DATA EVALUATION RECORD

1. **CHEMICAL:** Metolachlor
Shaughnessey No. 108801

2. **TEST MATERIAL:** Metolachlor Technical 97.0% purity; Ciba Geigy; FL800106; brownish black liquid.

3. **STUDY TYPE:** 72-3(A) Acute Estuarine/Marine Toxicity Fish and 72-4(a) Early Life Stage Fish. Species Tested: Sheepshead Minnow Cyprinodon variegatus.

4. **CITATION:** Ward, S., 1980. Effects of Metolachlor (Dual) on Survival, Growth, and Development of Sheepshead Minnows (Cyprinodon variegatus). Conducted by EG&G, Bionomics Marine Research Laboratory, Pensacola, Florida. Study No. BP-80-5-80. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 430446-02

5. **Reviewed By:**

Conchi Rodríguez **Signature:** Conchi Rodriguez
Biologist 3/11/94
Ecological Effects Branch **Date:**
Environmental Fate and Effects Division

6. **Approved By:**

Harry Craven **Signature:** Harry Craven
Supervisor 3/11/94
Ecological Effects Branch **Date:**
Environmental Fate and Effects Division

7. **CONCLUSIONS:** Both studies are classified as supplemental and the results of the studies may be used in the risk assessment. The LC50 for the sheepshead minnow was calculated as 7.9 mg/l. The MATC ranged between 1.0 mg/l - 2.2 mg/l (geometric mean of 1.48) based on length which was the most sensitive parameter.

8. MATERIALS AND METHODS:

A. Test Animals:

Acute Fish Toxicity: Juveniles sheepshead minnow (Cyprinodon variegatus) reared at BMRL in natural sea water. The fish were 7-10 mm SL and 6-22 mg wet weight.

Fish Early Life Stage: Embryos were obtained by stripping eggs from adult fish collected at Big Lagoon, a Gulf of Mexico estuary adjacent to BMRL. Egg production was enhanced by the addition of human chorionic gonadotrophin hormone (A.P.L., Ayerst Laboratories Incorporated, New Jersey) for two consecutive days. A sperm suspension made from macerated testes was added to fertilize the eggs.

B. Test System:

The dilution water was seawater pumped from Big Lagoon. The intake was at approximately 80 m offshore and 3 m deep. The water was pumped by a #316 stainless steel pump through PVC pipes into an elevated fiberglass reservoir. The water passed sandfilled fiberglass filters, charcoal beds, 10 μm polypropylene core filters. The water was heated, aerated and flowed by gravity into the laboratory. Salinity was artificially maintained at >15 parts per million (during periods of low salinity) by the addition of Rila Marine Mix.

Acute Fish Toxicity: Test vessels were glass aquaria (29 cm x 30 cm 91 cm) holding 48 liters of test solution. The test system consisted of proportional flow diluter (Mount and Brungs, 1967) that provided 2.5 volume additions in 24 hours. A photoperiod of 12 hours dark and 12 hour light was provided.

Early Life Stage: The test system consisted of proportional flow diluter (Mount and Brungs, 1967) that provided 2.5 volume additions in 24 hours. Embryos were placed in incubator cups (Pyrex tubing 5.1 cm diameter and 75 cm in length). One end of the cup was covered with a 315 μm mesh size nylon screen. The cup were placed in an incubator holding cup chambers. Daily, embryos were removed and counted and the cups cleaned. Surviving fish were transferred to a glass growth chamber (14 x 20.5 x 26 cm with a 425 μm mesh size over one end).

C. Dosage:

Acute Fish Toxicity: Ninety six hour flow through test. Five nominal concentrations were chosen based on a range finding test. The concentrations are 0.62, 1.2, 2.5, 5, 10 mg/l. A control and solvent control (TEG) were used.

Fish Early Life Stage: Based on the 96-hour study, the concentrations were the same as for the acute fish toxicity. A control and a solvent control (TEG 130 mg/l) were used.

