

DATA EVALUATION RECORD

1. **CHEMICAL:** 70 Orchard Spray
2. **TEST MATERIAL:** clear, colorless liquid CAS No. 64742-55-8
3. **STUDY TYPE:** §72-2 Acute Aquatic Invertebrate Toxicity
4. **CITATION:**

Author: Rausina, G.A. and L.S. Glenn
Title: 48-Hour Aquatic Toxicity Study in Daphnia with
70 Orchard Spray
Laboratory Report #: 82-069
Any Other Study #:
Sponsor: Gulf Refining and Marketing Co.
Laboratory: Gulf Life Sciences Center, PA
MRID No.: 413688-33

5. **REVIEWED BY:**

Conchi Rodríguez
Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: *Conchi Rodríguez*

Date: 6/16/94

6. **APPROVED BY:**

Harry Craven
Supervisory Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: *Harry Craven*

Date: 6/16/94

7. **CONCLUSIONS:** The study is not scientifically sound and does not meet the guideline requirements for an Acute Aquatic Invertebrate Toxicity. The study is classified as invalid. Polypropylene containers were used and the concentrations of the test material were not measured. The concentration at which the organisms were really exposed is not known.
8. **RECOMMENDATIONS:** A new study with the active ingredient is required. The active ingredient has to be identified.
9. **BACKGROUND**

10. MATERIALS AND METHODSA. Test Organisms:

Guideline Criteria	Reported Information
Species (Scientific Name)	<u>Daphnia magna</u>
All organisms should be approximately the same size and weight.	Daphnia were derived from a clone.
Immature organism should be used. Daphnids 1 st (<24hrs). Amphipods, stoneflies, and mayflies in 2 nd instar; midges 2 nd & 3 rd instar	All organisms for the test were first instar approximately 24 hours old.
Supplier	Sea Plantations, Inc, MA
All organisms from same source (yes or no)	Yes
Other Comments	N/A

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 7 days)	Not reported. However animals were received 4 months before the beginning of the test.
Wild caught 7 day quarantine (yes or no)	No
Check for signs of disease or injury (yes or no, if yes describe)	Not reported
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	N/A
No feeding during the study (When last fed)	Not reported
<3% mortality 48 hours prior to testing (% mortality, if any)	Not reported

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (prefer soft reconstituted water)	Charcoal filtered municipal water
Does water support test animals without observable signs of stress?	Not reported.

Was dechlorinated water used (not recommended)	No
Water Temperature (Daphnia-20°C) (Amphipods and mayflies-17°C) (Midges and mayflies-22°C) (Stoneflies-12°C)	20.8 to 21.3 °C
pH	8.3 - 8.6
Dissolved Oxygen (Static 1 st 48 hrs 40%; 2 nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	DO ranged during the study from 83.8% (7.3 mg/l) to 89.6% (7.8 mg/l) saturation
Total hardness (40 to 48 mg/L as CaCO ₃ well water)	130 - 148 mg/l as CaCO ₃
Total Alkalinity	46 - 48 mg/l as CaCO ₃
Specific Conductance	420 - 445 µmhos/cm
Test Aquaria 1. Material (glass or stainless steel) 2. a. Small organisms (3.9 L (1 gal) with 2 to 3 L solution) b. Daphnids and midges (250 ml glass beakers 200 mls of test solution)	1. Polypropylene container inside a vessel 2. a. N/A b. Vessel was 3.0 l
Type of Dilution System (Reproducible supply of toxicant)	Flow through proportional diluter system calibrated to deliver 100 ml of test water every 2.5 min/cycle.
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	System was examined daily. Flow rate was 9.6 vol/24 hours.
Biomass Loading Rate Static: < 0.8 g/L at ≤ 17°C; 0.5 g/L > 17°C Flow-through: 1 g/L/24 & must not be >10 g/L at any time at or below 17°C or 5 g/L at higher temperatures.	Not reported
Photoperiod (16 L & 8 D with a 15-30 min transition)	12 hours light, 12 hours darkness
Solvents 1. (Do not exceed 0.5 ml/L for static tests) 2. (Do not exceed 0.1 ml/L for flow- through)	No solvents

