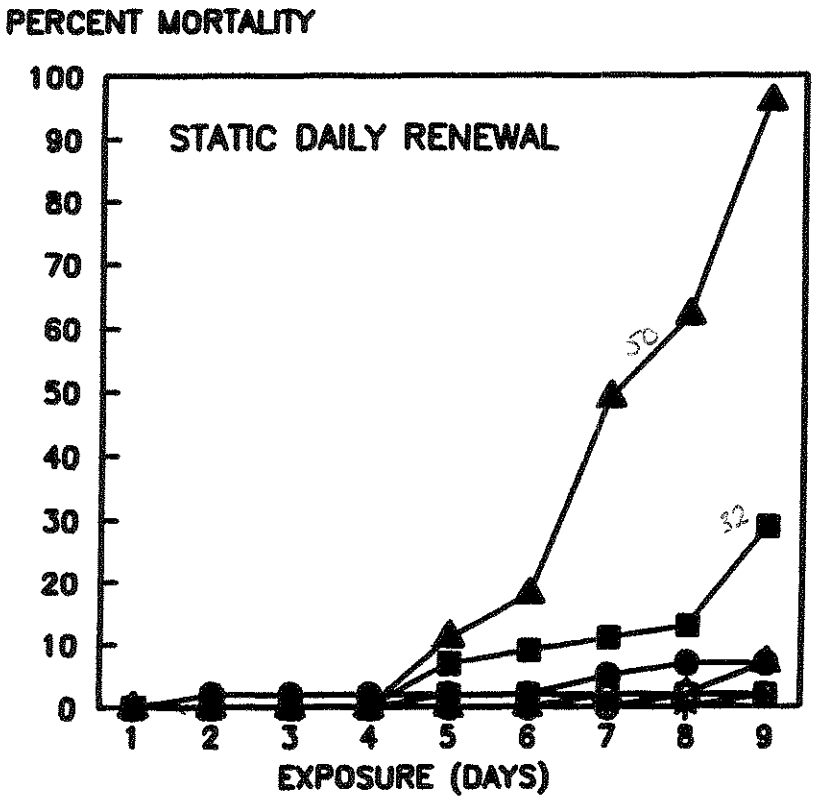




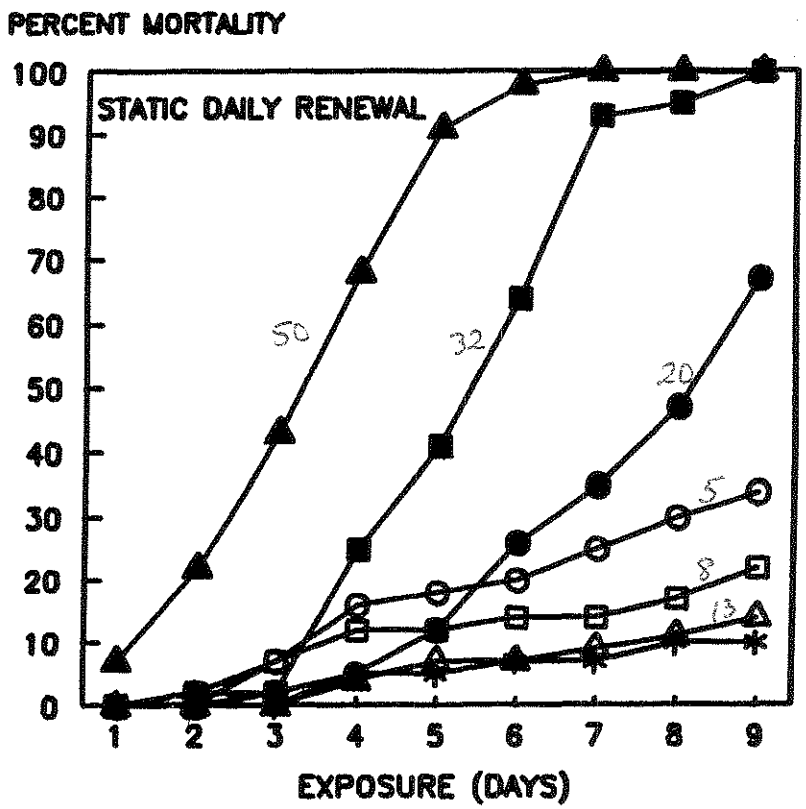
* Pulsed to ≤ 0.5 mg/L

