DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
§ 72-4(c)

1. **CHEMICAL:** Pyraclostrobin PC Code No.: 099100

2. **TEST MATERIAL:** BAS 500 F Purity: 99.7%

3. **CITATION:**

Authors: Ward, T.J., *et al.*

Title: BAS 500 F: A Flow-Through Life-Cycle Toxicity Test with the Saltwater Mysid, *Americamysis bahia*

Study Completion Date: February 18, 2004

Laboratory: T.R. Wilbury Laboratories, Inc.
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4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/2/04

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Signature: **Date:** 9/7/04

5. **APPROVED BY:** Lewis Brown, Biologist, OPP/EFED/ERB - I

Signature: Lewis R Brown **Date:** 2/10/05

6. STUDY PARAMETERS:

Scientific Name of Test Organisms: *Americamysis bahia*

Age of Test Organism: < 24 hours old

Definitive Test Duration 28 days

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

In a 28-day life-cycle test, *Americamysis bahia* neonates were exposed under flow-through conditions to BAS 500 F (pyraclostrobin) at nominal concentrations of 0 (negative and solvent controls), 0.28, 0.52, 1.0, 2.0, and 4.0 ppb. Mean-measured concentrations were <0.074 (LOQ, controls), 0.27, 0.50, 0.93, 1.9, and 3.6 ppb a.i.

There were 60 mysids/level: 15 mysids/compartment, 2 compartments/aquarium, and 2 replicate test aquaria/level. On Day 14, up to 20 pair/level were isolated for individual matings; the remainder of first-generation mysids were group retained by sex. First-generation mysids were observed for mortality and signs of abnormal behavior once daily throughout the study. Once daily during the reproduction period (Days 16-28), second-generation mysids were counted and discarded. Data endpoints included percent survival of unaffected (no sub-lethal effects) first-generation mysids at study termination (Day 28; combined sexes), number of young produced per female, length, and wet and dry weight of surviving first-generation mysids (Day 28; combined sexes).

A statistically-significant effect on overall survival of mysids was observed at the mean-measured 0.93, 1.9, and 3.6 ppb a.i. test levels. The percent alive and unaffected (no sub-lethal effects) mysids after 28 days of exposure averaged 88 and 80% for the negative and solvent controls, respectively, and 83, 82, 73, 52, and 0% for the mean-measured 0.27, 0.50, 0.93, 1.9, and 3.6 ppb a.i. treatment groups, respectively. The NOEC And LOEC for survival of first generation mysids were 0.50 and 0.93 ppb a.i., respectively. Lethargy was observed in surviving mysids from the mean-measured 3.6 ppb a.i. test group between 4 and 12, and 27-28 days.

The number of young per female was statistically-reduced at the 1.9 ppb a.i. treatment level, and averaged 7.5, 7.1, 6.0, 11.4, 7.6, and 2.0 for the negative control, solvent control, 0.27, 0.50, 0.93, and 1.9 ppb a.i. test groups, respectively. The NOEC and LOEC values for the number of young per female were 0.93 and 1.9 ppb a.i., respectively. The 3.6 ppb a.i. treatment level was not included in the statistical analyses for reproduction, terminal length, and wet and dry weight due to the 100% mortality observed by 28 days.

Terminal growth was also affected by exposure to BAS 500 F. Total length averaged 7.4, 7.3, 7.4, 7.2, 7.3, and 7.1 mm for the negative control, solvent control, 0.27, 0.50, 0.93, and 1.9 ppb a.i. test levels, and was statistically-reduced at the 1.9 ppb a.i. level compared to pooled controls. The NOEC and LOEC values for terminal length were 0.93 and 1.9 ppb a.i., respectively. No statistically-significant differences in wet or dry weights were observed up to an including the 1.9 ppb a.i. treatment level (the highest treatment level included in the statistical analyses). Consequently, the NOEC and LOEC values were 1.9 and >1.9 ppb a.i., respectively, for terminal wet and dry weights.

