6-15-00

MRID No. 445050-10

DATA EVALUATION RECORD
§ 72-4 - AQUATIC INVERTEBRATE LIFE CYCLE TEST

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

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

3. **CITATION:**

Authors:	D.S. Morris, H. Kelso, N. Shillabeer, N.J. Williams, and S.J. Wallace
Title:	ZA1296: Chronic Toxicity to *Daphnia magna*
Study Completion Date:	January 31, 1996
Laboratory:	Brixham Environmental Laboratory, Brixham, Devon, UK
Sponsor:	ZENECA Ag Products, Wilmington, DE
Laboratory Project ID:	BL5832/B
MRID No.:	445050-10
DP Barcode:	D245475

4. **REVIEWED BY:** Mark Mossler, M.S., 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 of Test Organism:	<24 hours
Definitive Test Duration:	21 days
Study Method:	Static renewal
Type of Concentrations:	Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a freshwater invertebrate life-cycle test.

Results Synopsis: Most sensitive endpoint: survival

NOEC: 180 mg/L LOEC: 300 mg/L MATC: 230 mg/L

8. ADEQUACY OF THE STUDY:

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. GUIDELINE DEVIATIONS:

1. The test design (1 daphnid/vessel, 10 replicates) was not the recommended design (7 vessels with one daphnid each and 4 vessels with 5 daphnids each).

2. The chemical analyses section was not reported fully.

3. The dilution water hardness (237 mg/L as $CaCO_3$) was greater than recommended (180 mg/L as $CaCO_3$).

10 SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms/Acclimation

EPA Guideline Criteria	Reported Information
Species *Daphnia magna*	*Daphnia magna*
Source Laboratory, commercial, or wild stock.	In-house cultures
Parental Acclimation Conditions Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Held at 20 ±2°C in dilution water
Parental Acclimation Period At least 21 days.	Continuous
Age of Parental Stock At least 10-12 days old at the beginning of the acclimation period.	15 ±1 days old

EPA Guideline Criteria	Reported Information
Food Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	Mixture of algae (*Chlorella vulgaris*) and microencapsulated food (Frippak Booster®)
Food Concentration 5 mg/L (dry wt.) of synthetic food or 10^8 cells/L of algae is recommended.	1.5×10^9 cells and 713 µg of Frippak Booster® per liter daily.
Were daphnids in good health during acclimation period?	Yes

B. Test System

EPA Guideline Criteria	Reported Information
Test Water Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)	Reconstituted water medium, aerated >2 hours prior to use
Water Temperature 20°C ±2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.	19.6-20.6°C
pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.	7.6-8.0 in the control and two lowest-concentration treatment solutions, 3.8-4.7 in the three highest-concentration treatment solutions
Total Hardness 160 to 180 mg/L as $CaCO_3$ is recommended.	235-247 mg/L as $CaCO_3$
Dissolved Oxygen Renewal: Must not drop below 50% for more than 48 hours. Flow-through: ≥60% throughout test.	≥89% of saturation during the test

EPA Guideline Criteria	Reported Information
Test Vessels or Compartments 1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.	Test compartments were glass beakers containing 80 mL of test solution.
Covers Renewal: Test vessels should be covered with a glass plate. Flow-through: Openings in test compartments should be covered with mesh nylon or stainless steel screen.	Beakers were covered with loose fitting lids
Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.	N/A
Renewal Rate Three times weekly.	Renewal on Mondays, Wednesdays, and Fridays
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.	New test solutions were stirred before addition to the test vessels
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. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.	Solvent: none Maximum conc.: N/A

C. Test Design

EPA Guideline Criteria	Reported Information
Duration 21 days	21 days
Nominal Concentrations Control(s) and at least 5 test concentrations; dilution factor not less than 50%.	Dilution water control and five treatment concentrations: 100, 180, 320, 560, and 1000 mg/L
Number of Test Organisms 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	10 chambers with one daphnid each
Test organisms randomly or impartially assigned to test vessels?	Randomly distributed
Renewal Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).	Treatment stock solutions were stirred for 48 hours prior to renewal. Parental daphnids were transferred to new test solutions three times a week.
Water Parameter Measurements 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	DO and pH were measured in the new solutions prior to dispensing and were also measured in two replicates of the old solutions containing live daphnids. Alkalinity, hardness, and conductivity were measured weekly in the dilution water control and once for the 180 mg/L treatment group. Temperature was measured daily in an abiotic replicate of the control and each treatment group that contained live daphnids. Temperature was also monitored continuously in the abiotic control replicate.

EPA Guideline Criteria	Reported Information
Chemical Analysis Needed 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.	Samples of new and old solutions from the control and treatment groups containing live daphnids were collected six times during the study. Test solutions were analyzed by HPLC.

