
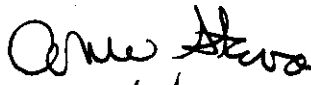


US EPA ARCHIVE DOCUMENT

MRID No. 427702-32

DATA EVALUATION RECORD

1. **CHEMICAL:** Pirate® (AC 303,630)
Shaughnessey No. 129093
2. **TEST MATERIAL:** AC 303,630; CAS No. 122453-73-0; Lot No. AC7504-59A; 94.5% purity; a tan powder.
3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Acute Flow-Through Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Ward, G.S. and J.D. Wisk. 1992. Acute Toxicity of AC 303,630 to *Daphnia magna* Under Flow-Through Test Conditions. Laboratory Project No. J9104006b. Performed by Toxikon Environmental Sciences, Jupiter, FL. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 427702-32.
5. **REVIEWED BY:**
 Anndrew C. Bryceland
 Fishery Biologist
 EEB/EFED Section 5
 USEPA
 Signature: 
 Date: 10/7/93
6. **APPROVED BY:**
 Ann Stavola, M.S.
 Supervisory Biologist
 EEB/EFED Section 5
 USEPA
 Signature: 
 Date: 10/7/93
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements. Under the conditions of the study, the 96-hour EC₅₀ was 5.83 µg ai/l which classifies AC 303,630 as very highly toxic to *Daphnia magna*. The NOEC was 2.52 µg ai/l.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
11. **MATERIALS AND METHODS:**
 - A. **Test Animals:** *Daphnia magna* were obtained from in-house

cultures originally acquired from the EPA. Prior to test initiation, a subculture of adults was isolated, maintained at 20°C, and fed a diet combination of *Selenastrum capricornutum* and a yeast/trout chow/cerophyll suspension. The adults were in good condition (no mortality within 48 hours prior to test initiation) and no ephippia were present. Neonates (<24 hours old) from these adults were collected for testing.

- B. **Test System:** The test was conducted under flow-through conditions using a proportional diluter with a 60% dilution factor. Test chambers were glass dishes (100 mm diameter; 50 mm height) with Nitex screen collars attached to the rims. The test chambers were positioned within glass chambers (42 x 21.5 x 12.5 cm) equipped with glass overflow tubes. The solution volume in each glass chamber was 300 ml and the solution depth was 5 cm. Sixty-five ml per cycle was delivered to each test chamber at a rate of 3.2 cycles per hour, resulting in 16.6 volume additions per day. The glass chambers were randomly positioned in a water bath.

The test was conducted under fluorescent lighting on a 16-hour light (intensity of 283-350 lux) and 8-hour dark photoperiod. Fifteen-minute dawn/dusk simulation periods were provided.

The dilution water was a blend of Town of Jupiter water and well water. The town water was aerated to remove chlorine, carbon and biofiltered (5 µm), and treated by reverse osmosis prior to use. The well water was also carbon filtered. At test initiation, the dilution water had a hardness and alkalinity of 108 and 74 mg/l as CaCO₃, respectively. The specific conductance was 801 µmhos/cm.

The stock solution was prepared with dimethylformamide (DMF). An injection volume of 54.7 µl of diluter stock solution [150 mg active ingredient (ai)/l] was added to a volume of 547 ml of dilution water for each cycle, providing the highest test concentration solution. The four lower concentration solutions were prepared by proportionally diluting the highest concentration solution.

- C. **Dosage:** Ninety-six-hour, flow-through test. Based on results of preliminary testing, nominal test concentrations selected for this study were 1.9, 3.2, 5.4, 9.0, and 15 µg ai/l. A dilution water control and

a solvent control were also included. The concentration of solvent in the solvent control and all exposure solutions was 100 μ l/l.

- D. **Design:** The test was initiated when ten daphnids were impartially added, by twos, to each of two duplicate chambers per treatment (i.e., 20 daphnids/treatment). The daphnids were fed a concentrated *Selenastrum capricornutum* culture three times a day during the study and the solutions were not aerated.

Observations of mortality and sublethal effects were noted daily. Dead or immobilized animals were removed at each observation. Temperature was monitored continuously in the water bath and hourly in the dilution water control. Dissolved oxygen concentrations (DO) and pH were measured daily in each chamber.

Analytical determination of AC 303,630 using high pressure liquid chromatography was performed on samples collected from each treatment and control solution on study days 0, 2, and 4. The diluter stock solution was also analyzed at these times.

- E. **Statistics:** The EC_{50} values and their 95% confidence intervals were calculated using probit analysis.