Based on significant reductions in survival of first generation mysids at study termination (most sensitive endpoint), the NOEC and LOEC values were 0.50 and 0.93 ppb a.i., respectively.

This study is scientifically sound. However, since the survival of male mysids following pairing was not monitored, and since offspring were not maintained and observed for 4 days, this study does not fulfill the guideline requirements for an aquatic invertebrate life-cycle toxicity test using the *Americamysis bahia* (72-4c), and is classified SUPPLEMENTAL.

Results Synopsis:

Endpoint	NOEC	LOEC
Adult Survival (Day 28)	0.50 ppb a.i.	0.93 ppb a.i.
Reproduction (no. young/ female)	0.93 ppb a.i.	1.9 ppb a.i.
Combined Length (mm)	0.93 ppb a.i.	1.9 ppb a.i.
Combined Dry Weight (mg)	1.9 ppb a.i.	>1.9 ppb a.i.
Combined Wet Weight (mg)	1.9 ppb a.i.	>1.9 ppb a.i.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Survival of male mysids following pairing were not provided, and second-generation mysids were not observed daily for at least 4 days for survival, development,

and behavior.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The parental stock were apparently not maintained separately from the brood stock.
2. The temperature (23.2-26.7°C) was slightly lower than recommended (27°C).
3. A high level of analytical variability was observed at the nominal 0.52 ppb test level, with a reviewer-calculated high-low ratio of 1.9.
4. Following pairing, the survival of males as well as reproductive females should have been recorded.
5. Only the number of offspring produced was recorded. Data regarding survival, development, and behavior of second-generation mysids for at least 4 days were not obtained.
6. Terminal growth endpoints should have been statistically evaluated by the study authors on a gender-specific basis.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of BAS 500 F (pyraclostrobin) to the mysid life cycle for the purpose of chemical registration (NU).

11. MATERIALS AND METHODS:

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species An estuarine shrimp species, preferably *Americamysis bahia*	*Americamysis bahia*

Guideline Criteria	Reported Information
Source/Supplier	Juveniles were collected from an in-house laboratory culture that resulted from the combination of two in-house cultures (in July 2001).
Age at Beginning of Test <24 hours old	<24 hours old
Parental Acclimation Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. Mysids should be in good health.	An isolated brood stock was apparently not maintained. The mysids were maintained under flow-through conditions, and were not treated for disease and were free of apparent disease, injuries, and abnormalities. Mortality was <3% during the 48 hours preceding the definitive study.
Parental Acclimation Period At least 14 days	Continuous
Brood Stock Test started with mysids from: - one brood stock, or - brood stock which has not obtained sexual maturity or had been maintained for >14 days in a laboratory with same food, water, temperature, and salinity used in the test.	At test initiation, juvenile mysids were collected from the culture stock that was maintained in the laboratory under the same conditions used in the definitive test.

B. Test System

Guideline Criteria	Reported Information
Source of Dilution Water May be natural (sterilized and filtered) or a commercial mixture; water must be free of pollutants.	Natural seawater collected at T.R. Wilbury Laboratories in Marblehead, MA, was adjusted to 15-17‰ salinity using deionized water. Diluted seawater was then aerated, passed through a 20-µm particle filter, an activated carbon filter, and an UV sterilizer prior to use. Results of chemical characterization of the dilution water (December 2002) are provided in Table 1, p. 12.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 27°C for mysids - At test termination, mean-measured temperature for each chamber should be within 1°C of selected test temperature. - Must be within 3°C of the mean of the time-weighted averages. - Must not differ by >2°C between chambers during the same interval.	Target: 25 ± 2°C Actual range: 23.2-26.7°C - Raw data not provided, so criteria were not assessed; however, the overall temperature range was within 2°C of the mean.
Salinity 15-30 ‰ - The difference between highest and lowest measured salinities should be less than 5 ‰.	15-17‰
pH 7.6 and 8.2	7.5-8.2
Dissolved Oxygen 60-100% saturation	5.0-7.9 mg/L (≥67% saturation).