D. Design:

Acute Fish Toxicity: The diluter system cycled for 24 hours prior the begining of the test. Ten fish were placed in each duplicate test container. Loading was 2 mg/l. There was no aeration and fish were not feed during the test. DO, pH, salinity, and temperature were measured daily. Observations were made every 24 hours. Measured concentrations (of all treatments) were taken from replicate A on day 0 and from replicate B at test termination.

Fish Early Life Stage: The diluter system cycled for 24 hours prior to the beginning of the test. After visual confirmation of fertilization, 25 embryos were selected and randomly placed in the incubator cups (4 cups per treatment, 100 embryos per treatment). Embryo mortality and time hatched were observed. Survival of juveniles was monitored for 22 days. At test termination standard length and weight were measured. Measured concentrations were done every 7 days alternating between replicates A and B. DO, pH, salinity and temperature were measured in all replicates initially. Later they were measured in one duplicate set of the test containers.

E. Statistics:

Acute Fish Toxicity: The 96-hour LC50 was graphically interpolated.

Fish Early Life Stage:
For hatch and survival, the control and solvent control were pooled. Fisher Exact Test (two tails) was used to compare the treatments to control. For length and weight, the control and solvent control were pooled. William's test was used to compare the treatments to the control.

9. REPORTED RESULTS AND CONCLUSIONS:

Acute Fish Toxicity: The mean measured concentrations ranged from 0.59 to 9.4 mg/l (83-95% of nominal) (Table 1). Mortality range from 0% in the control, solvent control and treatments <4.4 mg /l to 70% mortality in the 9.4 mg/l treatment. The NOEL was 4.4 mg/l.

Loading was calculated as 2 mg/l. Salinity ranged from 20 to 24 part per million. Temperature ranged from 25-26° C. DO concentrations were ≥93% of saturation. pH ranged from 7.8 to 8.1.

Fish Early Life Stage: The mean measured concentrations ranged from 0.55 to 8.6 mg/l (82-89% from nominal) (Table 2). Hatching was not affected at concentrations less than 8.6 mg/l. Hatching was >90% complete by day 7 in all treatments. No delay in hatch was observed. Several embryonic development abnormalities were observed but were not attributed to metolachlor.

The test was terminated at day 26 because fish were dying as a result of the artificial salt introduced in the system. Mortality was significant at concentrations ≥ 4.1 mg/l after 8, 15, and 22 days posthatch. One surviving fish at the 1.0 mg/l treatment had one eye.

Length was significantly affected at the 4.1 mg/l treatment. Weight was not affected. A trend in decreasing length and weight was observed.

The MATC for embryos and juveniles of sheepshead minnows was 2.2 - 4.1 mg/l.

Salinity ranged from 13-22 part per million. Temperature was 25±1°C. DO ranged from 1.4 to 8.1 mg/l (19-108 % of saturation). pH range from 6.7 to 7.9.

10. QUALITY ASSURANCE MEASURES:

"The study was not conducted under current EPA Good Laboratory Practice Regulations: however, it was audited by the Quality Assurance Unit employe by the laboratory at that time. The signature of the quality assurance auditor may be found on page 24."

11. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure was generally in accordance with protocols recommended by the guidelines but deviate from the SEP as follows:

There are no description of the holding tank, no observations, such as mortality, pH, DO, salinity were made during the holding time or acclimation period.

The number of volume additions per day (2.5) was less than recommended (5-10).

The photoperiod of 12 light/12 hours dark is not recommended. The recommended photoperiod is 16 hour light/8 hours dark.

In the fish early life stage study the pH varied more than 0.8 of a unit.

Fish for the toxicity test were larger than recommended, 6-22 grams wet weight. Fish weight is recommended to be between 0.1 to 5.0 grams.

The concentration of solvent used in the fish toxicity test was not reported.

No behavioral and deformities observations were included in the report.