12. **REPORTED RESULTS:** Mean measured concentrations for the 96-hour period were 1.4, 2.5, 3.9, 6.3, and 10.7 μ g ai/l which represent 70-78% of nominal concentrations (Table 1, attached). The stock solution remained stable during the exposure and was 97-119% of nominal.

By test termination, no mortality was observed in the controls and mortality in the exposure solutions ranged from 0 to 100% (Table 2, attached). Based on mean measured concentrations, the 96-hour EC_{50} (95% confidence interval) for AC 303,630 was 5.8 (5.0-6.8) μ g ai/l. The slope of the probit curve was 6.5. The no-observed-effect-concentration (NOEC) was 2.5 μ g ai/l, based on the lack of mortality and sublethal effects.

During the 96-hour test period, the test solutions had a pH of 7.9-8.3, a temperature of 18.0-19.7°C, and a DO of 2.2-8.6 mg/l (23-91% of saturation). The lower DO in the treatment and solvent control solutions was thought to be due to the presence of DMF. At test termination, the dilution water had a hardness and alkalinity of 100 and 79 mg/l as $CaCO_3$, respectively. The specific conductance was 2640 μ mhos/cm.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions other than those stated above were made by the study authors.

Statements of quality assurance and good laboratory practice compliance were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory Practice Regulations (40 CFR Part 160).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedures were generally in accordance with the SEP, but deviated as follows:

During the study, the DO levels in all but the negative control solutions were low (Table 5, attached). It is recommended that DO remain above 60% during the entire experiment when using a flow-through system.

According to the Subdivision E Guidelines 72-2(b)(4) flow-through studies are run for 96 hours. This longer exposure requires that the daphnia be fed.

- B. **Statistical Analysis:** Mean measured concentrations from 0 to 96 hours were 1.5, 2.5, 3.86, 6.31, and 10.7 $\mu\text{g ai/l}$. The reviewer used mean measured concentrations and the mortality data to calculate the 96-hour EC_{50} using EPA's Toxanal program. The results indicated that the EC_{50} was 5.83 $\mu\text{g ai/l}$ with a 95% confidence interval of 5.0-6.8 $\mu\text{g ai/l}$ (see printout, attached). The NOEC was 2.5 $\mu\text{g ai/l}$, based on the lack of mortality or sublethal effects.

- C. **Discussion/Results:** Although reduced oxygen levels were noted during the study, the solvent control daphnids (exposed to the lowest DO concentration) demonstrated no mortality. Therefore, the response observed in this study is believed to be largely due to the toxicant. However, the dilution water should have been vigorously aerated immediately prior to introduction into the diluter system.

Since the purpose of an acute daphnid test is to determine the toxicant's effect on the organism, a listing of sublethal effects should have been given. It was stated in the results that the NOEC was 2.52 $\mu\text{g ai/l}$ due to the lack of mortality and sublethal effects, but this was the only treatment level for which it was stated

that there were no observed effects.

This study is scientifically sound but and meets the guideline requirements. Under the conditions of this study, the 96-hour EC₅₀ was 5.83 µg ai/l which classifies AC 303,630 as very highly toxic to *Daphnia magna*. The NOEC was 2.5 µg ai/l.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, 9-3-93.

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Pages 6 through 8 are not included.

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***** 76 H14 LC50 FIT *****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10.7	20	20	100	9.536742E-05
6.31	20	9	45	41.19014
3.86	20	4	20	.5908966
2.52	20	0	0	9.536742E-05
1.4	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3.86 AND 10.7 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 6.533901

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	5.266251E-02		5.687084 5.026641
6.535903			

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
6	.1268264	1
.2838351		

SLOPE = 6.506743
 95 PERCENT CONFIDENCE LIMITS = 4.189517 AND 8.823969

LC50 = 5.835974
 95 PERCENT CONFIDENCE LIMITS = 5.059078 AND 6.794667

LC10 = 3.723327
 95 PERCENT CONFIDENCE LIMITS = 2.798361 AND 4.388057

MOSSLER AC 303630 DAPHNIA MAGNA 7-15-93

48-hr LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
11.5	20	20	100	9.536742E-05
7.1	20	4	20	.5908966
4.3	20	1	5	2.002716E-03
2.7	20	0	0	9.536742E-05
1.5	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 7.1 AND 11.5 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 8.248437

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	6.572952E-02	7.756318	6.953333	8.766265

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	1.298554	2.671903	4.568821E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 9.375468

95 PERCENT CONFIDENCE LIMITS = -1.308265 AND 20.0592

LC50 = 7.814987

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.720954

95 PERCENT CONFIDENCE LIMITS = 0 AND 7.729192