Guideline Criteria	Reported Information
Photoperiod 16-hr light/8-hr dark (14-hr light/10-hr dark also acceptable)	16 hours light, 8 hours dark, with a 15-minute transition period. The light intensity was approximately 38 footcandles.
Test Chambers 1. Material: All glass, No. 316 stainless steel, or perflorocarbon plastic 2. Size: Typically 30 x 45 x 15 cm (20.25 L) 3. Fill depth: 10 cm 4. Were chambers identical and covered during the test?	1. Glass aquaria 2. 21 x 40 x 25 cm (20 L) 3. 4- to 9-cm (up to 7 L fill volume). 4. Yes, loosely covered
Test Compartments (within chambers) - 250-mL glass beakers with side cutouts covered with nylon mesh or stainless steel screen, or - 90- or 140-mm id glass Petri dish bottoms with collars made of 200-250 µm mesh screen	- Test compartments were 10-cm diameter glass Petri dishes with 12-cm high collar of Nitex® screen. - Reproductive compartments were 6-cm glass petri dishes with 12-cm high collar of Nitex® screen.
Type of Dilution System Intermittent flow proportional diluters or continuous flow serial diluters should be used.	An intermittent-flow proportional diluter was used to deliver each concentration of the test substance, a negative (saltwater) control, and a solvent (dimethylformamide) control.

Guideline Criteria	Reported Information
Toxicant Mixing 1. Mixing chamber is recommended but not required; aeration should not be used for mixing. 2. If a mixing chamber was not employed, was it demonstrated that the test solution was completely mixed before introduction into the test system? 3. Was flow splitting accuracy within 10%?	1. Not reported. 2. The diluter was in operation for approximately 24 hours prior to the introduction of mysids. 3. Not reported.
Flow Rate 1. 5-10 volume additions per 24 hours. 2. Did the flow rate maintain the toxicant level and the DO at ≥60% of saturation? 3. Were the meter systems calibrated before study and checked twice daily during test period?	1. 13.6 volume additions/day 2. Yes 3. Yes
Solvents - Acceptable solvents include triethylene glycol, methanol, acetone, and methanol. - Solvent should not exceed 0.1 mL/L in a flow-through system.	Dimethylformamide, 0.1 mL/L
Aeration Dilution water should be vigorously aerated, but the test tanks should not be aerated.	The dilution water was aerated prior to use. The test chambers were not aerated.

C. Test Design

Guideline Criteria	Reported Information
Duration of the Test Approximately 28 days. Was the test terminated within 7 days of the median time of first brood release in the controls?	28 days No, the study duration was adequate. The first brood release occurred on Day 17 (p. 15).
Nominal Concentrations Negative control, a solvent control (when applicable), and at least five treatment levels, one of which must adversely affect a life stage and one must not affect any life stage. The dilution factor should not be >50%.	Nominal test concentrations were 0 (negative and solvent controls), 0.28, 0.52, 1.0, 2.0, and 4.0 ppb.
Distribution Number of mysids before pairing: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level. Number of mysids after pairing: ≥20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).	60 mysids/level: 15 mysids/compartment, 2 compartments/aquarium, and 2 replicate test aquaria/level. Up to 20 pair/level: 1 pair/compartment, up to 10 compartments/aquarium, and 2 replicate test aquaria/level. Extra, unpaired mysids were maintained in two extra compartments per aquarium.
Pairing Should be conducted when most of the mysids are sexually mature, usually 10-14 days after test initiation. All pairing should occur on the same day.	Female and male adults were paired on Day 14 and reproduction was monitored from Days 17 (first offspring produced) through 28.
Test organisms randomly or impartially assigned to test vessels?	Yes
Were treatments randomly assigned to individual test chamber locations?	Yes