B. **Statistical Analysis:** The Binomial Test was used to calculate the LC50 in the acute toxicity test. For the fish early life stage study, William's Test was used to determined the LOEC and NOEC values.

C. **Results\Discussion:**

Acute Fish Toxicity: Many deficiencies were found in the study as mentioned in the deviations section. Based on the data presented, the LC50 value was calculated as 7.9 mg/l with confidence values of 4.4 to infinity. This study is scientifically sound. The study is classified as supplemental and the results may be useful in the risk assessment.

Fish Early Life Stage:

Hatch Data: In the reanalysis of the data, the registrant reported a NOEL value of 1 ppm and clarified that the abnormal fish were not included in the data. However, in the print out for the hatch data (page 37) it seems that the abnormal fry were included. The reviewer calculated the NOEL value as 4.1 mg/l. In this data set the abnormal fry were included. This is a more conservative value than the registrant NOEL. It is not very clear how the registrant calculated the NOEL for this parameter.

Survival Data: The study was terminated at day 26 because of mortalities caused by the addition of the artificial salt. The data does not show unusual mortalities in the control and solvent control neither at the lowest two concentrations tested. The reviewer does not consider the mortalities as caused by the addition of artificial salt but as caused by the chemical. Based on the data of the study, the NOEL for survival was 2.5 mg/l and the LOEL was 5.0 mg/l.

Length and Weight: Length was affected at concentrations of ≥2.2 mg/l. The NOEL is 1.0 mg/l. Weight was not significantly affected, but there is a decreasing trend as the concentration of metolachlor increases.

The water quality in the study was not the best. There was a variation of more than 1 unit in pH (6.7-7.9) during the

period of the study and the salinity ranged from 13 to 22 part per million.

The study is classified as supplemental and the results may be useful in the risk assessment.

D. **Adequacy of the Study:**

Acute Fish Toxicity and Fish Early Life Stage

1. **Classification:** Supplemental

2. **Rationale:** The studies do not fulfill the guideline requirements because of the many deficiences found in the studies. However, new studies will not be required. The results may provide useful information for a risk assessment.

3. **Repairability:** None

TABLE 1. Nominal and measured metolachlor (Dual®) concentrations during a 96-hour exposure of sheepshead minnows (Cyprinodon variegatus) in flowing, natural seawater. Salinity was 20-24 °/₀₀ and temperature was 25-26°C.

Nominal concentration (mg/ℓ;ppm)	Measured concentration (mg/ℓ;ppm)				
	0 h	96 h	Average	% of nominal	# samples
Control	ND[a]	ND	---	---	2
Sol. control	ND	ND	---	---	2
0.62	0.56	0.62	0.59	95	2
1.2	0.95	1.1	1.0	83	2
2.5	2.0	2.4	2.2	88	2
5.0	4.4	4.3	4.4	88	2
10	8.7	10	9.4	94	2
80,822 (stock)	82,000	-	-	101	1

[a]Not detected.

TABLE 2. Nominal and measured metolachlor (Dual®) concentrations during an early life stage test of sheepshead minnows (Cyprinodon variegatus) in flowing, natural seawater. Salinity was 17±2 °/oo and temperature was 25±4°C.

Nominal concentration (mg/ℓ;ppm)	Measured concentration (mg/ℓ;ppm)				
	Mean	S.D.[a]	Range	% of nominal	# samples
Control	ND[b]	---	---	---	6
Sol. control	ND	---	---	---	6
0.62	0.55	±0.04	0.50-0.60	89	6
1.2	1.0	±0.1	0.86-1.2	83	6
2.5	2.2	±0.2	2.0-2.4	88	6
5.0	4.1	±0.4	3.5-4.7	82	6
10	8.6	±0.4	8.2-9.0	86	4
80,825 (stock)	80,000	±6,000	72,000-91,000	99	6

[a]Standard deviation.
[b]Not detected.

