

DATA EVALUATION RECORD

1. CHEMICAL: Paclobutrazol

2. FORMULATION: PP. 333 Technical, 92.4% a.i.; and
GFU-029 W.P. (50% a.i.)

3. CITATION: Hammer, M.J. and I.R. Hill. 1982. Toxicity of the
technical material and formulation GFU 029 to first
instar Daphnia magna. Submitted by ICI Americas, Inc.,
Wilmington, Del.; under Reg. No. 10182-TT; Accession No.
248689.

4. REVIEWED BY: John J. Bascietto
Wildlife Biologist
EEB/HED

5. DATE REVIEWED: 1-20-83

6. TEST TYPE: Acute Toxicity - Freshwater aquatic invertebrate

A) Test species: water flea, Daphnia magna

7. REPORTED RESULTS:

48-hr EC₅₀ (technical PP. 333) = 33.2 (25.8-53.0) mg a.i./l
(95% c.i.)

48-hr EC₅₀ (formulation GFU 029) = 94 (65-135) mg ai/l*

*(only 16 mg ai/l in solution)

8. REVIEWER'S CONCLUSIONS: The study is scientifically sound and
with a 48-hr. EC₅₀ = 33.2 mg/l technical PP 333 is considered
"slightly toxic" to Daphnia magna. The study fulfills the guideline
requirements for acute toxicity to a freshwater aquatic invertebrate
for the technical compound and the formulated product.

9. Materials/Methods

A. Test Procedures:

animals - *Daphnia magna*, less than 24 hrs old, cultured in their lab, kept in a dense algal suspension to maintain parthenogenetic reproduction with only low production of males and ehippia.

Methods - as per EPA guidelines for the most part dilutions made with reconstituted water ("hard"). 250 ml glass beakers with 200 ml of test solutions for the technical tests - each conc. replicated x3

For the formulated product - a "suspension" was maintained over the 48 hrs of testing by testing 100 ml test volumes in 250 ml flasks, agitated on a rotary shaker at about 90 rpm. Each conc. replicated x 3

Each conc. tested 10 animals per replicate. Two tests were conducted for each chemical (I & II) totaling four tests.

Environmental conditions were closely held within recommendations by conducting in controlled temperature rooms with 16 hour (of light) photoperiod. Temp = 20 + 1°C throughout. pH and D.O. were monitored at beginning and end of each test. Zero hour measurements were made in the absence of the *Daphnia* from separate aliquots.

Each conc. was made from stock solutions of test substances in water only. No solvents were used, reducing the obtainable solubility to that of the lower limits of the technical and formulated products.

Analytical chemistry - (By HPLC)

Technical - samples taken from each replicate at zero hour and from one replicate after 48 hours. In addition at 0-hour samples of the two highest doses were passed sequentially through two filters under vacuum: 1) MF Millipore filter - 0.1 µm; 2) Diaflo Ultrafilter Um 10, retentivity approx. >10,000 MW. Samples of filtrates were taken after each stage and analyzed by HPLC. It was considered likely that PP 333 passing through the Diaflo filter was in solution.

Formulated Product (GFU 029) -

Sampled each test conc. at zero hour and from one replicate of the vessels containing *Daphnia* after 48 hours. Vessels without animals which contained 400, 100 and 12.5 mg ai/l were also sampled at 4, 10, 24 and 48 hours.

In addition 5 ml samples were taken from the 400, 100, and 12.5 mg ai/l conc. at zero hour and from vessels without Daphnia at 24 and 48 hours. These were centrifuged at about 5000 g for 20 min. The supernatant was sampled and analyzed for PP 333 to indicate the conc. of PP 333 in solution.

B. Statistical Analysis:

"EC₅₀ and 95% c.i. were calculated using the weighted linear regression of log conc. plotted against logit transformation of the Daphnia response. A combined EC₅₀ was calculated as the logarithmic mean of the EC₅₀'s for the individual tests."

10. Results

Daphnia Response

— See Tables 5 & 6 attached.

Analytical Chemistry —

(See Tables 1 & 2 attached)

Tech. — measured conc. of PP. 333 were within 10% PP. 333 of nominal except for the 35 mg/l conc. in both tests I + II.

Analysis of filtered solutions (Table 1) indicated that the majority of PP 333 was present in solution.

Formulation (Refer to Table 2.0

GFU 029 — measured concentrations of PP 333 showed considerable variation. The level of agitation could not be raised high enough to maintain uniform distribution of the powder without harming the animals.

Analysis of supernatant of the centrifuged samples showed that the majority of PP 333 at high concentrations was not in solution at 400 mg ai/l the PP 333 in the supernatant did not exceed the solubility of PP 333. At lower concentrations, 12.5 mg ai/l, most of the PP 333 as in solution

PH and D.O. determinations are shown in tables 5 & 6.

Paclobutrazol scientific review

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Pages 4 through 7 are not included in this copy.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients
 - ☐ Identity of product impurities
 - ☐ Description of the product manufacturing process
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Results of Statistical treatments (EC₅₀'s)

Technical PP 333

Time (hours)	EC ₅₀ (mg/l)	
	Test I	Test II
4	>35(a)	>35(a)
10	>35(a)	>35(a)
24	>35(a)	>35(a)
48	>35(a)	33.2 (25.8 - 53.0)

(a) EC₅₀ higher than aqueous solubility level, the highest concentration tested.

Formulation GFU029-

EC ₅₀ (mg ai/l)			
Time (hours)	Test I	Test II	Mean (I and II)
4	>400(a)	>400 (a)	>400
10	224(186-277)	484(b) (332-1038)	330
24	170(133-228)	241 (176-377)	203
48	105(82-135)	85 (65-111)	94

(a) EC₅₀ higher than range tested and not calculable

(b) EC₅₀ higher than range tested, but calculable

11. Reviewer's Evaluation

A. Test Procedure:

The only obvious procedural shortcoming was their failure to use a solvent to get the material (especially the formulation) into solution. This laboratory had done so with reported fish LC_{50} studies with PP 333. It is not clear why they chose not to do so with Daphnia, but they did do very extensive analytical work to mitigate the deficiency.

B. Statistical Analysis--

These were performed appropriately, considering the partial responses obtained. The results of the analyses are considered valid, and do reflect the raw data obtained.

C. Results

Unfortunately they do not have the required (definitive) five (5) concentrations in the technical study (Test II) upon which the calculated 48 LC_{50} = 33.2 (25.8 - 53.0) mg/l is based. Although they did test five doses, the highest dose was shown to have "particulate matter" attached to the Daphnia. This may invalidate the high dose where the 50% mortality is found. Only 30% mortality was observed in test I of this series, at the high dose of 35 mg/l (which also saw the "particulate matter"). Based on both observations we are willingly to concede that the dose is probably valid for statistical evaluation ("particulate matter" didn't necessarily cause the 50% mortality in test II). The results of the formulation testing are acceptable as reported due to the extensive analyses.

D. Conclusions

1. Category: Core for technical . Core for formulation
2. Rationale: apparent low toxicity and extensive "optional" testing mitigate the procedural "error" of not using a solvent.
3. Repair: N/A