Guideline Criteria	Reported Information
Feeding Mysids should be fed live brine shrimp nauplii at least once daily. 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.	Mysids were fed newly-hatched live brine shrimp *Artemia salina* nauplii, *ad libitum*, 3 times/day (150 to 600 *Artemia* per mysid per day) during the test, except during the final 24 hours of the test.
Counts Live adult mysids should be counted at initiation, at pairing, and daily after pairing. Live young must be counted and removed daily. Missing or impinged animals should be recorded.	Yes Yes N/A
Controls Negative control and carrier control (when applicable) are required.	Negative saltwater and solvent (DMF) controls were included.
Water Parameter Measurements 1. Temperature should be monitored daily in one chamber and at least three times in all chambers. 2. Salinity should be measured daily in at least one test vessel. 3. pH should be measured at the beginning, the end, and at least weekly during the test in the control vessels and highest test level. 4. Dissolved oxygen must be measured at each concentration at least once a week.	1. Temperature was measured daily in each replicate test vessel, and continuously in one negative control test vessel. 2.- 4. Salinity, pH, and DO were measured daily in each replicate test vessel.

Guideline Criteria	Reported Information
Chemical Analysis Toxicant concentration must be measured in one chamber at each toxicant level every week.	Samples for HPLC analysis were collected from alternating replicate test vessels on Days 0, 7, 14, 21, and 28.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Chemical Analysis For all test groups, a) the measured concentration of the test material should not be <50% of the time-weighted average measured concentration for >10% of the duration of the test, and b) the measured concentration should not be >30% of the time-weighted average measured concentration for >5% of the duration of the test.	Mean-measured concentrations were <0.074 (<LOQ, controls), 0.27, 0.50, 0.93, 1.9, and 3.6 ppb a.i. (Table 2, p. 19). A low level of variability existed among results obtained for all but the nominal 0.52 ppb test level (with reviewer-calculated high-low ratios of ≤1.5). At the 0.52 ppb level, the high low ratio was 1.9.
Controls - Survival of the paired first-generation controls must be ≥70%. - ≥75% of the paired first-generation female controls produced young, or - The average number of young produced by the first-generation female controls was ≥3.	- All criteria met.

Guideline Criteria	Reported Information
Data Endpoints Must Include 1. Survival of first-generation mysids, gender specified 2. Number of live young produced per female 3. Dry weight and length of each first generation mysid alive at the end of the test, gender specified **Data Endpoints Should Also Include** 4. Incidence of morphological findings. 5. Survival, development, and behavior of second-generation mysids for at least 4 days.	1. Total survival of first-generation mysids, and of females paired for reproduction. 2. Number of live young produced per female. 3. Weights (wet and dry) and length of each first generation living at end of test, but not gender specific (raw data were gender-specific; however, data were combined by the study authors for statistical analyses). 4. Incidence of sub-lethal effects pertaining to behavior or appearance. 5. Endpoint not assessed.
Raw data must include 1. Survival of first-generation mysids, gender specified 2. Number of live young produced per female 3. Terminal weight and length measurements, individual and gender specified	1. Daily survival of first-generation mysids, not gender-specific, and of reproductive females (Days 16-28). 2. Number of live young produced (Days 16-28). 3. Terminal weight (wet and dry) and length of individuals, individual and gender specified.

Effects Data

Concentration (ppb a.i.)		% Survival Day 28 (Alive and Unaffected)	Reproduction, Days 16-28	
Nominal	Mean Measured (% nominal)	♂ and ♀	Total No. of Young[1]	No. Young Per Female
Negative Control	<0.074	88	150	7.5
Solvent Control	<0.074	80	128	7.1
0.28	0.27 (96)	83	120	6.0
0.52	0.50 (96)	82	228	11.4
1.0	0.93 (93)	73*	141	7.6
2.0	1.9 (95)	52*	31	2.0*
4.0	3.6 (90)	0*	0	0*

[1] Reviewer-calculated from replicate offspring production data, Table A.4, p. 30.
*Statistically different ($\alpha = 0.05$) from the pooled control.

