Active Ing. Name
Checked in OPPIN's 2/14/05 BGM

MRID No. 439876-01

DATA EVALUATION RECORD
§ 72-2 - ACUTE EC_{50} TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** ~~Tebufenozide~~ Gokilaat Cyphenothrin — PC Code No.: ~~129026~~ 129013

2. **TEST MATERIAL:** S-1668 — Purity: 96.3%

3. **CITATION:**

Authors: Jane Bowman and John Bucksath
Title: Acute Flow-Through Toxicity of S-1668 to *Daphnia magna*
Study Completion Date: October 9, 1995
Laboratory: ABC Laboratories, Inc., Columbia, MO
Sponsor: Sumitomo Chemical Company, Ltd., Osaka 541, Japan
Laboratory Report ID: 42280
MRID No.: 439876-01
DP Barcode: D237130; D237151; D237155

4. **REVIEWED BY:** Thomas M. Steeger, Ph.D., Fishery Biologist, EFED, ERB IV

Signature: Thomas M Steeger **Date:** 3/25/98

APPROVED BY: Mike Rexrode, Fishery Biologist, EFED, ERB IV

Signature: M. Rexrode **Date:** 9-8-98

6. **STUDY PARAMETERS:**

Age of Test Organism:	<24 hours
Definitive Test Duration:	48 hours
Study Method:	Flow-Through
Type of Concentrations:	Mean measured

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill guideline requirements for an acute toxicity test with a freshwater invertebrate. Based on mean measured concentrations, the 48-hour EC_{50} value of 0.92 ppb ai classifies S-1668 as very highly toxic to *Daphnia magna*. The NOEC was determined to be 0.14 ppb ai. This study is classified as supplemental but upgradeable to core provided the registrant demonstrates that neither water hardness nor

pH affect the solubility or toxicity of S-1668

Results Synopsis

EC_{50}: 0.92 ppb ai 95% C.I.: 0.78 - 1.11 ppb ai

NOEC: 0.14 ppb ai Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental

B. Rationale: pH and hardness exceeded the recommended range

C. Repairability: Upgradeable to core provided the registrant demonstrates that neither pH nor hardness affect solubility or toxicity of S-1668.

9. **GUIDELINE DEVIATIONS:** The reported pH (8.1-8.3) was higher than recommended (7.2-7.6). Hardness (138 - 150) exceeded the recommended range of 40 - 48 ppm.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Daphnia magna*
All organisms are approximately the same size and weight?	Not reported
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3rd instar.	1st instar (<24 h)
Supplier	In-house cultures

Guideline Criteria	Reported Information
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Cultures were maintained under conditions similar to testing.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No signs of sickness or injury were observed
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study.	No feeding during the study
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water.	Hard blended water, biologically aged prior to use.

Guideline Criteria	Reported Information
Does water support test animals without observable signs of stress?	Yes
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20.3-21.0°C
pH Prefer 7.2 to 7.6.	8.1-8.3
Dissolved Oxygen Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%.	≥60% during the test, with the exception of the 48 hour DO measurement in the highest treatment, which was 54% of saturation.
Total Hardness Prefer 40 to 200 mg/L as $CaCO_3$.	138-150 mg/L as $CaCO_3$
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 mL (daphnids and midges) or 2-3 L.	 Glass 1 L 0.9 L
Type of Dilution System Must provide reproducible supply of toxicant.	Proportional diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	10 vol/24 hrs.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.	1 daphnid per 90 ml solution

Guideline Criteria	Reported Information
Photoperiod 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	0.1 mL/L DMF

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L, then no definitive test is required.	Several range finding tests were conducted: nominal concentrations of 0.1, 0.4, and 1.6 µg/L resulted in 5%, 10%, and 10% immobilization; a static test with nominal concentrations of 0.5, 2.5, and 5.0 µg/L resulted in 0, 70, and 100% immobility; a flow through test with nominal concentrations of 0.36, 0.72, 1.5, 3.0, and 6.0 µg/L resulted in 0, 0, 5, 11, and 100% mortality.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	Negative control, solvent control, 0.36, 0.72, 1.5, 3.0, and 6.0 µg/L
Number of Test Organisms Minimum 20/level, may be divided among containers.	20 per level, 10 per replicate
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	Temperature, DO, and pH were measured at test initiation and termination in replicates A and B of each control and treatment, and measured at 24 hours in replicates C and D of each control and treatment.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Solutions collected at 0 and 48 hours and analyzed for S-1668 using GLC.

12. **REPORTED RESULTS:**

A. **General Results**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Static: ≤10% Flow-through: ≤5%	0% mortality in the control
Percent Recovery of Chemical	Range 31-43%
Raw data included?	Yes

Mortality/Immobilization

Nominal Concentration (μg/L)	Mean Measured Concentration (μg ai/L)	Number of Daphnids	Cumulative Number Immobile/Dead	
			24-hr	48-hr
Control	Control	20	0	0
Solvent Control	Solvent Control	20	0	0
0.36	0.14	20	1	1
0.72	0.30	20	0	0
1.5	0.48	20	1	1
3.0	0.91	20	0	8
6.0	1.93	20	8	20

Other Significant Results: Signs of toxicity included quiescent daphnids, laying on the bottom of the test vessel, trailing extraneous material, and immobilization. Some or all of these signs were observed in the four highest test concentrations (see reviewer's comments).

B. Statistical Results:

Method: Binomial method

48-hr EC_{50}: 1.0 μg/L (0.96 μg ai/L)

95% C.I.: 0.50 - 2.0 μg/L (0.48 - 1.93 μg ai/L)

Probit Slope: N/A

NOEC: 0.15 μg/L (0.14 μg ai/L)

13. **VERIFICATION OF STATISTICAL RESULTS:**

Method: Moving average method

48-hr EC_{50}: 0.92 ppb ai

95% C.I.: 0.78 - 1.11 ppb ai

Probit Slope: N/A

NOEC: 0.14 ppb ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound; however, it does not fulfill the guideline requirements, and is thus classified as supplemental. Based on mean measured concentrations, the 48-hour EC_{50} for *Daphnia magna* exposed to S-1668 was 0.92 ppb ai, which classifies this compound as very highly toxic to the daphnid. The NOEC was determined to

be 0.14 ppb ai.

Despite the instability of S1668 in water, the compound remains very highly toxic to daphnids in acute flow-through toxicity tests. Mean measured concentrations for each of the treatment levels were: 0.15, 0.31, 0.50, 0.94, and 2.0 µg/L. These represented 42%, 43%, 33%, 32% and 33% of the nominal concentrations. The report concludes that based on the large difference between nominal and measured test concentrations, the test material did not appear to be stable in water.

DO ranged from 4.6 to 7.8 ppm representing 54-92% saturation at 21°C. DO was significantly dependent on dose ($P<0.01$) with DO levels dropping from 7.8 to 4.6 ppm in controls versus the 2.0 µg/L treatment.

Water hardness (138-150 ppm) and pH (8.1 - 8.3) exceeded the recommended ranges of 40 - 48 ppm and 7.2 - 7.6, respectively. Based on this deviation from the guideline, this study is classified as supplemental but can be upgraded to core provided the registrant demonstrate that neither pH nor water hardness affect the solubility or toxicity of S-1668.