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

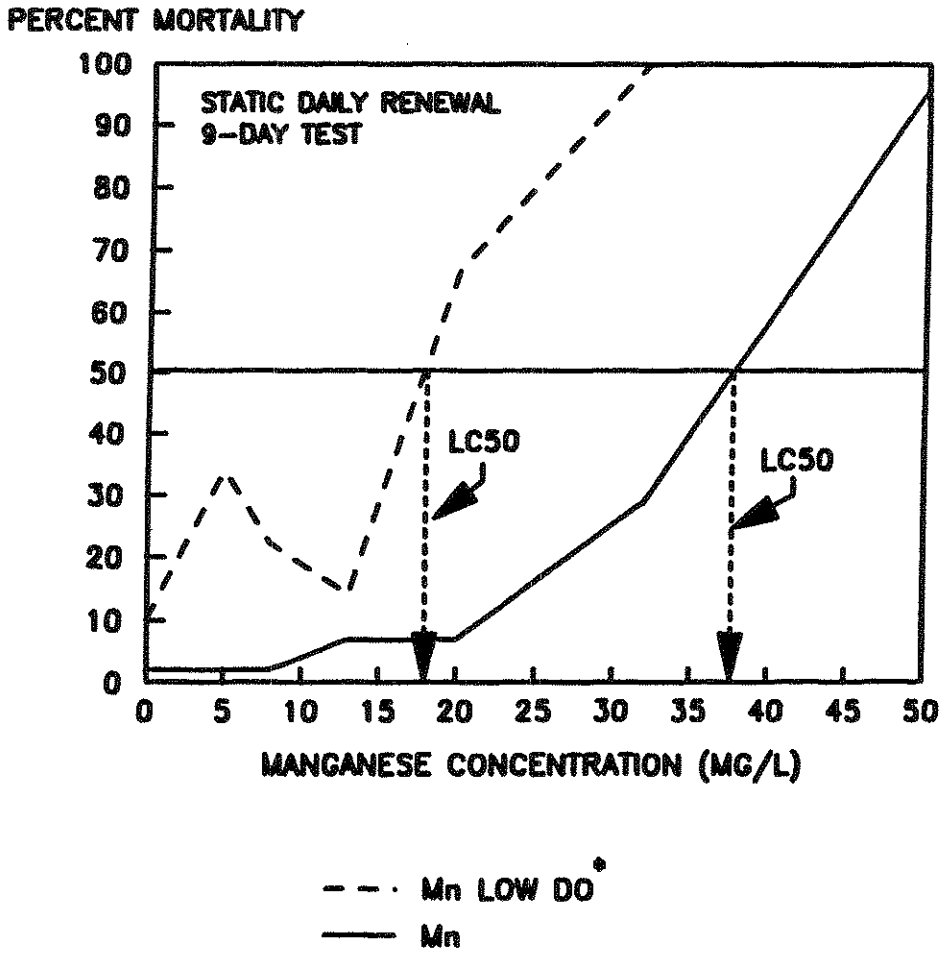
OXYGENATED CONDITIONS (DO > 7 MG/L)



