DATA EVALUATION RECORD
MIDGE CHRONIC TOXICITY STUDY
Non Guideline (US EPA)

1. **CHEMICAL:** Prothioconazole PC Code No.: 113961

2. **TEST MATERIAL:** JAU6476 (tech) Purity: 98.6%

Common name: Prothioconazole
Chemical:
IUPAC name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS No.: 178928-70-6
Synonyms: JAU6476 Technical

3. **CITATION:**

Author: Hendel, B.

Title: Influence of JAU6476 (tech) on Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System

Study Completion Date: September 14, 2000

Laboratory: Bayer AG Crop Protection Business Group
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Laboratory Report ID: E 4161775-1

MRID No.: 46246131

DP Barcode: D303488

4. **REVIEWED BY:** Gregory Hess, Staff Scientist, Dynamac Corporation

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APPROVED BY: Teri Myers, Staff Scientist, Dynamac Corporation

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5. **APPROVED BY:** Kevin Costello, Geologist, OPP/EFED/ERB-III

Signature: **Date:**

6. **SECONDARY REVIEW BY:**

Émilie Larivière, EAD, PMRA, HC
Signature: **Date:** October 19, 2005
10-19-05

Christopher J. Salice, OPP/EFED/ERB-IV
Signature: **Date:** August 15, 2005
8-15-05

7. STUDY PARAMETERS:

Age of Test Organism:	1st Instar, <2-3 days old
Definitive Test Duration:	28 days
Study Method:	Static
Type of Concentrations:	Nominal

8. CONCLUSIONS:

The 28-day chronic toxicity of JAU6476 (Prothioconazole) to the midge, *Chironomus riparius*, was studied under static conditions in water-spiked exposures. Endpoints that were assessed by the study author and verified by the reviewer included total emergence, development time (days to emergence), and development rate (all endpoints assessed with combined sexes because the number of male and female organisms were not controlled at test initiation, so it was not appropriate to analyze emergence separately by sex). Survival (actual larval survival) and ash-free dry weights were not assessed in this study.

The nominal test concentrations were 0 (negative and solvent controls), 1.14, 2.29, 4.57, 9.14, 18.3, 36.6, and 57.1 ppm a.i. Mean-measured concentrations were not determined for all treatment levels. In overlying water at the nominal 1.14, 9.14, and 57.1 ppm a.i. treatment levels, measured concentrations were 216.67, 101.71, and 9.84% of nominal levels on Day 0, 1.05, 10.08, 17.17% on Day 7, and <LOQ (<0.011 ppm a.i.), 0.25%, and 1.94% on Day 28, respectively. In pore water at 1.14, 9.14, and 57.1 ppm a.i., measured concentrations were 0.17, 0.33, and 0.03% of nominal levels on Day 0, <LOQ, 0.17%, and 0.20% on Day 7, and <LOQ, 0.08%, and 0.09% on Day 28, respectively. Given the large drop in measured concentrations, the geometric mean was used for toxicity endpoints. For the nominal 1.14, 9.14, and 57.1 ppm a.i. treatment levels the geometric mean concentrations were 0.017, 0.308, and 4.04 ppm a.i. and 0.007, 0.0162, and 0.985 ppm a.i. for the overlying water and pore water, respectively. The NOAEC is 4.04 and 0.985 ppm a.i. for the overlying water and pore water, respectively.

This study was designed to fulfill proposed OECD Draft Guideline 219 (February 2000) and does not fulfill any current U.S. EPA guideline. There were no treatment-related effects at any of the nominal overlying water treatment concentrations by 28-days. However, it is difficult to determine actual exposure concentrations since concentrations dropped significantly through the course of the experiment. In addition, JAU 6476 (technical) was added to overlying water, not sediment. This study is scientifically sound but does not fulfill EPA requirements for a sediment toxicity test and is therefore classified as SUPPLEMENTAL.