12. REPORTED RESULTS:

A. General Results

EPA Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality ≤30%	No mortality in the control group
Did daphnids in each control produce at least 40 young after 21 days?	Yes
Were no ephippia produced in any of the controls?	Not reported
Data Endpoints - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs.	- Survival of parental daphnids - Mean live offspring per adult daphnid - Length of surviving first-generation daphnids - Dry weight of surviving first-generation daphnids - Observations of F0 and F1 daphnids
Raw data included?	Yes

Effects Data

Toxicant Concentration (mg/L)		No. (%) Dead or Immobile (21 Days)	Mean Live Young/ Adult Daphnid	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Measured				
Control	<0.49	0/10 (0%)	92	4.0	0.50
100	97	2/10 (20%)	96	4.0	0.27
180	180	0/10 (0%)	90	4.0	0.41
320	300	10/10 (100%)	0	N/A	N/A
560	340	10/10 (100%)	0	N/A	N/A
1000	370	10/10 (100%)	0	N/A	N/A

Toxicity Observations: Mortality was the only sign of test material toxicity.

B. Statistical Results

Endpoint	Method	NOEC (mg/L)	LOEC (mg/L)
21-Day Survival	21-day LC_{50} of 230 mg/L (95% C.I.= 190 - 340 mg/L) by moving average angle analysis	N/A	N/A
Mean live young/ adult daphnid	Wilcoxon Rank Sum test with Bonferroni's adjustment	180	300
Length	visual interpretation	180	300
Dry weight	Wilcoxon Rank Sum test with Bonferroni's adjustment	<97	97

13. VERIFICATION OF STATISTICAL RESULTS:

Endpoint	Method	NOEC (mg/L)	LOEC (mg/L)
21-Day Survival	visual interpretation	180	300
Reproduction	Williams' test	180	CNBD*
Length	Wilcoxon Rank Sum test with Bonferroni's adjustment	180	CNBD
Dry weight	Williams' test and t-test	180	CNBD

*Could not be determined due to complete mortality at treatment levels above 180 mg/L.

14. REVIEWER'S COMMENTS: It was stated in the materials section that the solubility of the test material was approximately 15,000 mg/L. However, the test solutions were reported to be suspensions at concentrations ≥320 mg/L. It is apparent from the recoveries that the material is soluble up to about 350 mg/L and that upon analysis, the undissolved material was somehow separated from the test substance that was in solution. It was not stated how this separation was accomplished.

When the control, 97 mg/L, and 180 mg/L dry weight data were analyzed together, the treatment values were significantly reduced. However, when control group dry weight was compared directly to the 180 mg/L group dry weight, there was no significant difference detected at the 95% or 99% level of certainty. Consequently, this study is scientifically sound, fulfills the guideline requirements, and can be classified as **Core**. Based on the most sensitive endpoint (survival), the MATC is between 180 and 320 mg/L. The geometric mean MATC is 230 mg/L.

Daphnid reproduction
File: dam Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	10	91.600	91.600	94.250
2	97 ppm	10	96.900	96.900	94.250
3	180 ppm	10	89.500	89.500	89.500

Daphnid reproduction
File: dam Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	94.250				
97 ppm	94.250	0.844		1.71	k= 1, v=27
180 ppm	89.500	0.669		1.79	k= 2, v=27

s = 7.023
Note: df used for table values are approximate when v > 20.

Daphnids length (in scale units)
File: dam2 Transform: NO TRANSFORMATION

WILCOXON'S RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	Control	49.000				
2	97 ppm	48.875	73.00	53.00	8	
3	180 ppm	48.900	102.00	78.00	10	

Critical values use k = 2, are 1 tailed, and alpha = 0.05

Daphnids weight
File: dam3 Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	10	0.504	-0.305	-0.305
2	97 ppm	8	0.274	-0.565	-0.477
3	180 ppm	10	0.412	-0.408	-0.477

Daphnids weight
File: dam3 Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	-0.305				
97 ppm	-0.477	3.686	*	1.71	k= 1, v=25
180 ppm	-0.477	3.909	*	1.79	k= 2, v=25

s = 0.099
Note: df used for table values are approximate when v > 20.

Daphnids weight
File: dam4 Transform: NO TRANSFORMATION

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.5040	CALCULATED t VALUE =	1.5458
GRP2 (~~BLANK CRTL~~ 180 PPM) MEAN =	0.4120	DEGREES OF FREEDOM =	18
DIFFERENCE IN MEANS =	0.0920		

TABLE t VALUE (0.05 (2),18) = 2.101 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),18) = 2.878 NO significant difference at alpha=0.01

Daphnids weight
File: dam4 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	10	0.504	0.504	0.504
2	180 ppm	10	0.412	0.412	0.412

Daphnids weight
File: dam4 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.504				
180 ppm	0.412	1.546		1.73	k= 1, v=18

s = 0.133
Note: df used for table values are approximate when v > 20.