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

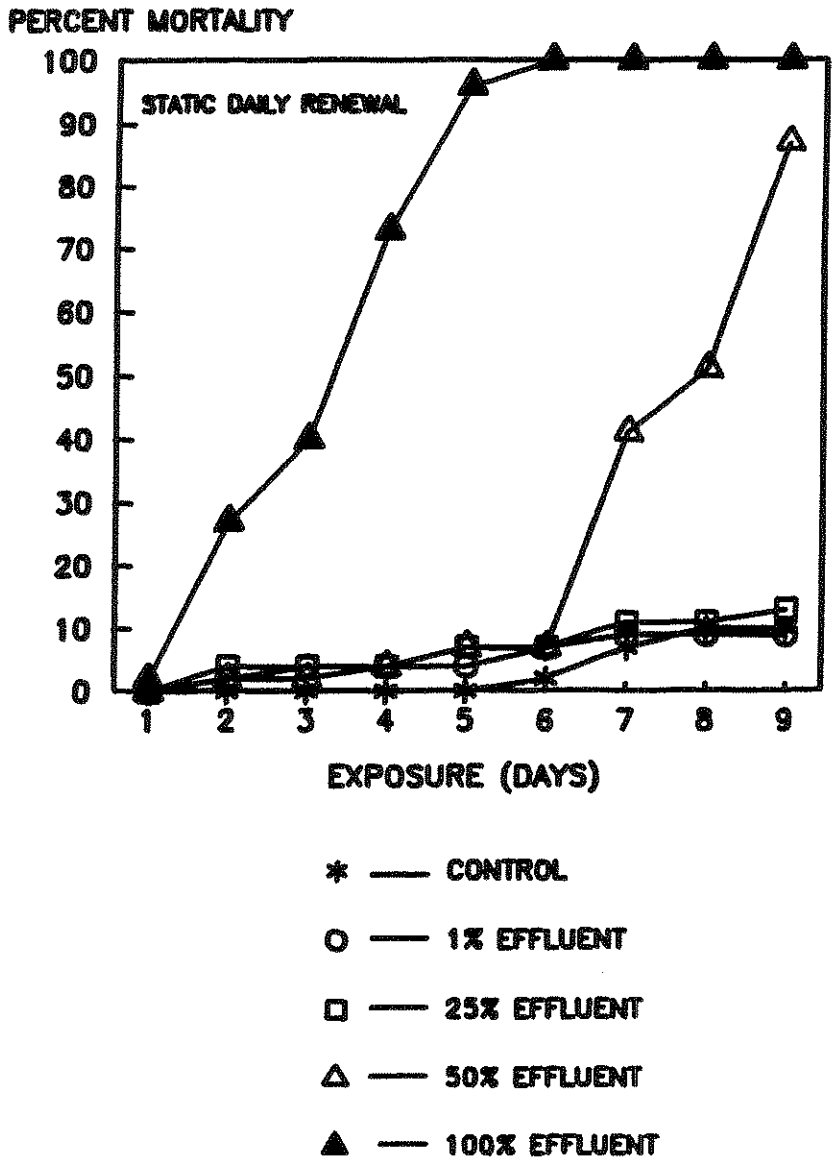
Oxygenated

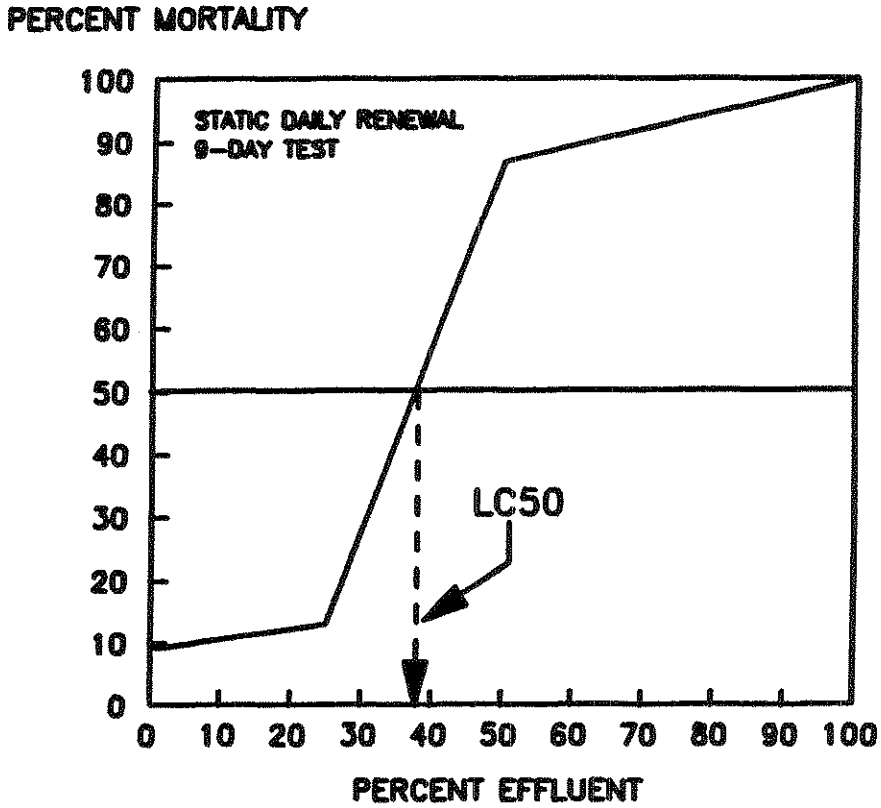
Point	Concentration	95% Confidence Limits	
	mg/L	Lower	Upper
LC1	23.07	17.99	26.31
LC50	36.19	33.56	38.88
LC99	56.77	50.11	71.76

Pulsed low DO

Point	Concentration	95% Confidence Limits	
	mg/L	Lower	Upper
LC1	15.66	15.13	16.24
LC50	19.55	18.77	20.42
LC99	24.40	23.27	25.66

