DATA EVALUATION RECORD
§ 72-3 – ACUTE EC$_{50}$ TEST WITH AN ESTUARINE/MARINE MOLLUSK EMBRYO/LARVAL STUDY

1. **CHEMICAL:** Mesotrione   <u>PC Code No.</u>: 122990

2. **TEST MATERIAL:** ZA1296 technical   <u>Purity</u>: 96.8%

3. **CITATION:**

<u>Authors</u>: S.J. Kent, N. Shillabeer, J.E. Caunter, and S.J. Wallace

<u>Title</u>: ZA1296: Acute Toxicity to Larvae of the Pacific Oyster (*Crassostrea gigas*)

<u>Study Completion Date</u>: February 29, 1996

<u>Laboratory</u>: Brixham Environmental Laboratory, ZENECA Ltd., Brixham, Devon, UK

<u>Sponsor</u>: ZENECA Ag Products, Wilmington, DE

<u>Laboratory Report ID</u>: BL5594/B

<u>MRID No.</u>: 445050-09

<u>DP Barcode</u>: D245475

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates Inc.

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: **Date:** 6/12/00

6. **STUDY PARAMETERS:**

Age of Test Organism: <1 hour post-fertilization
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements. Based on nominal concentrations, the 48-hour EC$_{50}$ was estimated to be 69 ppm, which classifies ZA1296 technical as slightly toxic to Pacific oyster larvae.

Results Synopsis

EC$_{50}$: 69 ppm 72   95% C.I.: 64 – 74 ppm
NOEC: 32 ppm   Probit Slope: N/A

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8. ADEQUACY OF THE STUDY:

A. **Classification:** Core

B. **Rationale:** N/A

C. **Repairability:** N/A

9. BACKGROUND:

10. GUIDELINE DEVIATIONS:

1. The pH of the seawater used (8.1-8.2) was slightly higher than recommended (7.7-8.0).
2. The salinity of the seawater used (32%) was greater than recommended (10-17%).
3. Test vessels (250-mL borosilicate beakers) were smaller than recommended (1-L glass beakers).

11. SUBMISSION PURPOSE:

12. MATERIALS AND METHODS:

A. **Test Organisms**

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster, the Eastern oyster, the mussel, or the Quahog.	*Crassostrea gigas*
Age of embryos Eggs should be tested within 3 hours of fertilization.	Embryos introduced into test solutions 15 minutes after fertilization
Supplier	Guernsey Sea Farms, Channel Islands, UK
Are all oysters from same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural seawater from an uncontaminated source or reconstituted water.	Aerated, filtered seawater from Tor Bay, Devon, UK
Does water support test animals without observable signs of stress? Not more than 10% abnormal embryos and not more than 30% mortality in 48 hours.	Yes
Salinity 10-17 % salinity, weekly range < 6 ‰	31.5‰
Water Temperature 20°-25° C, ±2°C	19.6 - 20.5°C
pH 7.7-8.0	7.22 - 8.19
Dissolved Oxygen ≥ 60% throughout	≥97% of saturation during the test
Total Organic Carbon	Not reported
Test Vessels Glass 1-liter beakers preferred.	250-mL borosilicate beakers with loose-fitting covers
Type of Dilution System Must provide reproducible supply of toxicant.	Static test
Flow rate Consistent flow rate.	N/A
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Aeration Not recommended.	No aeration during the test
Solvents Not to exceed 0.5 mL/L.	Solvent: none Maximum conc.: N/A

C. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L, then no definitive test is required.	No range finding tests reported
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series.	Dilution water control as well as 6 treatment levels of 10, 18, 32, 56, 100, and 180 mg/L. Treatments replicated twice.
Number of Controls Four replicates of each control or 10% of the total number of treatment replicates.	Dilution water control replicated four times
Number of Test Organisms 20,000 to 30,000 embryos/L per treatment level and in each control.	22,000 embryos/L
Biological observations made? Occurrences of misshapen or malformed shells should be reported.	Yes

Water Parameter Measurements
1. Temperature
   Measured hourly in at least one chamber.
2. DO and pH
   Measured at beginning of test and at 48 h in the high, medium, and low doses and in the control.
	Temperature was measured daily in two control and one treatment replicate(s) and continuously in a surrogate vessel.
	
