123-2 w/ Lemna gibba

DATA EVALUATION REVIEW

5/3/1991

1. Chemical: Tilt

2. Test Material: CGA-64250, 90.7% MRID 00133363

3. Study type: Nontarget Area Phytotoxicity, aquatic plant growth- Lemna gibba

4. Study ID: Hollister, T. (1981). The effect of CGA-64250 to duckweed, Lemna gibba. An unpublished study prepared by EG&G Bionomics for Ciba-Geigy Corp. ACC# 4324-012-02

5. Reviewed By: Dana Barrett
Biologist
EEB
Signature: Dana Barrett
Date: 5-3-91

6. Approved By: Charles Lewis
Head, Section III
EEB
Signature: Charles Lewis
Date: 5/3/91

7. Conclusions:

The study does not follow the guidelines for the aquatic plant nontarget phytotoxicity test - Lemna gibba. This study is classified as "supplemental"; see 14a.

8. Recommendations:

Under new guidelines, the Lemna gibba study is not required as part of the aquatic plant nontarget phytotoxicity.

9. Background:

This study was submitted in support of registration.

10. Discussion of Individual Tests or Studies:

11. Materials and Methods:

a. Test Organism - The freshwater aquatic plant Lemna gibba was obtained from the Department of Botany, Howard University, Washington, D.C. Upon initiation of the study the plants were seven days old.

Test System - 250 mL culture dishes covered with glass tops, each of which contained 100 mL of test medium. The cultures were incubated at 24C under continuous 10,300 lux illumination.

b. Dosage - The following measured concentrations were used: control, solvent control (grade A acetone), 2.54, 6.50, 12.9, 23.4, and 46.9 ppm. Three replicates, five plants per replicate, were utilized at each concentration. See Table 1.

c. Description - Observations were made on test days 1, 2, 3, 4, 7, 8, 9, 10, 11, and 14. Frond production was recorded; every frond visibly projecting beyond the edge of the parent frond was counted.

d. Statistics Employed - Frond production was subjected to analysis of variance (ANOVA) and Williams' method (Williams 1971) to locate significant differences among treatment means. Test concentrations were converted to a logarithm and the corresponding percentage decrease of frond production was converted to a probit (Finney, 1971).

12. Reported Results:

The 14 day EC50 was determined to be 9.02 ppm with 95% confidence limits of 4.88 - 13.18 ppm. Flowering was not observed among any of the control plants or plants exposed to the test material during the 14-day exposure. See Table 2.

13. Study Authors Conclusions -

"Measured concentrations of CGA-64250 >6.50 ppm significantly affected growth of Lemna gibba after 14 days of exposure. After 14 days, inhibition of frond production was from 4% in cultures exposed to 2.54 ppm to 93% in those exposed to 46.9 ppm" (excerpt from study).

14. Reviewer's Discussion -

a. Test Procedures -

The study deviated from the protocol outlined in "Subdivision-J-Guidelines". The following discrepancies were noted:

- The light intensity was 10,300 lux instead of the recommended 5000.
- Photoperiod data was not submitted.
- pH data was not submitted.
- Initial (parental) frond count data was not submitted.
- No-Effect-Level (NOEL) was not determined.
- Lowest dose tested was below the NOEL level that was determined by EEB.

b. Statistical Analysis -

Using the Moving Average Method, the EC50 was determined to be 4.82766 ppm with the confidence limits of 4.202795 - 5.464939 ppm. (Independent statistical analysis is attached). Analysis of variance (ANOVA) determined the NOEL to be <2.54 ppm.

c. Conclusion -

1) Classification - Supplemental

2) Rationale - discrepancies outlined in 14a.

3) Repairability - Not repairable.

211201 tilt Aquatic Vasc. Plant

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
46.9	100	94	94	0
23.4	100	92	92	0
12.9	100	87	87	0
6.5	100	71	71	0
2.54	100	15	15	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.638915

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.0303092	4.82766	4.202795	5.464939

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.6055093	8.219996	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 2.20134
95 PERCENT CONFIDENCE LIMITS = .4883788 AND 3.914301

LC50 = 5.114936
95 PERCENT CONFIDENCE LIMITS = .6698352 AND 10.32052

LC10 = 1.354936
95 PERCENT CONFIDENCE LIMITS = 2.39034E-03 AND 3.438102

Table 1. Results of chemical analyses of CGA-64250 at day 0 of a 14-day exposure of Lemna gibba to CGA-64250.

Nominal concentration (mg/l (ppm)	Measured concentration (mg/l)	% Nominal
Control	ND[a]	—
Solvent control	ND	—
3	2.54	85
6	6.50	108
12	12.9	108
25	23.4	94
50	46.9	94
50,000 (stock)	47,104	94

[a] Not detected.

(Excerpt: Original Study).

Table 2. Results of a 14-day exposure of duckweed, Lemna gibba to CGA-64250. Percentage change is stimulation (+) or inhibition (-) of frond production in exposed cultures as compared to the solvent control.

Concentration (ppm, mg/l)	PERCENT CHANGE (DAY)									
	1	2	3	4	7	8	9	10	11	14
Control	0	+4	0	+2	+8	+7	+18	+28	+24	+13
Solvent Ctrl.	-	-	-	-	-	-	-	-	-	-
2.54	0	-4	-8	-23	-18	-19	-12	-1	-1	-4
6.50	+5	0	-3	-26	-47	-52	-55	-61	-62	-67*
12.9	-5	-8	-14	-32	-54	-61	-46	-70	-76	-85*
23.4	-5	-19	-26	-42	-63	-67	-72	-77	-81	-90*
46.9	-10	-35	-48	-62	-75	-79	-79	-83	-88	-93*

* Significantly less ($P<0.05$) than the solvent control.

(Copied from Study).