Results Synopsis:

Based on Geometric Mean Concentrations in the Porewater
Total Emergence, Development Time (Date to emergence), Development Rate
NOAEC: 0.985 ppm a.i. (4.04 ppm a.i. in overlying water)
LOAEC: > 0.985 ppm a.i. (>4.04 ppm a.i. in overlying water)
EC_{50}: > 0.985 ppm a.i. (>4.04 ppm a.i. in overlying water)

Endpoints affected: None

9. ADEQUACY OF THE STUDY:

A. Classification: SUPPLEMENTAL

B. Rationale: This study was not designed to fulfill any current U.S. EPA guideline.

C. Repairability: N/A

10. GUIDELINE DEVIATIONS:

The following sources were used as guidance in evaluating this study, and deviations from these guidance documents are listed below:

OECD 2000. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental, Health and Safety Publications, Series on Testing and Assessment No. 23.

OECD 2004. Test Guideline 219. Sediment-Water Chironomid Toxicity Test Using Spiked Water.

U.S. EPA. 1996. Ecological Effects Test Guidelines, OPPTS 850.1735 (Public Draft), EPA-712-C-96-354. April 1996.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates. Office of Research and Development and Office of Water, Washington, D.C. EPA/600/R-99/064. March 2000.

1. The study was initiated with <2-3-day old larvae (first instar), whereas 10-day old larvae (second to third instar) are recommended.

2. Pre-test mortality of the larvae were not reported.

3. Initial measurements of length and weight should have been provided for a sub-set, and terminal ash-free dry weights should have been determined at study termination.

4. The water temperature of 19.5-20.0°C was slightly lower than the recommended 22-24°C.

5. The dissolved oxygen content was not provided in terms of percent saturation.

6. Sediments were not analyzed for total volatile sulfides, which is a required analysis. In addition, sediments were not analyzed for BOD, COD, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, oil and grease, and petroleum hydrocarbons; these analyses are suggested in the guidance documents.

7. The test chemical was mixed into stock solutions and added to the overlying water instead of the sediment as recommended.

8. The test vessels were covered by clear plastic plates instead of glass covers as recommended for static tests.

9. The overlying water was not renewed during testing.

10. Only three replicate vessels were used to collect biological data, instead of the eight recommended.

11. Concentrations of Jau6476 (tech.) in the sediment were not assessed. Furthermore, overlying and pore water concentrations were not analyzed at every nominal level.

11. <u>SUBMISSION PURPOSE</u>: This study was submitted to provide information on the toxicity of JAU6476 (Prothioconazole) to sediment-dwelling chironomids for the purpose of pesticide registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Chironomus tentans *Other species which can be used are Hyalella azteca, Chironomus riparius, Daphnia sp., Ceriodaphnia sp.* (Specific criteria for these species are not listed in this report)	*Chironomus riparius*
Life Stage Second to third instar larvae (about 10 d old larvae with at least 50% at third instar.	1st instar, <2-3 days old.
Supplier Brood stock can be obtained from laboratory, commercial, or government sources.(Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	In house culture maintained since 1991. Originally obtained in the 1960's from a water butt in the back garden of a house in Nottingham, UK.
All organisms from the same source?	Yes.

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Brood stock must be acclimated to culture water gradually from transport water to 100% culture water; water temperature exchange rate not to exceed 2°C within 24 hr; Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	Continuous breeding cultures were maintained in gently-aerated reconstituted M7 medium at a temperature of 20±2 °C. The larvae used in this study were removed from a 21- to 28-day old synchronous culture.

Guideline Criteria	Reported Information
Feeding Feeding should begin on day 0 and continue through day 9 unless food is not being eaten.	During rearing, midges were fed with green algae and a suspension of Tetra Phyll®.
Pretest Mortality A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	Not reported.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (Overlying water) and sediment Soft reconstituted water or water from a natural source, **not** de-chlorinated tap water. [Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details)].	Overlying water was from the same source as the culture water (reconstituted M-7 medium prepared with deionized water, mineral salts, and vitamins; Table 1, p. 8). The sediment was prepared in the laboratory by combining 74% fine quartz sand, 5% dried, finely-ground sphagnum peat, 20% kaolin, and 1% calcium carbonate.
Does water support test animals without observable signs of stress?	Midges have successfully survived and reproduced over several generations in the dilution water.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	No problems were observed.