Mean-Measured Concentration (ppb a.i.)	Growth, Day 28		
	Mean Length, mm	Mean Wet Weight, mg	Mean Dry Weight, mg
	♂ and ♀	♂ and ♀	♂ and ♀
Neg. control	7.4	3.58	0.74
Solvent control	7.3	3.48	0.76
0.27	7.4	3.55	0.78
0.50	7.2	3.46	0.76
0.93	7.3	3.41	0.77
1.9	7.1*	3.19	0.75
3.6	—*	—*	—*

* Statistically-different (α=0.05) from the pooled control (only combined sexes data were statistically analyzed by the study authors).
— Only mysids from one replicate survived to the end of the study, and a statistically-significant difference was assumed.

Toxicity Observations: A statistically-significant effect on overall survival of mysids was observed at the mean-measured 0.93, 1.9, and 3.6 ppb a.i. test levels (Table 3, p. 20). The percent alive and unaffected (no sub-lethal effects) mysids after 28 days of exposure

averaged 88 and 80% for the negative and solvent controls, respectively, and 83, 82, 73, 52, and 0% for the mean-measured 0.27, 0.50, 0.93, 1.9, and 3.6 ppb a.i. treatment groups, respectively. A 28-day LC_{50} was not calculated by the study authors. The NOEC for survival of unaffected mysids was 0.50 ppb a.i. (Table 4, p. 21).

Lethargy was observed in surviving mysids from the mean-measured 3.6 ppb a.i. test group between 4 and 12, and 27-28 days (comparison of Tables A.1 and A.2, pp. 25-28).

Reproduction in terms of the number of young per female was statistically-reduced at the 1.9 and 3.6 ppb a.i. treatment levels, and averaged 7.5, 7.1, 6.0, 11.4, 7.6, 2.0, and 0 for the negative control, solvent control, 0.27, 0.50, 0.93, 1.9, and 3.6 ppb a.i. test groups, respectively (Table 3, p. 20). The NOEC for the number of young per female was 0.93 ppb a.i. (Table 4, p. 21).

Terminal growth was also affected by exposure to BAS 500 F. Total length averaged 7.4, 7.3, 7.4, 7.2, 7.3, and 7.1 mm for the negative control, solvent control, 0.27, 0.50, 0.93, and 1.9 ppb a.i. test levels, and was statistically-reduced at the 1.9 ppb a.i. level compared to pooled controls (Table 3, p. 20). No statistically-significant differences in wet or dry weights were observed up to an including the 1.9 ppb a.i. treatment level; however, for all growth endpoints, a statistically-different assumption was made for the 3.6 ppb a.i. test level, as only one replicate had surviving mysids. The NOEC for total length and wet or dry weights were 0.93 and 1.9 ppb a.i., respectively (Table 4, p. 21).

B. Statistical Results:

Statistical analyses were performed on survival of unaffected (no sub-lethal effects) first-generation mysids (Day 28), the number of young per surviving female, mean terminal length (combined sexes), and mean terminal wet and dry weights (combined sexes) via TOXSTAT statistical software (Version 3.3; Gulley et al., 1990). Analyses included Bartlett's Test (evaluation of homogeneity) and Chi square test (assessment of normality). ANOVA and Dunnett's or William's tests were then used to compare treatments to the pooled controls. The NOEC and LOEC were determined from significance data. The MATC was calculated as the geometric mean of the NOEC and LOEC. Mean-measured concentrations were used for all estimations.

Most sensitive endpoint: Survival of first-generation mysids

Results Synopsis

Endpoint	Method[2]	NOEC	LOEC	MATC
Survival (Day 28)[1]	ANOVA, Dunnett's or Williams	0.50 ppb a.i.	0.93 ppb a.i.	0.68 ppb a.i.
Reproduction (no. young/ female)	ANOVA, Dunnett's or Williams	0.93 ppb a.i.	1.9 ppb a.i.	1.3 ppb a.i.
Length (mm)[1]	ANOVA, Dunnett's or Williams	0.93 ppb a.i.	1.9 ppb a.i.	1.3 ppb a.i.
Wet Weight (g)[1]	ANOVA, Dunnett's or Williams	1.9 ppb a.i.	3.6 ppb a.i.	2.6 ppb a.i.
Dry Weight (g)[1]	ANOVA, Dunnett's or Williams	1.9 ppb a.i.	3.6 ppb a.i.	2.6 ppb a.i.

