62623
MRID No. ~~92189-010~~

DATA EVALUATION RECORD

1. **CHEMICAL:** Triclopyr Triethylamine
Shaughnessey No. 116002

2. **TEST MATERIAL:** M-3724 Herbicide; 43.8% active ingredient (3,5,6-trichloro-2-pyridyloxyacetic acid, triethylamine salt); 56.2% inert ingredients; Lot No. N-1207; a clear pink liquid.

3. **STUDY TYPE:** Marine Invertebrate Static Acute Toxicity Test. Species Tested: eastern oyster (Crassostrea virginica), pink shrimp (Penaeus duorarum), and fiddler crab (Uca pugilator).

4. **CITATION:** Anonymous. 1979. Acute toxicity of M-3724 to larvae of the eastern oyster, pink shrimp, and fiddler crabs. Performed by Bionomics-EG&G, Inc., Pensacola, FL. Submitted by Dow Chemical Company, MI. EPA MRID No. ~~92189-~~62623 ~~010~~. 12-3(b)

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist II
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 2/5/91

3.7.91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 2/5/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: H. T. Craven
Date: 3/15/91

7. **CONCLUSIONS:** This study is scientifically not sound. Mortality and water quality were not measured in the eastern oyster test. In the pink shrimp test, the dissolved oxygen dropped as low as 24% of saturation. The fiddler crab is not an acceptable test species. Together with other deviations from the guidelines, uncertainties in the results limit their usefulness. Based on nominal concentrations, the 48-hour EC_{50} value of M-3724 Herbicide to eastern oyster larvae was 55.7 ppm (v/v). The 96-hour LC_{50} values for pink

16.5 hrs

shrimp and fiddler crab were 957.7 and >1000 ppm (v/v), respectively. A descriptive classification could not be assigned to the test material because the concentration was given as a volume to volume ratio.

8. RECOMMENDATIONS: N/A

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals:

1. Adult oysters were collected from the field and held in flowing sea water for approximately 6 months. Larvae were obtained by induced spawning of adults. Individual, sexually mature female oysters, held in 1-liter glass chambers, were induced to spawn by increasing the temperature in the chambers from 23°C to 33°C over a 30-minute interval in the presence of sperm (excised from the gonad of a sexually mature male oyster). Density of the larvae was determined by hemacytometer counts.

2. Shrimp and crabs were acclimated to the laboratory for a minimum of seven days following collection from the field. Shrimp were 4-6 cm long (rostrum to telson) and crabs were 2-3 cm wide (carapace width). Mortality was less than 3% during the acclimation period.

B. Test System:

1. Oyster Larvae Test. Filtered (5 µm) natural sea water was adjusted to 23 ppt using commercial sea salts. Test concentrations were obtained by pipetting appropriate amounts of the test material, dissolved in distilled water, into one-liter glass jars containing 900 mL dilution water. The temperature was 20°±1°C.

2. Shrimp and Crab tests. The tests were conducted in 19-liter uncovered glass jars containing 15 liters of filtered (5 µm) natural sea water. The test material was pipetted directly into the test containers. Salinity was adjusted to 24 ppt for the pink shrimp test and 20 ppt for the fiddler crab test; temperature was 15°±1°C for both tests.

No aeration was used and the animals were not fed during the tests.

C. **Dosage:** Forty-eight-hour static test using eastern oyster larvae. Ninety-six-hour static test using pink shrimp and fiddler crabs.

D. **Design:** Based on preliminary test results, oyster larvae (20,000-30,000) were added to five nominal concentrations (8.7, 18, 32, 56, and 87 ppm, v/v) and a dilution water control. The criterion for effect was failure of the larvae to develop normally to the straight hinge stage within 48 hours. After 48 hours of exposure, the larvae in each vessel were collected with a sieve, washed into glass vials and preserved with 3% formalin. The number of normal 48-hour straight-hinge larvae that occurred in a 200-larvae total count was microscopically determined for each test concentration. The percentage of abnormally developed larvae was calculated for each duplicate container and averaged.

Based on preliminary tests, pink shrimp were tested at 750, 870, 1000, 1200, and 1400 ppm (v/v) and a control. Five shrimp were added to each test container. Mortality and dissolved oxygen (D.O.) were determined every 24 hours.

Five fiddler crabs were added to each test container. Based on preliminary tests, the crabs were tested at 320, 560, 750, 870, and 1000 ppm (v/v) and a control. Complete loss of equilibrium was determined by prodding with a glass rod every 24 hours.

Two replicate containers were used for each concentration and control. The pH of the test solutions was determined at the beginning and end of the test.

E. **Statistics:** The 48-hour median effective concentration (EC_{50}) for eastern oysters was given as an interval based on inspection. The 96-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) for pink shrimp were determined by the probit method. No LC_{50} could be calculated in the fiddler crab test due to insufficient mortality.

12. **REPORTED RESULTS:** The 48-hour EC_{50} interval for eastern oyster larvae was given as being between 56 and 87 ppm (v/v). The 96-hour LC_{50} was given as 895 ppm, v/v, (C.I. =

759-1055) for pink shrimp. The 96-hour LC_{50} for the fiddler crab was greater than 1000 ppm (v/v).