Guideline Criteria	Reported Information
Water Temperature 23°C ± 1°C. Daily mean test temperature Must not deviate more than ±1°C and instantaneous temperature must be within ±. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	Test water temperature was maintained at 19.5-20.0°C, and was measured on days -1, 6, 13, 21, and 28 from one replicate of the negative control and on days -1 and 28 in one replicate from each treatment group (Table 2, p. 15).
pH Not specified, but should be appropriate to the test species and should not deviate more than 0.4 pH units.	8.0-8.7, with increasing levels with increasing time duration; measured on days -1, 6, 13, 21, and 28 from one replicate of the negative control and on days -1 and 28 in one replicate from each treatment group (Table 2, p. 15).
Dissolved Oxygen Should be measured at the beginning and end of short term tests. DO should be >40 percent and <100 percent saturation.	DO ranged from 7.1-8.5mg/L, and was measured on Days -1, 6, 13, 21, and 28 from one replicate of the negative control and on days -1 and 28 in one replicate from each treatment group (Table 2, p. 15).
Total Hardness Prefer 40 - 200 mg/L as $CaCO_3$.	195.8 mg/L $CaCO_3$; measured in the dilution water prior to introduction into the test vessels.
Conductivity Not specified, but should be amenable to the test species.	597 µS/cm.
Sediment Characterization All sediment must be characterized for: pH, organic carbon content (TOC), total volatile sulfides, particle size distribution (% sand, silt, clay), and percent water content.	pH: 6.4 (Table 4, p. 16) TOC: 2.4% Total volatile sulfides: Not reported Particle size distribution: 77.3% sand, 9.7% silt, 13.0% clay Water holding capacity: 43.1 g water/100 g dry weight sediment

Guideline Criteria	Reported Information
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Cation Exchange Capacity: 9.0 meq/100 g sediment
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	The test substance, JAU6476 (tech) (Prothioconazole; Batch no. Fl. 6233/0031) was adequately characterized. Description: white crystalline Purity = 98.6%
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	The test material (1014.5 mg) was dissolved in dimethylformamide (DMF, 1.0 mL) to obtain the pre-stock solution. Negative (dilution water) and solvent (DMF, 0.10 mL/L) controls were used in the test.
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	Not applicable, as the sediment was not spiked. Test concentrations for the overlying water-spike were selected in order to define the EC_{15} (p. 9). Applications were made to the overlying water, not the sediment. Aliquots of the stock solution were applied just below the water surface, and the dilution water was gently mixed.

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100ml of sediment and 175 ml of overlying water.	1. Glass beakers 2. 0.6 L (diam. 8 cm) containing a 1.5-cm layer of sediment and 6.0 cm of overlying water. The volume of water was ~0.38 L.
Covers Static: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Test vessels covered by clear plastic plates.
Type of Dilution System Must provide reproducible supply of toxicant.	N/A - Static system.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A - Static system.
Aeration Dilution water should be vigorously aerated so that dissolved oxygen in the overlying water remains above 40% saturation. In static systems, overlying water may be gently aerated through a 1-mL pipet located not closer than 2 cm from the sediment surface; Test organisms should not added 12 to 24h; Water quality characteristics should be measured before test organisms are added.	Gentle aeration was provided through glass Pasteur pipette 2.5 cm above the sediment layer during testing.

Guideline Criteria	Reported Information
Photoperiod 16 hours light, 8 hours dark with a 15-30 min transition period and illuminance of about 100 to 1000 lux.	16 hours light, 8 hours dark with 30 minutes of dawn and dusk within an environmental chamber. Light intensity averaged 1500 lux.
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Dimethylformamide, 0.10 mL/L

