DATA EVALUATION RECORD

1. Chemical: PP321

2. Test Material: PP321 - 96.5% ai

3. Study Type: Toxicity to the Green Algae

   Species Tested: Selenastrum capricornutum

4. Study ID: Thompson, R.S.; Williams, T.D. (1985) PP321: Toxicity to the Green Algae Selenastrum capricornutum. Submitted by Imperial Chemical Industries PLC, prepared by Brixham Laboratory, Brixham, Devon. EPA Accession No. 073989.

5. Reviewed By: Candy Brassard, Environmental Protection Specialist, EEB/HED — Signature: Candy Brassard — Date: 10/6/87

6. Approved By: Douglas J. Urban, Head, Section III, EEB/HED — Signature: Douglas J. Urban — Date: 10/7/87

7. Conclusions

The study appears to be scientifically sound, however there are major discrepencies that detract from the study. This study is classified as "supplemental". Therefore, the Guideline Requirement Reference No. 123-2, Tier II aquatic plant nontarget phytotoxicity test is not fulfilled.

Based on available information, the EC_{50} appears to be > 310 ppb (mean measured concentration).

8. Recommendations:

The comapny should review the discrepencies outlined in Section 14 prior to submitting another aquatic plant nontarget phytotoxicity study.

9. Background:

This study was submitted prior to registration of PP321 or Karate 1E Insecticide for use on cotton.

10. Discussion of Individual Test: N/A

11. Materials and Methods:

a. Test Organisms - The unicellular green alga Selenastrum capricornutum Printz, (Strain ATCC 22662) were obtained from laboratory cultures maintained under uncontaminated conditions.

A culture of the alga in the exponential growth phase was used as inoculum for the test. The culture was grown in the medium and under the environmental conditions described for the test.

b. Test System - 250 mL Borosilicate glass conical flasks with polyurethane foam bunges, containing 100 mL test solution. The cultures were incubated at 24 ± 1 °C under continuous illumination of ≈ 8000 Lux, with orbital shaking at 100 rpm.

The pH of each test solution was measured at start of test. The pH of two of the replicate test solutions (containing algae) from each control and test concentration was also recorded.

Temperature was measured daily. Light intensity was measured once during the study.

The pH ranged from 7.2 to 7.4 at the start of the test and 7.7 to 9.0 at end of test.

The temperature ranged from 23.5 to 23.9 °C. The Light intensity was reported to be 8500 Lux.

c. Dose - The following nominal exposure concentrations were used: control, two solvent controls (0.1 mL/L and 0.156 mL/L), 0.056, 0.10, 0.18, 0.32, 0.56, and 1.0 mg PP321/L.

Measured concentrations were reported at the start of the test for each test solution. At the end of the test, each blank solution was sampled and analyzed in the same manner. Analysis of the test material was not possible in solutions containing algal cells. See Table 5 for analytical results.

Excerpted from submission -

"The measured concentrations at the start of the test ranged from 38% to 100% of the nominal values. The measured concentrations after 96 hours ranged from 3% to 11% of the nominal values. The variation in the percent nominal values at the start of the

test, and the decrease in the measured concentrations between the start and finish, were attributed to the low water solubility of the test substance."

d. Study Design - Three controls, one of each solvent control, and each test concentration were used. One blank vessel (without algae inoculum) for each control and treatment was incubated concurrently. Test vessels were randomly placed in the incubator.

Excerpted from submission -

"Each replicate test vessel was inoculated with 0.55 ml of the inoculum culture to give algae density of 1.1×10^4 cells/ml based on the measured cell density of the inoculum culture. This value was used for the growth calculations.

"The algae cell densities of the inoculum and test cultures were determined by electronic particle counting, using a Coulter Counter Model 3B, counting at a lower threshold equivalent spherical diameter of $\sim$ 2.8 um.

"After 24, 48, 72, and 96 hours, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density."

e. Statistics

- Biological Data

  Reported algal cell densities are in Table 1.

- Area under the growth curve (days 0 to 4) was used for each replicate culture, according to the formula given by OECD--see ATTACHMENT A.

  These areas were examined by ANOVA and Dunnett's procedure. See Table 2 for mean growth areas under the growth curve and percentages of the solvent control. See Attachment A for method used to determine area under growth curve and growth rate.

12. Reported Results:

Results of area under growth curve. Excerpted from submission:

"A significant difference was identified only at the lowest concentration tested (nominal 0.056 mg/l) at which the area under the growth curve was reduced by 17% compared with the solvent control. Since the areas obtained at the next higher nominal concentration (0.1 mg/l) and the maximum nominal concentration tested (1.0 mg/l) were within 2% of the solvent control, the statistical significance at 0.056 mg/l was not considered biologically significant."

Results of growth rate. Excerpted from submission:

"These data were analyzed as described for the area method. The mean growth rates and the significant differences identified are given in Table 3, together with the rates expressed as percentages of that of the (pooled) solvent control. A statistically significant reduction in growth rate was identified only for the lowest concentration tested (nominal 0.056 mg/l). The reduction was 5%, and was not considered biologically significant, since the next higher concentration and the maximum concentrations tested resulted in growth rates within 1% of the pooled solvent control."

13. Study Author's Conclusions/QA Measures:

With regard to the area under growth curve:

"No significant effect concentration (P = 0.05) 1.0 mg/l (nominal)
Median effective concentration, biomass (EC50) > 1.0 mg/l (nominal)

"The nominal concentration of 1.0 mg/l had an initial measured concentration of 0.58 mg/l and a mean of the initial and final measured concentrations of 0.31 mg/l."