PAPER MILL WHOLE EFFLUENT TOXICITY TO FRESHWATER MUSSELS (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%

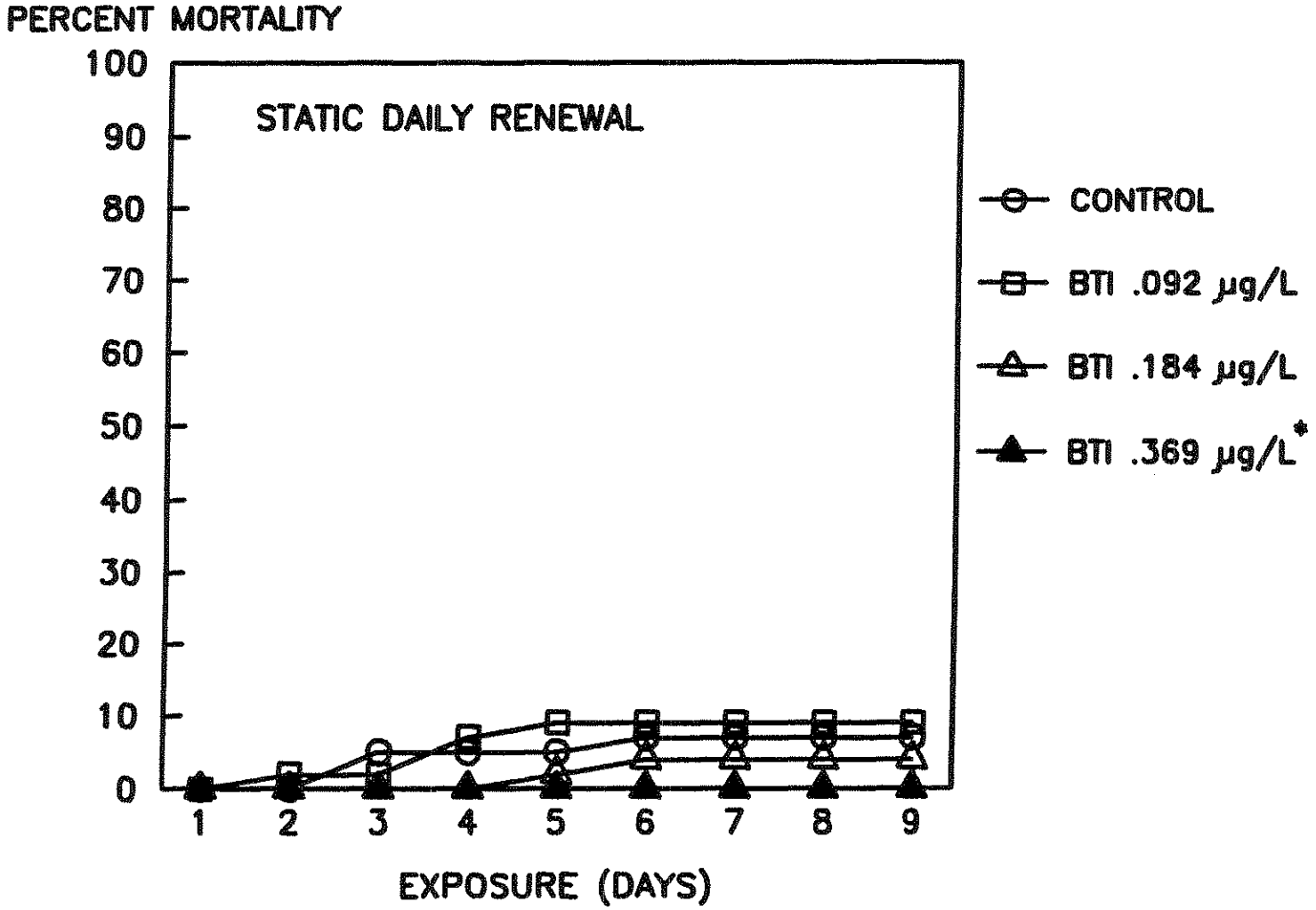
CHRONIC TESTS*

	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 greater than Ceriodaphnia for some toxicants. These factors indicate that juvenile mussels are promising test organisms for a wide range of ecotoxicological studies.

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

1. TEST TYPE:	Solid phase, static renewal
2. TEMPERATURE:	24 $\pm^{o}$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 cililary action or empty shells

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).