[1] Toxicity values based on survival, length, and wet and dry weight results were determined relative to combined sexes rather than for separate sexes.
[2] The statistical method was either the Dunnett's or the William's Test (not specified).

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Endpoints statistically assessed included percent survival (Day 28), reproduction (number of young per female), terminal lengths, and wet and dry weights. Survival, length, and wet and dry weight data were analyzed for combined sexes because mean replicate data were either not provided or were not statistically analyzed by the study authors. With the exception of percent survival and terminal length (combined sexes), data for all endpoints were determined to be normally distributed and the variances were homogeneous. The NOEC and LOEC for these endpoints were determined using ANOVA, and if necessary, followed by Bonferroni's tests. The NOEC and LOEC for data which did not meet the assumptions of ANOVA (and which could not be transformed to satisfy these assumptions) were determined using the non-parametric Kruskal-Wallis test. These analyses were conducted using TOXSTAT statistical software using mean-measured concentrations. All analyses were performed using pooled control data based on the results of t-tests, which indicated no significant differences. The 3.6 ppb a.i. treatment group was excluded from all but the percent survival statistical analysis due to 100% mortality for all adult mysids by 28 days.

Most sensitive endpoint: Reproduction

Results Synopsis

Endpoint	Method	NOEC	LOEC
Survival (Day 28)[1]	ANOVA, Dunnett's or Williams	3.6 ppb a.i.	>3.6 ppb a.i.
Reproduction (no. young/ female)	ANOVA, Dunnett's or Williams	0.93 ppb a.i.	1.9 ppb a.i.
Length (mm)[1]	ANOVA, Dunnett's or Williams	1.9 ppb a.i.	>1.9 ppb a.i.
Wet Weight (g)[1]	ANOVA, Dunnett's or Williams	1.9 ppb a.i.	>1.9 ppb a.i
Dry Weight (g)[1]	ANOVA, Dunnett's or Williams	1.9 ppb a.i.	>1.9 ppb a.i.

[1] Toxicity values based on survival, length, and wet and dry weight results were determined relative to combined sexes rather than for separate sexes.

14. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were similar to those of the study authors. The reviewer determined NOEC and LOEC values for first generation survival (3.6 and >3.6 ppb a.i., respectively) were higher than those of the study authors (0.50 and 0.93 ppb a.i., presumably due to the different statistical methods used. The more conservative values determined by the study authors is reported in the Conclusion section of this DER. The reviewer and study authors NOEC and LOEC values for reproduction (number of young per female) were identical. The study authors' NOEC and LOEC values for terminal length were lower (0.93 and 1.9 ppb a.i., respectively) than those of the reviewer (1.9 and >1.9 ppb a.i., respectively) due to the statistical methods used (parametric for the study authors and non-parametric for the reviewer). Consequently, the more conservative values determined by the study authors is reported in the Conclusion section of this DER. The reviewer determined LOEC value (>1.9 ppb a.i.) for terminal wet and dry weight was lower than that of the study authors (3.6 ppb a.i.) because the reviewer did not include the 3.6 ppb a.i. treatment level in the statistical analyses due to the significant treatment related effects on percent survival. Consequently, the reviewer considered the LOEC values for wet and dry weight to be greater than the 1.9 ppb a.i., the highest treatment level included in the reviewer's statistical verification. The more conservative reviewer-determined LOEC value is reported in the Conclusion section of this DER.

This study is scientifically sound. However, deviations from FIFRA Guideline §72-4c included the failure to report survival of first-generation male mysids (following pairing), and failure to observe second-generation mysids for 4 days. As a result, this study does not fulfill the guideline requirement for an aquatic invertebrate life-cycle toxicity test using an estuarine species and is classified SUPPLEMENTAL.

No insoluble test material was observed in any vessel during the study (p. 18).

Test concentrations were adjusted for the purity of the test material (p. 11).

