11 ✓ 6-12-00

MRID No. 449017-04

DATA EVALUATION RECORD
§ 72-2 - ACUTE EC_{50} TEST WITH A FRESHWATER INVERTEBRATE

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

2. **TEST MATERIAL:** 2-amino-4-methylsulfonylbenzoic acid (AMBA)
A metabolite of mesotrione - 99% purity

3. **CITATION:**

Authors:	S.E. Magor and N. Shillabeer
Title:	R044276 (AMBA): Acute Toxicity to *Daphnia magna*
Study Completion Date:	April 8, 1998
Laboratory:	Brixham Environmental Laboratory, Devon, England
Sponsor:	ZENECA Ag Products, Wilmington, DE
Laboratory Report ID:	BL6392/B
MRID No.:	449017-04
DP Barcode:	D259964

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

Signature: [signature] **Date:** 12/21/99

APPROVED BY: Max Feken, M.S., Environmental Toxicolgist,
Golder Associates Inc.

Signature: [signature] **Date:** 12/21/99

5. **APPROVED BY:** James Goodyear, USEPA

Signature: [signature] **Date:** 6/12/99 + ER

6. **STUDY PARAMETERS:**

Age of Test Organism:	<24 hours
Definitive Test Duration:	48 hours
Study Method:	Static
Type of Concentrations:	Nominal

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements. The 48-hour EC_{50} value of 160 ppm classifies the test material as practically non-toxic to *Daphnia magna*.

FOR AMBA

Results Synopsis:

EC_{50}: 160 ppm	95% C.I.: 140-190 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:** No deviations of consequence were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Daphnia magna*
All organisms are approximately the same size and weight?	Not reported
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3rd instar.	1st instar (<24 h)
Supplier	In-house cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Neonates were collected from 19 ±1 day old parents
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No

Guideline Criteria	Reported Information
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.	No feeding during the study
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	Not reported

C. **Test System**

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water.	Elendt's M4 *Daphnia* medium, aerated for >2 hours prior to use
Does water support test animals without observable signs of stress?	Yes
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20 ±1°C
pH Prefer 7.2 to 7.6.	5.9 - 7.9
Dissolved Oxygen Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.	≥95% of saturation
Total Hardness Prefer 40 to 200 mg/L as $CaCO_3$.	230 mg/L as $CaCO_3$

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 mL (daphnids and midges) or 2-3 L.	 Glass 250-mL 200 mL
Type of Dilution System Must provide reproducible supply of toxicant.	N/A
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.	1 daphnid/40 mL
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.	Solvent: none Maximum conc.: N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L, then no definitive test is required.	No range finding test reported
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Control, 18, 32, 56, 100, and 180 ppm

Guideline Criteria	Reported Information
Number of Test Organisms Minimum 20/level, may be divided among containers.	20, 5 per replicate
Test organisms randomly or impartially assigned to test vessels?	Yes
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, 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 continuously in a surrogate vessel and at 0, 24, and 48 hours in one test vessel. DO of the dilution water and pH of the bulk solutions at time 0 as well as in two replicates at test termination were recorded.
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	Samples of the bulk dosing solutions taken at time 0 and samples from one replicate of each group at 48 hours were collected and analyzed by HPLC.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes, but compliance was to UK GLP
Control Mortality Static: ≤10% Flow-through: ≤5%	No immobilization in the control group
Percent Recovery of Chemical: 1) % of nominal; 2) Procedural recovery; 3) Limit of determination (LOD)	94-100% of nominal, proc. recovery not reported, LOD = 0.043 or 0.044 ppm

Guideline Criteria	Reported Information
Raw data included?	Yes

Analytical results

Nominal concentration (ppm)	Measured concentration (ppm)	
	Hour of Study	
	0	48
Negative control	<LOD	<LOD
18	17	17
32	32	32
56	55	56
100	87	100
180	170	180

Mortality

Concentration (ppm)		Number of Organisms	Cumulative Number Immobilized	
			Hour of Study	
Nominal	Mean Measured		24	48
Control	<LOD	20	0	0
18	17	20	0	0
32	32	20	0	0
56	56	20	0	0
100	94	20	0	0
180	180	20	0	13

Other Significant Results: No signs of toxicity were reported.

B. Statistical Results

Method: moving average angle

48-hr EC_{50}: 160 ppm
95% C.I.: 140-190 ppm
Probit Slope: N/A
NOEC: 100 ppm

13. VERIFICATION OF STATISTICAL RESULTS:

Method: binomial probability

48-hr EC_{50}: 160 ppm
95% C.I.: not determined
Probit Slope: N/A
NOEC: 94 ppm

14. REVIEWER'S COMMENTS: This study is scientifically sound, fulfills the guideline requirements, and can be classified as **Core.** The 48-hour EC_{50} was determined to be 160 ppm, which classifies this material as practically non-toxic to *Daphnia magna*.

MOSSLER MESOTRIONE DAPHNIA MAGNA 9-10-99

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
180	20	13	65	13.1588
94	20	0	0	9.536742E-05
56	20	0	0	9.536742E-05
32	20	0	0	9.536742E-05
17	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 0 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 160.4681

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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