	DO and pH were measured at initiation and termination in two control and one treatment replicate(s).

Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes, initial and terminal solutions analyzed by HPLC.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% abnormal embryos and not more than 30% mortality in 48 hours.	8% abnormal embryos and 19% mortality in the dilution water control by test termination
Recovery of Chemical	90-106% of nominal
Raw data included?	Yes
Signs of toxicity (if any) were described?	Only reported as abnormal development

Larval mortality

Concentration (ppm)		Number of Normal Larvae per sample	Percentage normal	Mean Percent Decrease in Normal Development
Nominal	Mean Measured			
Control	<0.52	16	75	-
10	9	17	79	0%
18	18	17	79	0%
32	32	17	79	0%
56	58	15	69	8%
100	100	0	0	100%
180	190	0	0	100%

*Abnormal larvae include dead and abnormally developed larvae

B. Statistical Results

Method: Moving average angle (based on nominal conc.)

48-hr EC$_{50}$: 69 ppm   95% C.I.: 64 - 74 ppm
Probit Slope: N/A   NOEC: 32 ppm

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14. <u>VERIFICATION OF STATISTICAL RESULTS</u>:

Parameter	Results[*]
Statistical Method for EC_{50}	Binomial
EC_{50} (95% C.I.)	72 ppm (could not be calculated)
Probit Slope	N/A
Statistical Method for NOEC	Bonferroni's test
NOEC	58 ppm

[*]Based on mean measured concentrations

15. <u>REVIEWER'S COMMENTS</u>: This study is scientifically sound and fulfills the guideline requirements. The study can be classified as **Core**.

Oyster larvae normal development
File: oys   Transform: ARC SINE(SQUARE ROOT(Y))

ANOVA TABLE
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SOURCE	DF	SS	MS	F
Between	6	2.891	0.482	138.756
Within (Error)	9	0.031	0.003	
Total	15	2.922		

Critical F value =   3.37   (0.05,6,9)
Since F > Critical F REJECT Ho: All equal

Oyster larvae normal development
File: oys   Transform: ARC SINE(SQUARE ROOT(Y))

BONFERRONI t-TEST   —   TABLE 1 OF 2   Ho:Control<Treatment
_____

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	1.046	0.748		
2	9 ppm	1.095	0.785	-0.960	
3	18 ppm	1.089	0.785	-0.854	
4	32 ppm	1.089	0.785	-0.854	
5	58 ppm	0.980	0.690	1.278	
6	100 ppm	0.079	0.000	18.940	*
7	190 ppm	0.079	0.000	18.940	*

Bonferroni t table value = 2.93   (1 Tailed Value, P=0.05, df=9,6)

NOEC= 58 ppm

Oyster larvae normal development
File: oys   Transform: ARC SINE(SQUARE ROOT(Y))

BONFERRONI t-TEST   —   TABLE 2 OF 2   Ho:Control<Treatment
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GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	4			
2	9 ppm	2	0.139	18.6	-0.037
3	18 ppm	2	0.139	18.6	-0.038
4	32 ppm	2	0.139	18.6	-0.038
5	58 ppm	2	0.139	18.6	0.057
6	100 ppm	2	0.139	18.6	0.748
7	190 ppm	2	0.139	18.6	0.748

⑦

Mossler   ZA1296   Crassostrea gigas   8-5-98
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
190	100	100	100	0
100	100	100	100	0
58	100	8	8	0
32	100	0	0	0
18	100	0	0	0
9	100	0	0	0

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 72.13574

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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