Dissolved oxygen was at saturation upon initiation of all tests. In the pink shrimp test, D.O. dropped below 40% of saturation in the 750, 870 and 1000 ppm (v/v) test vessels between 48 and 96 hours after initiation (Table 4, attached). Oxygen saturation remained above 60% of saturation in all containers in the fiddler crab test. Dissolved oxygen was not measured in the eastern oyster larvae test.

The final pH was 8.0±0.5 for all test concentrations and controls in all tests.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

No conclusions were made by the author.

No statements were made regarding quality assurance and good lab practice.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines with the following deviations:

1. Eastern Oyster Larvae Test

The D.O. of the test solutions was not measured during and after the test. It cannot be assumed that D.O. sags are not responsible for the abnormal growth noted. In the two accompanying studies, D.O. decreases were found.

Mortality was not observed during the study. Mortality is an endpoint in the test and must be reported. The SEP recommends that no more than 30% mortality occur in the test.

The salinity (23 ppt) of the dilution water differed from recommended values of 30-34 ppt for marine tests and 10-17 ppt for estuarine tests.

The age of the larvae was not noted in the report.

The NOEC was not determined.

2. Pink Shrimp and Fiddler Crab Tests

The fiddler crab is not an acceptable test species and no data were in the report to support its use. The results indicate that it was not as sensitive as the pink shrimp.

The average weight of the test animals was not included in the report. Loading could not be determined in either test.

The D.O. dropped as low as 24% saturation sometime between 48 and 96 hours in the pink shrimp test. The SEP recommends that oxygen saturation remain above 40% during the last 48 hours of the test.

The test temperature (15°±1°C) was much lower than recommended (22°±1°C).

The salinity in the pink shrimp test (24 ppt) and fiddler crab test (20 ppt) differed from recommended test solution salinities (previously noted).

The shrimp and crabs were held for a minimum of seven days prior to testing. A period of at least ten days is recommended. The report did not state whether the test organisms were maintained under actual test conditions for at least 48 hours before the test as recommended in the SEP.

3. All Tests

Treatment concentrations were reported as µL/L or ppm. The density of the test material, a liquid, was not given in the report so conversion of the endpoints to milligrams of product per liter of test solution is not possible. The registrant needs to submit this information.

Rangefinding tests were mentioned as being performed but no results were given in the report.

The photoperiod was not reported.

Actual water quality parameters measured were not included in the report.

The temperature was not monitored continuously and the temperature maintenance system was not described in the report.

The inert ingredients in the formulation were not tested separately.

B. Statistical Analysis:

The reviewer used EPA's Toxanal program to determine the 48-hour EC_{50} for eastern oyster as 55.7 ppm, v/v (95% C.I. = 53.2-58.2) by the moving average method (printout, attached). Raw data from the oyster larval test was not submitted therefore, no NOEC could be determined.

The 96-hour LC_{50} for pink shrimp was 957.7 ppm, v/v (95% C.I. = 871.0-1047.2 ppm) by probit analysis (printout attached). The slope of the probit line was 12.3. An LC_{50} value for fiddler crabs could not be generated.

C. Discussion/Results:

1. Eastern Oyster Larvae Test.

It is the opinion of the reviewer that variable water quality can affect the toxicity of chemicals. Monitoring water quality is an essential part of every aquatic toxicity test for this reason. Mortality was not measured, so the reviewer has no way to determine the effect of water quality on the oyster larvae. The uncertainty under which the test was performed limits the usefulness of the EC_{50} generated.

2. Shrimp and Crab Tests.

Control D.O. was at 45% saturation after 96 hours in the pink shrimp test. Dissolved oxygen appears to decrease with increasing toxicant concentration (Table 4, attached). The test material may have a high biological oxygen demand when mixed with water, but this is not supported by the crab data (i.e. greater than 60% oxygen saturation in all containers). A more plausible explanation may be that loading in the pink shrimp test chambers was excessive and that the higher mortality seen at higher concentrations is due to increased oxygen usage by the toxicant stressed animals, followed by suffocation. If the test would

have been run at the recommended temperature (22°C), increased sensitivity may have led to a lower LC_{50}. The crabs do not appear to be as sensitive to the test material as the pink shrimp or perhaps loading in the chambers was less excessive.

D. **Adequacy of the Study:**

(1) Classification: Invalid

(2) Rationale: 1) Eastern oyster larval test: Mortality in the chambers was not determined; water quality measurements were not made. 2) Shrimp and crab tests: the fiddler crab is not an acceptable species; significant deviations from the guidelines existed (temperature, dissolved oxygen, salinity). 3) All tests: The concentration of the test material was given in µL/L, but the density was not provided; there were too many significant deviations from the guidelines.