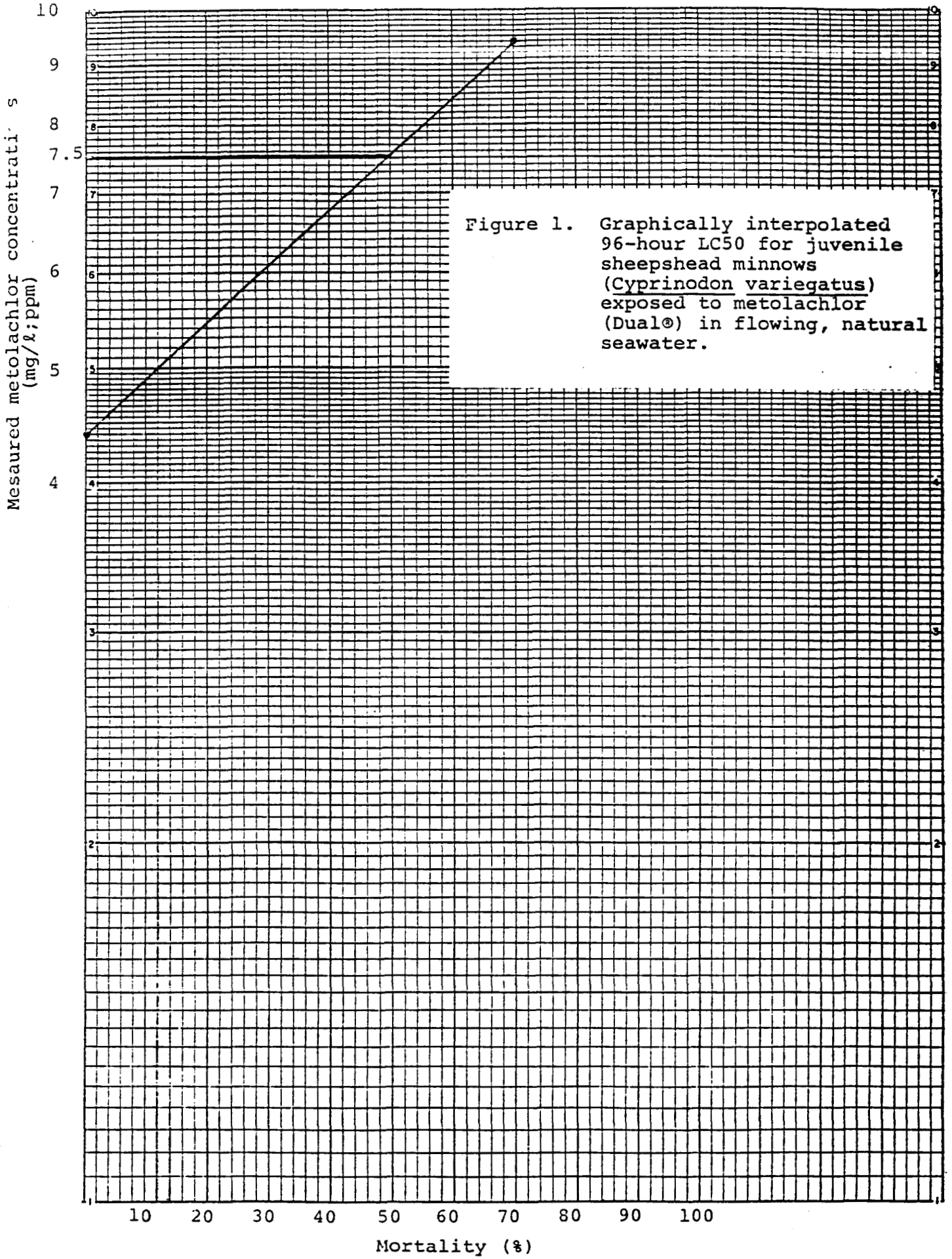

Figure 1. Graphically interpolated 96-hour LC50 for juvenile sheepshead minnows (Cyprinodon variegatus) exposed to metolachlor (Dual®) in flowing, natural seawater.