The method was validated by fortifying samples of dilution water with BAS 500 F at 0.2 ppb and 5.0 ppm (p. 16). The 5.0 ppm samples were analyzed before and after centrifugation. Recoveries averaged 0.17 ppb (85%) for the 0.2 ppb samples, and 4.30 (86%) and 2.22 ppm (44%) for the uncentrifuged and centrifuged 5.0 ppm samples, respectively. The LOD was 0.00443 ppb a.i. (erroneously reported in terms of ppm), and the LOQ was 0.074 ppb a.i. (p. 17).

Each batch of samples collected was accompanied by a laboratory control sample prepared in dilution water, a dilution water blank, and two matrix spike samples prepared in water collected from a control vessel (p. 15). Recovery from the 1.0 ppb laboratory controls samples averaged 110% of nominal, and recovery from the 1.0 ppb matrix spike samples averaged 100% of nominal (Table 2, p. 19). No test material was detected (LOQ 0.074 ppb a.i.) in the dilution water blanks.

Based on the Certificate of Analysis, the IUPAC name for pyraclostrobin was methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-N-methoxy carbamate, and the CAS No. is 175013-18-0 (p. 51). The test substance is expected to be stable for at least 4 years when stored refrigerated (≤4°C).

This study conformed with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part. 160. A Quality Assurance Statement was provided.

15. REFERENCES:

ASTM. 1990. Guide for Conducting Life-Cycle Toxicity Test with Saltwater Mysids. Designation E 3.61-90.

Gulley, D.D., *et al.* 1990. TOXSTAT Version 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, Wyoming.

U.S. EPA. 1985. Standard Evaluation Procedure, Fish Early Life Stage. Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.

U.S. EPA. 1988. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. 72-4. Fish Early Life Stage and Aquatic Invertebrate Life-Cycle Studies. Ecological Effects Branch, Hazard Evaluation Division, Office of

Pesticide Programs, Washington, D.C. Draft, March 1988.

U.S. EPA. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rule.

16. RESULTS OF STATISTICAL VERIFICATION:

Adult percent survival (combined sexes)
File: 7601sd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	84.250	84.250	45.000
2	0.27	83.000	83.000	20.000
3	0.50	81.500	81.500	18.000
4	0.93	73.500	73.500	12.000
5	1.9	51.500	51.500	7.000
6	3.6	0.000	0.000	3.000

Calculated H Value = 10.524 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Adult percent survival (combined sexes)
File: 7601sd Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 4 3 2 1
6	3.6	0.000	0.000	\
5	1.9	51.500	51.500	. \
4	0.93	73.500	73.500	. . \
3	0.50	81.500	81.500	. . . \
2	0.27	83.000	83.000	. . . . \
1	GRPS 1&2 POOLED	84.250	84.250	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

Reproduction young per female by day 28
File: 7601rd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	91.958	22.989	19.938
Within (Error)	7	8.072	1.153	
Total	11	100.030		

Critical F value = 4.12 (0.05,4,7)
Since F > Critical F REJECT Ho:All groups equal

Reproduction young per female by day 28
File: 7601rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	7.325	7.325		
2	0.27	6.000	6.000	1.425	
3	0.50	11.400	11.400	-4.382	
4	0.93	7.650	7.650	-0.349	
5	1.9	2.000	2.000	5.726	*

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Reproduction young per female by day 28
File: 7601rd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.27	2	2.643	36.1	1.325
3	0.50	2	2.643	36.1	-4.075
4	0.93	2	2.643	36.1	-0.325
5	1.9	2	2.643	36.1	5.325

Reproduction young per female by day 28
File: 7601rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	7.325	7.325	8.012
2	0.27	2	6.000	6.000	8.012
3	0.50	2	11.400	11.400	8.012
4	**0.93**	**2**	**7.650**	**7.650**	**7.650**
5	1.9	2	2.000	2.000	2.000

Reproduction young per female by day 28
File: 7601rd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

GRPS 1&2 POOLED	8.012				
0.27	8.012	0.739		1.89	k= 1, v= 7
0.50	8.012	0.739		2.00	k= 2, v= 7
0.93	**7.650**	**0.349**		**2.04**	**k= 3, v= 7**
1.9	2.000	5.726	*	2.06	k= 4, v= 7

s = 1.074
Note: df used for table values are approximate when v > 20.