(3) Repairability: No

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 01-24-91.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

LOUIS M. RIFICI TRICLOPYR CRASSOSTREA VIRGINICA 1-20-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
87	97	97	100	9.765625E-02
56	97	37	38.1443	9.765625E-02
32	97	2	2.0619	17.1875
18	97	2	2.0619	17.1875
8.7	97	0	0	5.46875

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 59.53199

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.064194E-02	55.65054	53.2161	58.22577

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
12	8.693159	100.0826	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 7.388232
95 PERCENT CONFIDENCE LIMITS =-14.39535 AND 29.17182

LC50 = 55.77085
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 37.54155
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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LOUIS M. RIFICI TRICLOPYR PENAEUS DUORARUM 1-20-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1400	10	10	100	9.765625E-02
1200	10	10	100	9.765625E-02
1000	10	3	30	17.1875
870	10	3	30	17.1875
750	10	2	20	5.46875

THE BINOMIAL TEST SHOWS THAT 0 AND 1200 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 1042.498

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.214883	945.8125	825.5075	1041.442

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
14	.2137714	1	.0997954

SLOPE = 12.27678
95 PERCENT CONFIDENCE LIMITS = 6.600559 AND 17.953

LC50 = 957.7228
95 PERCENT CONFIDENCE LIMITS = 871.0012 AND 1047.265

LC10 = 754.7318
95 PERCENT CONFIDENCE LIMITS = 593.6161 AND 838.3421

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TABLE 4. Measured concentrations of dissolved oxygen during a 96-hour exposure of pink shrimp (Penaeus duorarum) to M-3724 in static sea water. Salinity was 24 °/oo and temperature, 15±1°C.

Nominal concentration (µl/l;ppm)	Dissolved oxygen (mg/l and % of saturation)		
	24 h	48 h	96 h
Control	6.8 (81)	5.1 (61)	3.8 (45)
750	6.8 (81)	5.3 (63)	3.0 (36)
870	6.2 (74)	5.3 (63)	2.5 (29)
1,000	6.3 (75)	5.2 (62)	2.0 (24)
1,200	6.2 (74)	5.1 (61)	-[a]
1,400	6.3 (75)	4.9 (59)	-[a]

[a]DO not measured because 100% mortality had occurred.

Laughlin No. 116002 Chemical Name Triclopyr. Triethylamine Chemical Class ____ Page 1 of 3

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
4-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
pecies	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
ab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
cc.		Comments:		
4-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)=		
pecies	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
ab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
cc.		Comments:		
-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
pecies		Slope= # Animals/Level= Age(Days)= Sex =	____	____
ab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
cc.		Comments:		
-Day Dietary LC_{50}		LC50 = ppm (95% C.L) [illegible]		
pecies		Slope= # Animals/Level= Age(Days)= Sex =	____	____
ab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
cc.		Comments:		
48-Hour LC_{50} Larvae test		LC50 = 55.7 ppm* (95% C.L. MOVING AVERAGE 53.2 - 58.2) Contr. Abnormal (%)= 3.0 Sol. Contr. Mort.(%)= N/a		
pecies Crassostrea VIRGINICA 43.8		Slope= N/A # Animals/Level= Temperature = 20°C LR 1/24/91 (INVALID		
ab Biohomics - EG+G		48-Hour Dose Level ppm/(Abnormal %Mortality) 8.7 (3), 18 (5), 32 (5), 64 (40), 87 (100)		
cc. MRID No. 92189-010		Comments: ppm in v/v Ratio nominal concentrations, mortality not measured		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mor(%)= Sol. Con. Mor.(%)=		
pecies		Slope= # Animals/Level= Temp.=	____	____
ab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
cc.		Comments:		
96-Hour LC_{50}		LC50 = pp__ (95% C.L) Con. Mort.(%)= Sol. Con. Mort.(%)=		
pecies		Slope= # Animals/Level= Temp.=	____	____
ab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
cc.		Comments:		

Shaughnessey No. 116002 Chemical Name Triclopyr Triethylamine Chemical Class ____ Page 2 of 3

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
96-Hour LC_{50}		LC50 = 957.7 ppm (95% C.L. Probit 871.0 - 1047.3) Con. Mort(%)=0 Sol. Con. Mort.(%)=N/A	LR 1/24/91	INVALID
Species Penaeus duorarum	43.8	Slope= 12.3 # Animals/Level= 10 Temp.= 15± 1°C		
Lab Bionomics EG&G		96-Hour Dose Level ppm/(%Mortality) 750 (20), 870 (30), 1000 (30), 1200 (100), 1400 (100)		
Acc. MRID # 92189-018		Comments: ppm IN V/V Ratio, nominal concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		

Haughnessey No. 116002 Chemical Name Triclopyr, Triethylamine Chemical Class ____ Page 2 of 3

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
96-Hour LC_{50}		LC50 = >1000 ppm* (95% C.L. N/A) Con. Mort(%) = 0 Sol. Con. Mort.(%)= N/A		
Species *Uca pugilator*	43.8	Slope= N/A # Animals/Level=10 Temp.= 16±1°C	LR 1/24/91	ACCEPTED
Lab *Bionomics EG+G*		96-Hour Dose Level ppm*/(%Mortality) 320 (0), 560 (0), 750 (0), 870 (0), 1000 (0)		
Acc. *MRID # 92189-010*		Comments: ppm w/v/v Ratio, nominal concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		