TABLE 3. Test concentrations and mortality of juvenile sheepshead minnows (Cyprinodon variegatus) exposed to metolachlor (Dual®) in flowing, natural seawater. Salinity was 20-24 °/oo and temperature was 25-26°C.

Mean measured concentration (mg/ℓ;ppm)	Mortality (%)			
	24 h	48 h	72 h	96 h
Control	0	0	0	0
Sol. control	0	0	0	0
0.59	0	0	0	0
1.0	0	0	0	0
2.2	0	0	0	0
4.4	0	0	0	0
9.4	0	5	25	70

TABLE 4. Hatching success of sheepshead minnow (Cyprinodon variegatus) embryos exposed to metolachlor (Dual®) in flowing, natural seawater.

Mean measured concentration (mg/ℓ;ppm)	Percentage hatch[a]				
	Treatment total	Rep. A1	Rep. A2	Rep. B1	Rep. B2
Control	98	96	96	100	100
Sol. control	100	100	100	100	100
0.55	100	100	100	100	100
1.0	97	100	96	96	96
2.2	92	92	76	100	100
4.1	95	88	100	96	96
8.6	97	96	100	92	100

[a] Percentage hatch was calculated by dividing the number of embryos which hatched by 25 for each of the replicates and 100 for the treatment.

TABLE 5. Percentage hatch of sheepshead minnows (Cyprinodon variegatus) embryos exposed to metolachlor (Dual®) in flowing, natural seawater as related to time.

Mean measured concentration (mg/ℓ;ppm)	Percentage hatch[a] Test Day 5	6	7	8
Control	11	84	100	100
Sol. control	0	53	98	100
0.55	7	55	99	100
1.0	1	46	98	99
2.2	1	59	99	99
4.1	0	35	92	99
8.6	0	32	93	100

[a]Percentage hatch was calculated by dividing the number of embryos which hatched on each test day by the total number of embryos which hatched.

TABLE 6. Mortality of juvenile sheepshead minnows (Cyprinodon variegatus) hatched in and exposed to metolachlor (Dual®) in flowing, natural seawater for 22 days posthatch.

Mean measured concentration (mg/ℓ;ppm)	Percentage mortality[a]				
	Treatment total	Rep. A1	Rep. A2	Rep. B1	Rep. B2
Control	6	4	4	8	8
Sol. control	3	4	0	4	4
0.55	8	4	12	12	4
1.0	4	12	4	0	0
2.2	8	4	11	4	12
4.1	40[b]	55	28	25	50
8.6	100[b]	100	100	100	100

[a]Percentage mortality was calculated by dividing the number of juveniles that died throughout the test by the number that hatched.
[b]Significantly greater ($P \leq 0.05$) than the solvent control.

TABLE 7. Growth of sheepshead minnows (Cyprinodon variegatus) exposed for 26 days to metolachlor (Dual®) in flowing, natural seawater. Mean standard length and standard deviation (S.D.) are given in millimeters (mm).

Mean measured concentration (mg/ℓ;ppm)	Mean standard length ± S.D.				
	Treatment total	Rep. A1	Rep. A2	Rep. B1	Rep. B2
Control	11±2	11±2	11±2	12±2	11±1
Sol. control	12±1	12±1	12±2	12±2	11±1
0.55	12±2	11±2	12±1	12±2	12±1
1.0	11±1	12±1	11±2	11±1	11±1
2.2	11±2	11±1	11±2	10±1	11±2
4.1	10±2[a]	11±1	10±2	10±2	12±1
8.6	-[b]	-	-	-	-

[a]Fish significantly ($P \leq 0.05$) smaller than control fish.
[b]No fish surviving.

TABLE 8. Growth of sheepshead minnows (Cyprinodon variegatus) exposed for 26 days to metolachlor (Dual®) in flowing, natural seawater. Wet weights are given in milligrams.