Terminal length (combined sexes)
File: 7601ld Transform: 1/Y (INVERSE)

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	0.136	7.375	18.500
2	0.27	0.135	7.400	7.000
3	0.50	0.139	7.200	19.000
4	0.93	0.138	7.250	14.500
5	1.9	0.141	7.100	19.000

Calculated H Value = 5.617 Critical H Value Table = 9.490
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Terminal length (combined sexes)
File: 7601ld Transform: 1/Y (INVERSE)

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 2 1 4 3 5
2	0.27	0.135	7.400	\
1	GRPS 1&2 POOLED	0.136	7.375	. \
4	0.93	0.138	7.250	. . \
3	0.50	0.139	7.200	. . . \
5	1.9	0.141	7.100	. . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,5) = 2.807 Unequal reps - multiple SE values

Terminal wet weight (combined sexes)
File: 7601wd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.178	0.045	0.918
Within (Error)	7	0.343	0.049	

Total	11	0.521

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Terminal wet weight (combined sexes)
File: 7601wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	3.523	3.523		
2	0.27	3.550	3.550	-0.143	
3	0.50	3.465	3.465	0.300	
4	0.93	3.420	3.420	0.535	
5	1.9	3.190	3.190	1.734	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Terminal wet weight (combined sexes)
File: 7601wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.27	2	0.545	15.5	-0.028
3	0.50	2	0.545	15.5	0.058
4	0.93	2	0.545	15.5	0.103
5	1.9	2	0.545	15.5	0.333

Terminal wet weight (combined sexes)
File: 7601wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	3.523	3.523	3.532
2	0.27	2	3.550	3.550	3.532
3	0.50	2	3.465	3.465	3.465
4	0.93	2	3.420	3.420	3.420
5	**1.9**	**2**	**3.190**	**3.190**	**3.190**

Terminal wet weight (combined sexes)
File: 7601wd Transform: NO TRANSFORMATION

SOURCE	DF	SS	MS	F
Between	4	0.0017	0.0004	0.333
Within (Error)	7	0.0083	0.0012	
Total	11	0.0100		

Critical F value = 4.12 (0.05,4,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Terminal dry weight (combined sexes)
File: 7601dd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.753	0.753		
2	0.27	0.780	0.780	-0.917	
3	0.50	0.760	0.760	-0.250	
4	0.93	0.770	0.770	-0.583	
5	1.9	0.745	0.745	0.250	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Terminal dry weight (combined sexes)
File: 7601dd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL

File: 7601dd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.753	0.753		
2	0.27	0.780	0.780	-0.917	
3	0.50	0.760	0.760	-0.250	
4	0.93	0.770	0.770	-0.583	
5	1.9	0.745	0.745	0.250	

Bonferroni T table value = 2.84 (1 Tailed Value, P=0.05, df=7,4)

Terminal dry weight (combined sexes)
File: 7601dd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL

1	GRPS 1&2 POOLED	4			
2	0.27	2	0.085	11.3	-0.028
3	0.50	2	0.085	11.3	-0.007
4	0.93	2	0.085	11.3	-0.017
5	1.9	2	0.085	11.3	0.008

Terminal dry weight (combined sexes)
File: 7601dd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	0.753	0.753	0.763
2	0.27	2	0.780	0.780	0.763
3	0.50	2	0.760	0.760	0.763
4	0.93	2	0.770	0.770	0.763
5	**1.9**	**2**	**0.745**	**0.745**	**0.745**

Terminal dry weight (combined sexes)
File: 7601dd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.763				
0.27	0.763	0.351		1.89	k= 1, v= 7
0.50	0.763	0.351		2.00	k= 2, v= 7
0.93	0.763	0.351		2.04	k= 3, v= 7
1.9	**0.745**	**0.251**		**2.06**	**k= 4, v= 7**

s = 0.034
Note: df used for table values are approximate when v > 20.