With regard to growth rate:

"Median effective concentration, growth rate (EC50) > 1.0 mg/l (nominal)"

"The conduct of this study has been inspected/audited in accordance with ICI's policies and procedures for Good Laboratory Practice . . . ."

14. Reviewer's Discussion and Interpretation of the Study Results:

The study deviated from the protocol outlined in the 1982 Guidelines--Subdivision J--Tier II aquatic plants and the SEP, "Nontarget Plants: Growth and Reproduction of Aquatic Plants Tiers 1 and 2, 1986, and the following discrepancies were noted: -

a. Test Procedures -

- No raw data were submitted.
- The light intensity was 8500 Lux instead of the recommended 4000.
- The study was only conducted for 4 days instead of the recommended 5 days.
- Each flask was inoculated with 11,000 cells/mL rather than 3000 cells/mL.
- The increase in pH in one of the replicates for the 0.32 mg/L dose from 7.7 to 9.0 causes concern, since this class of chemicals is pH sensitive with regards to hydrolysis.
- The measured concentrations were only 3 to 11 percent of the nominal concentrations after 96 hours. The study author reported that this was attributed to the low water solubility of the test substance.
- The photoperiod was not reported.
- The culture medium included $Na_2EDTA.2H_20$ at a level of 0.150 mg. Since EDTA is a known chelator, it should not be used in the culture medium. See Subdivision J, Pesticide Assessment Guidelines.
- The pH should be measured in the test vessels with the inoculum as well.

b. Statistical Analysis - EEB determined that there was an effect seen at the intermediate levels 0.18 and 0.32 mL/L (nominal concentration). However, the statistical validity is questionable since the two highest levels were not statistically different from the solvent control (0.156 mL/L). A satisfactory dose-response relationship was not achieved. See Attachment B. A Stephan's program was not conducted since the data in Table 2 and Table 3

show that an EC_{50} was not achieved at any treatment level.

c. Discussion/Results - The maximum application rate for PP321 on cotton is expected to be 0.03 lb ai/A. This could result in a concentration of 22 ppb if applied to a 6-inch water column.

The estimated concentration in a 6-inch water column is less than the measured concentrations of the highest level tested. Based on the available information, there was no effect at the highest dose tested, and it appears that this application rate will not pose a hazard to the aquatic green alga Selenastrum capricornutum.

Therefore, the EC_{50} appears to be > 1.0 mg/L nominal concentration or > 310 ppb mean measured concentration.

d. Adequacy of Study

1) Classification - Supplemental

2) Rationale - Based on the discrepencies outlined in Section 14, this study is classified as supplemental.

3) Repairability - This study can not be repaired.

Attachment

ATTACHMENT A

Area Under Growth Curve

where N_0 = Cell density at start of test (x 10^4 cells/mL).
N_1 = Cell density at t_1.
N_n = Cell density at t_n.
t_1 = Time (days) of first measurement after start of test.
t_n = Time (days) of n^{th} measurement after start of test.

Growth Rates

The growth rates of the cultures were relatively constant over the first 3 days of the test but declined slightly during the final 24 hours (see Figure 1). Growth rates were calculated therefore, for each replicate culture, for Days 0 to 3, according to formula:

$$\text{Growth rate} = \frac{\ln (N_2/N_1)}{t}$$

Where N_1 = Cell density at start (x 10^4 cells/mL).
N_2 = Cell density at Day 3 (x 10^4 cells/mL).
t = Time interval = 3 days.

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_____ Identity of product inert ingredients.

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_____ Description of the product manufacturing process.

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OBS	TRT	RESP1	RESP2	RESP3	RESP4	RESP5	RESP6	RESP7	RESP8	RESP9	RESP10	RESP11	RESP12	RESP13	RESP14	RESP15	RESP16	RESP17	RESP18	RESP19	RESP20
1	A	446	443	449	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	B	437	361	406	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	C	485	467	457	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	D	394	425	359	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	E	419	335	379	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	F	443	431	408	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	G	448	458	440	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

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GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
TRT	7	A B C D E F G

NUMBER OF OBSERVATIONS IN DATA SET = 160

NOTE: ALL DEPENDENT VARIABLES ARE CONSISTENT WITH RESPECT TO THE PRESENCE OR ABSENCE OF MISSING VALUES. HOWEVER, ONLY 21 OBSERVATIONS CAN BE USED IN THIS ANALYSIS.

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GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESP

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	
MODEL	6	20484.66666667	3414.11111111	4.85	0.0070	0.675334	6.
ERROR	14	9848.00000000	703.42857143		ROOT MSE		RESP
CORRECTED TOTAL	20	30332.66666667			26.52222787		423.3333

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE	PR
TRT	6	20484.66666667	4.85	0.0070	6	20484.66666667	4.85	0.(

(14)

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GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: RESP
NOTE: THIS TEST CONTROLS THE TYPE I COMPARISONWISE ERROR RATE.
NOT THE EXPERIMENTWISE ERROR RATE

ALPHA=0.05 DF=14 MSE=703.429

NUMBER OF MEANS	2	3	4	5	6	7
CRITICAL RANGE	46.3605	48.6142	50.1507	50.9815	51.6123	52.0839

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

DUNCAN GROUPING	MEAN	N	TRT
A	469.67	3	C
A			
B A	448.67	3	G
B A			
B A	446.00	3	A ←
B A			
B A C	427.33	3	F
B C			
B C	401.33	3	B
C			
C	392.67	3	D
C			
C	377.67	3	E