

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

S 72-2(b) - ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

1. CHEMICAL: Glyphosate S# No.: 417300
2. TEST MATERIAL: Glygran WDG Purity: 80%
3. CITATION
Authors: R.L. Boeri, J.P. Magazu, T.J., Ward
Title: Acute Toxicity of Glygran WDG to Daphnid, *Daphnia magna*
Study Completion Date: August 6, 1996
Laboratory: T.R. Wilbury Laboratories, Inc.
Sponsor: Lewis and Harrison for Industria Prodotti Chimici
Laboratory Report ID: 1009-LP
MRID No.: 44125706
DP Barcode: D231056
4. REVIEWED BY: Dennis J. McLane, Wildlife Biologist, EEB, EFED
Signature: *D J McLane* Date: 4-14-97
5. APPROVED BY: Les W. Touart, Head of Section 1, EEB, EFED
Signature: *L W Touart* Date: 4/27/97
6. STUDY PARAMETERS
Age of Test Organism: <24 hours old
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured

7. CONCLUSIONS:

The study is scientifically sound but does not fulfill guideline requirements. However, based rationale in REVIEWER'S COMMENTS on page 7 no further testing is required provided the company accepts EC50 >22 mg/L rather than the statistically derived value in the study.

Results Synopsis

LC₅₀: >22 ppm a.i. 95% C.I.: N/A
NOEL: >22 ppm a.i.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: The hardness of water may have changed the



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results.

C. Repairability: Provide another study or agreed that the EC50 is >22 mg/L.

9. Guideline Deviations

1. The report failed to if the organisms were of the same weight and size.
2. Source of the water was not provide in terms of well or tap water.
3. The loading rate was not reported.
4. The raw data was not reported.
5. The pH range was highly variable 6.4 to 8.4 when 7.2 to 7.6 is suggested.
6. The hardness was very high 160 versus 40 to 48 range.
7. The statistical method was not reported.

10. SUBMISSION PURPOSE:

Registration of an old chemical.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
All organisms are approximately the same size and weight?	Not Reported
<u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 th instar.	<24 hours old
<u>Supplier</u>	Aquatic Biosystems, Inc.

Guideline Criteria	Reported Information
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	34 days
Wild caught organisms were quarantined for 7 days?	No
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
<u>Feeding</u> No feeding during the study.	"Daphnids were not fed during the test."
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	0% mortality 48 hours preceding the test.

C. Test System:

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	The report did not say if the water was from a well or a public water system.
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	19.1 to 20.3°C

Guideline Criteria	Reported Information
pH Prefer 7.2 to 7.6.	6.4 to 8.4
Dissolved Oxygen Static: $\geq 60\%$ during 1 st 48 h and $\geq 40\%$ during 2 nd 48 h, flow-through: $\geq 60\%$.	48 hours lowest % D.O. is 100%
Total Hardness Prefer 40 to 48 mg/L as CaCO_3 .	160 mg/L as CaCO_3
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.	1. glass beakers 2. 300 mL 3. 250 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 $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow- through: ≤ 1 g/L/day.	not reported
Photoperiod 16 hours light, 8 hours dark.	16 hours of light & 8 hours of dark
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	Water

D. Test Design:

Guideline Criteria	Reported Information
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<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L, then no definitive test is required.	Results: 100% survival at 0.098, 0.98, and 9.8 mg/L; 10% survival at 98 mg/L; 0% at 980 mg/L
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	13, 22, 36, 60 and 100 mg/L of a.i.. This meets the 60% increase criteria.
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	Twenty level ten per replicate.
<u>Test organisms randomly or impartially assigned to test vessels?</u>	"Twenty daphnids were indiscriminately and equally distributed among two replicates."
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary $> 1^{\circ}C$. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	1. "...measured and recorded daily in each test vessel that contained live animals. The temperature in a beaker of water incubated among the test vessels was recorded continuously during the test. 2. "...measured and recorded daily in each test vessel that contained live animals.
<u>Chemical Analysis</u> 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	None of these items applies but measured conc. were made.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes

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<u>Control Mortality</u> Static: ≤10% Flow-through: ≤10%	0%
<u>Percent Recovery of Chemical</u>	95%
<u>Raw data included?</u>	no

Mortality

Concentration (ppm)		Number of Organ- isms	Cumulative Number Dead	
Nominal	Mean Measured		Hour of Study	
			24	48
Control		20	0	0
13	12.4	20	0	0
22	22.1	20	0	0
36	36.7	20	0	0
60	56.8	20	0	0
101	101	20	4	15

B. Statistical Results

Method:

48-hr LC₅₀: 86.5 ppm a.i.;
95% C.I.: 56.8-101 ppm a.i.
Probit Slope: N/A
NOEC: 56.8 ppm a.i.

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	86.5 (56.8-101) ppm a.i.
Moving Average Angle LC ₅₀ (95% C.I.)	Toxanal provided the following statement: 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.
Probit LC ₅₀ (95% C.I.)	
Probit Slope	
NOEC	56.8 ppm a.i.

14. REVIEWER'S COMMENTS:

EEB believes that the acid conditions caused by the active ingredient were buffered by the increased water hardness. The pH range was highly variable and outside the acceptable range (7.2-7.6). In this study the range was 6.4-8.4. Also, the hardness was 160 mg/L as CaCO₃, which much higher than the require range 40 to 48 mg/L as CaCO₃, and higher than the companion fish studies (MRID Nos.44125704 and 44125705) (44 mg/L as CaCO₃). As shown in both fish studies, when active ingredient concentration increases the pH as the concentration decreases producing more acidic solution. In this test it appears the pH did not fall as low as the fish studies because of the hardness of the water raised the pH. The lowest control pH was 8.2 at 48 hours which is above the acceptable range. EEB believes that it is the low pH value or acidity that is causing the mortality. However, in this study, the acid was buffered because of the increase in the hardness. Based on the fish studies, if the test had been performed within the required hardness range the pH may have dropped sooner and at lower concentrations. The fish studies show a combined pH range of 6.3 to 7.3 at the 22 mg/L level. Because EEB believes the acid would have little effect at these pH levels. EEB would accept that the EC50 as greater 22 mg/L and not require an additional acute daphnid study.

MCLANE GLYPHOSATE DAPHNIA MAGNA 48 EC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
101	20	15	75	2.069473
56.8	20	0	0	9.536742E-05
36.7	20	0	0	9.536742E-05
22.1	20	0	0	9.536742E-05
12.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 56.8 AND 101 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 86.51186

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.