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> (LC ₅₀ >100 mg/L with 30 fish, no definitive test required.)	No data included
<u>Definitive Test</u>	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	5 concentrations 1.00, 1.8, 3.2, 5.6, 10.0 mg/l a negative control and a positive control (hexavalent chromium)
Controls (Minimum control mortality; static 10%; flow-through 5%)	5% mortality (1/20)
Number of Test Organisms; (Minimum 20/level can be divided among containers)	20 /level, 2 vessels per test concentration
All organisms must be randomly assigned to test vessels. (yes or no, describe if no)	Yes
Biological Observations	No
Water Parameter Measurements 1. Temperature - record every 6 hrs; >1°C. 2. D.O. beginning, 48 hrs, end for control high, medium, and low dose. 3. pH beginning, 48 hrs, end for control, high, medium, and low dose.	1. Recorded daily in each vessel 2. Recorded daily in each vessel. 3. Recorded daily in each vessel
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	Not performed

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Mean Measured Concentrations (report conc.)	Not measured
Recovery of Chemical (% recovery)	N/A
Mortality & Observations (Describe observations & attach mortality tables)	Mortality ranged from 100% in the highest concentration to 10 % in the lowest (see table)

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EC50 value (type of analysis)	2.20 mg/l (Litchfield-Wilcoxon)
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12. **STUDY AUTHOR'S CONCLUSIONS / QUALITY ASSURANCE MEASURES:**

"The 48-hour EC50 was 2.2 mg/l nominal concentration of 70 Orchard Spray. The maximum saturated test concentration achievable in water under the conditions of the study could not be determined. The results of the hexavalent chromium positive control study indicated that the parameters were within acceptable limits."

The study was performed prior to the effective date of the Environmental Protection Agency's Good Laboratory Practice Standard (40 CFR 160, October 16, 1989) and thus do not comply with 40 CFR 160.

13. **REVIEWER'S DISCUSSION AND INTERPRETATION**

A. **Test Procedure:**

The following items did not meet the guideline criteria:

1. The purity of the test material was not reported.
2. The photoperiod was not as recommended
3. The test vessels material was polypropylene. This is not a recommended material.

B. **Statistical Analysis**

Test	Results
Binomial	EC50 = 2.4 mg/l (95% CI 1 - 5.6)
Moving Average Method	EC50 = 2.38 mg/l (95% CI 1.8 - 3.07)
Probit Method	EC50 = 2.36 mg/l (95% CI 1.8 - 2.9)

C. **Discussion/Results:** The major fault of the study is that the organisms were placed into polypropylene containers. The recommended materials for test vessels are glass or stainless steel. The test solution must have been analyzed to know the exact amount of the test material that the organisms were exposed to. It is known that some materials can adsorb the pesticide tested.

The purity of the test material was not reported. It is not

know if this test material is a typical end use product or the technical grade of the active ingredient.

The study is not scientifically sound and does not meet the guideline requirements for an acute aquatic invertebrate toxicity. Based on the conditions of this study, the EC50 for Daphnia magna is 0.41 mg/l. This classifies 100 Paraffin Oil as moderately toxic. A new study is required.

D. Adequacy of the Study:

1. Classification: Invalid
2. Rational: Polypropylene containers were used and the concentrations of the test material were not measured
3. Repairability: No

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

Rodriguez 70 Orchard Spray EC50 Daphnia

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10	19	19	100	1.907348E-04
5.6	19	17	89.4737	3.643036E-02
3.2	19	13	68.42111	8.353423
1.8	19	6	31.5789	8.353423
1	19	2	10.5263	3.643036E-02

THE BINOMIAL TEST SHOWS THAT 1 AND 5.6 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 2.4

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1357467	2.388013	1.850083	3.077528

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	.1125373	1

GOODNESS OF FIT PROBABILITY
.942728

SLOPE = 3.599218
95 PERCENT CONFIDENCE LIMITS = 2.391804 AND 4.806633

LC50 = 2.361692
95 PERCENT CONFIDENCE LIMITS = 1.873035 AND 2.924905

LC10 = 1.048018
95 PERCENT CONFIDENCE LIMITS = .6277577 AND 1.398516

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