25 6-15-00

MRID No. 445050-07

DATA EVALUATION RECORD
§ 72-3 - ACUTE LC_{50} TEST WITH A ESTUARINE/MARINE FISH

1. **CHEMICAL:** Mesotrione PC Code No.: 122990

2. **TEST MATERIAL:** ZA1296 Purity: 96.8%

3. **CITATION:**

Authors:	S.J. Kent, N. Shillabeer, J.E. Caunter, S.J. Wallace
Title:	ZA1296: Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*)
Study Completion Date:	February 16, 1996
Laboratory:	Brixham Environmental Laboratory, Brixham Devon, UK
Sponsor:	ZENECA Ag Products, Wilmington, DE
Laboratory Report ID:	BL5592/B
MRID No.:	445050-07
DP Barcode:	D245475

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist, Golder Associates Inc.

Signature: [signature] **Date:** 8/25/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature: P. Kosalwat **Date:** 8/25/98

5. **APPROVED BY:**

Signature: [signature] **Date:** 6/15/00

6. **STUDY PARAMETERS:**

Age or Size of Test Organism:	25-36 mm
Definitive Test Duration:	96 hours
Study Method:	Static
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. The 96-hour LC_{50} of ~~410~~ 520 ppm classifies ZA1296 as practically non-toxic to the sheepshead minnow. The NOEC was 100 ppm.

Results Synopsis

LC_{50}: 410 ppm 95% C.I.: 340 - 510 ppm

NOEC: 100 ppm Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The nominal concentrations of two highest treatment levels (560 and 1000 mg/L) appeared to be higher than the solubility of the test compound under the conditions of this test. Therefore, a solvent should have been used.

2. The pH of the 560 and 1000 mg/L nominal test solutions (4.46 and 4.02, respectively) were much lower than recommended (7.7 - 8.0).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the silverside (*Menidia* spp.).	*Cyprinodon variegatus*
Mean Weight 0.1-5 g	Mean: 0.86 g Range: 0.44 - 1.35 g
Mean Standard Length Longest not > 2x shortest	Mean: 31 mm Range: 25-36 mm
Supplier	In house culture
All fish from same source?	Yes

Guideline Criteria	Reported Information
All fish from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Fish culture maintained under conditions similar to test
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study	Not fed 48 hours prior to or during testing.
Pretest Mortality < 3% mortality 48 hours prior to testing	<1% mortality 3 weeks prior to the study.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Reconstituted seawater or seawater from a natural source.	Seawater mixed with dechlorinated tap water (50/50) that was filtered, sterilized, and treated with sodium thiosulphate.
Does water support test animals without observable signs of stress?	Yes
Salinity Weekly range should not deviate by more than 6%.	17.5 ±1‰
Water Temperature 22°C	21.0 - 21.9°C

Guideline Criteria	Reported Information
pH Monthly range must not deviate by more than 0.8 unit. Euryhaline: 7.7-8.0 Stenohaline: 8.0-8.3	3.95 - 8.11
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	>65% throughout test
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	 Glass 40 L 30 L
Type of Dilution System Must provide reproducible supply of toxicant	Static test
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.29 g/L
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	No solvent was used

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	None
Nominal Concentrations of Definitive Test Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Control and 5 treatment levels: 100, 180, 320, 560, and 1000 mg/L.
Number of Test Organisms Minimum 10/level for static test, 20/level for flow-through, may be divided among containers	10 fish per treatment level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature was measured daily in all aquaria and hourly in the control. DO and pH were measured every 24 hours in all test chambers.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Yes, solutions collected and analyzed at test initiation, 48 hours, and 96 hours.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	48-100% of nominal; 55-100% of nominal when one measurement at the highest treatment level is excluded.
Control Mortality Not more than 10% control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg/L)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control	<0.45	10	0	0	0	0
100	100	10	0	0	0	0
180	180	10	0	0	0	0
320	310	10	0	0	0	0
560	520	10	0	3	4	5
1000	550*	10	5	10	10	10

*Mean of 48 and 96 hour measured concentrations only.

Other Significant Results: The 100 mg/L nominal concentration was observed to be a clear pale yellow solution. A darker yellow color was observed in the 180 and 320 nominal treatment levels with a small number of undissolved particles on the surface and bottom of the test solutions. The 560 and 1000 mg/L solutions were even darker yellow to brown and were cloudy. All particles appeared to be dissolved in the 180 and 320 mg/L test solution after 24 hours. Some particles remained and settled in the 560 and 1000 mg/L test solutions over the duration of the test. All samples were centrifuged before analysis.

The concentration of test material in the initial (0 hour) sample from the highest treatment level was only 35% of nominal (350 mg/L). While the 48 and 96 hour samples contained 550 and 540 mg/L of test material, respectively. The authors decided to exclude the initial measurement from calculated mean measured concentration since during "the majority of the study the measured concentrations in the nominal 1000 mg l^{-1} were closer to those values obtained at 48 and 96 hours than the 0 hour concentration" and "50% of the nominal 1000 mg l^{-1} test solution fish were dead at 24 hours whereas no fish were dead in the nominal 560 mg l^{-1} test solution at this time.

Sublethal effects including sounding, loss of balance, weakness, rapid respiration and ceased swimming were observed in fish from all but the lowest concentration (100 mg/L).

B. Statistical Results

Method: Moving average angle

96-hr LC_{50}: 410 mg/L 95% C.I.: 340 - 510 mg/L

Probit Slope: N/A NOEC: 100 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Method: Binomial method

96-hr LC_{50}: 520 ppm 95% C.I.: 310 - 550 ppm

Probit Slope: N/A NOEC: 100 ppm

14. **REVIEWER'S COMMENTS:** Although the authors expected the test material to be soluble in water at a higher concentration than the concentrations selected, it appears that the two highest treatment levels (560 and 1000 mg/L nominal) were indeed higher than the solubility of this compound under the conditions of this test. The authors, therefore, should have used a solvent. In addition, the pH of the two highest treatment levels were much lower than the recommended pH for maintaining sheepshead minnows. The low pH may have been directly responsible for the toxicity of the treatment solutions to sheepshead minnows. Nevertheless, it appears that this compound is practically non-toxic (LC_{50} > 100 ppm) to sheepshead minnows regardless of whether or not this study was performed using a solvent or a buffer (to raise the pH).

This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using an estuarine fish. The 96-hour LC_{50} of 410 ppm classifies ZA1296 as practically non-toxic to the sheepshead minnow. The NOEC was 100 ppm. This study is classified as **Core**.

MAX FEKEN ZA1296 SHEEPSHEAD 08-04-98

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
550	10	10	100	9.765625E-02
520	10	5	50	62.30469
310	10	0	0	9.765625E-02
180	10	0	0	9.765625E-02
100	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 310 AND 550 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 519.9999

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.

****