Mean measured concentration (mg/ℓ;ppm)	Mean wet weight				
	Treatment total	Rep. A1	Rep. A2	Rep. B1	Rep. B2
Control	34	33	32	39	33
Sol. control	40	39	40	48	31
0.55	39	36	46	42	35
1.0	37	43	39	33	34
2.2	35	36	38	29	38
4.1	31	35	31	22	45
8.6	-[a]	-	-	-	-

[a]No fish survived.

Conchi Rodriguez Metolachlor LC50 Sheepshead Minnow

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
9.399999		20	14	70
5.765915				
4.4	20	0	0	9.536742E-05
2.2	20	0	0	9.536742E-05
1	20	0	0	9.536742E-05
.59	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 4.4 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.926247

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Sheepshead Minnow Length - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Solvent Control	4	11.750	11.750	11.750
2	Control	4	11.250	11.250	11.500
3	0.59 mg/l	4	11.750	11.750	11.500
4	1.0 mg/l	4	11.250	11.250	11.250
5	2.2 mg/l	4	10.750	10.750	10.750
6	4.4 mg/l	4	10.750	10.750	10.750

Sheepshead Minnow Length - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Solvent Control	11.750				
Control	11.500	0.588		1.73	k= 1, v=18
0.59 mg/l	11.500	0.588		1.82	k= 2, v=18
1.0 mg/l	11.250	1.177		1.85	k= 3, v=18
2.2 mg/l	10.750	2.353	*	1.86	k= 4, v=18
4.4 mg/l	10.750	2.353	*	1.87	k= 5, v=18

s = 0.601
Note: df used for table values are approximate when v > 20.

Sheepshead Minnow Weight - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	36.838	36.838	37.717
2	0.59 mg/l	4	39.475	39.475	37.717
3	1.0 mg/l	4	37.200	37.200	37.200
4	2.2 mg/l	4	35.125	35.125	35.125
5	4.4 mg/l	4	33.450	33.450	33.450

Sheepshead Minnow Weight - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	37.717				
0.59 mg/l	37.717	0.236		1.73	k= 1, v=19
1.0 mg/l	37.200	0.097		1.81	k= 2, v=19
2.2 mg/l	35.125	0.460		1.84	k= 3, v=19
4.4 mg/l	33.450	0.911		1.85	k= 4, v=19

s = 6.075
Note: df used for table values are approximate when v > 20.

eepshead Minnow Survival - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	94.750	94.750	94.750
2	0.62 mg/l	4	92.000	92.000	93.875
3	1.2 mg/l	4	95.750	95.750	93.875
4	2.5 mg/l	4	93.250	93.250	93.250
5	5.0 mg/l	4	42.500	42.500	42.500

Sheepshead Minnow Survival - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	94.750				
0.62 mg/l	93.875	0.200		1.73	k= 1, v=19
1.2 mg/l	93.875	0.200		1.81	k= 2, v=19
2.5 mg/l	93.250	0.344		1.84	k= 3, v=19
5.0 mg/l	42.500	11.966	*	1.85	k= 4, v=19

s = 7.130
Note: df used for table values are approximate when v > 20.

Sheepshead Minnow Hatching - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	0.985	0.985	0.990
2	0.55 mg/l	4	1.000	1.000	0.990
3	1.0 mg/l	4	0.950	0.950	0.950
4	2.2 mg/l	4	0.910	0.910	0.920
5	4.1 mg/l	4	0.930	0.930	0.920
6	8.6 mg/l	4	0.840	0.840	0.840

Sheepshead Minnow Hatching - Metolachlor
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.990				
0.55 mg/l	0.990	0.106		1.72	k= 1, v=22
1.0 mg/l	0.950	0.740		1.80	k= 2, v=22
2.2 mg/l	0.920	1.374		1.83	k= 3, v=22
4.1 mg/l	0.920	1.374		1.84	k= 4, v=22
8.6 mg/l	0.840	3.065	*	1.85	k= 5, v=22

s = 0.077
Note: df used for table values are approximate when v > 20.