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be throughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment	Test containers were prepared with sediment and overlying water 7 days prior to treatment (p. 9). The sediment was covered by a sheet, and the test water poured slowly into the beaker; the sheet was removed carefully thereafter.
Renewal of Overlying Water: Renewal is required and flow rates should not differ by more than 10% in any two test chambers and should begin on day -1.	None performed.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	On Day -1, the larvae were carefully allocated to the test vessels using a blunt pipette.
Range Finding Test	None described.
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels observed at least three times per week for behavior differences between test and control organisms. The sex, time and number of emerged adults were recorded daily during the emergence period.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 1.14, 2.29, 4.57, 9.14, 18.3, 36.6, and 57.1 ppm a.i..
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	For biological testing 20 larvae/replicate; 3 replicates/treatment and control. For analytical purposes parallel replicates were prepared for the nominal 1.14, 9.14, and 57.1 ppm a.i. treatment groups (the only treatment levels assessed analytically); additionally 1 replicate/control and 2 replicates/level for the nominal 1.14, 9.14, and 57.1 ppm a.i. treatment groups were prepared (pp. 9-10).
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding Midges in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin® suspension daily. A drop in d.o. level below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until d.o. levels increase.	During exposure, midges were fed a Tetra Phyll® suspension (1g/20 mL of water) at the rate of 0.5 mg Tetra Phyll/larvae/day for the first 10 days and 1.0 mg Tetra Phyll/larvae/day until test termination. Food was added to test vessels on Days -1, 0, 2, 3, 6, 7, 8, 10, 13-17, 20-24, and 27.

Guideline Criteria	Reported Information
Water Parameter Measurements Overlying Water Quality should measure conductivity, hardness, pH, alkalinity, and ammonia in all treatments at beginning and end of a test and should not vary by more than 50% within a treatment during the test.	The pH was measured on days -1, 6, 13, 21, and 28 from one replicate of the negative control and on days -1 and 28 in one replicate from each treatment group (Table 2, p. 15). DO was measured on Days -1, 6, 13, 21, and 28 from one replicate of the negative control and on days -1 and 28 in one replicate from each treatment group (Table 2, p. 15). Water total hardness, alkalinity, conductivity, pH and dissolved oxygen were also measured in the overlying water on Day -6 (Table 3, p. 16).
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. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	One parallel replicate vessel was sampled from the nominal 1.14, 9.14, and 57.1 ppm a.i. treatment groups on days 0, 7, and 28; the concentrations of JAU6476 (tech) were determined in the overlying test water and the pore (interstitial) water. Sediment concentrations were not determined.

13. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes.

Guideline Criteria	Reported Information
Control Mortality Must be ≤ 30% in the sediment at end of the test.	Negative control: 5% mortality (3/60) Solvent control: 10% mortality (6/60) These values were reviewer-interpreted from emergence data (Table 11, p. 23). Mortality data were not reported.

Guideline Criteria	Reported Information
Percent Recovery of Chemical: 1) % of nominal;	1. In overlying water at day 0: nominal 1.14 ppm a.i. = 217% 9.14 ppm a.i. = 102% 57.14 ppm a.i. = 9.84% at day 7: 1.14 ppm a.i. = 1.05% 9.14 ppm a.i. = 10.08% 57.14 ppm a.i. = 17.17% at day 28: 1.14 ppm a.i. = <LOQ; 0% 9.14 ppm a.i. = 0.25% 57.14 ppm a.i. = 1.94% (Table 21, p. 32). In pore water at day 0: nominal 1.14 ppm a.i. = 0.17% 9.14 ppm a.i. = 0.33% 57.14 ppm a.i. = 0.03% at day 7: 1.14 ppm a.i. = <LOQ; 0% 9.14 ppm a.i. = 0.17% 57.14 ppm a.i. = 0.20% at day 28: 1.14 ppm a.i. = <LOQ; 0% 9.14 ppm a.i. = 0.08% 57.14 ppm a.i. = 0.09% (Table 21, p. 32).
2) Procedural recovery;	2. Although method validation (procedural recovery) samples were run concurrently with the sample analysis, results were not converted from area counts and therefore recoveries in terms of percent nominal were not provided (Table 3 of the Analytical Report, p. 45).
3) Limit of quantitation (LOQ)	3. LOQ = 0.011 ppm a.i.

Guideline Criteria	Reported Information
Data Endpoints - Survival of Larvae - Ash-free dry weight (AFDW) should be determined by pooling all living organisms from a replicate and drying to a constant weight (e.g. 60°C for 24 h)	- Number emerged per sex -Total number emerged - Emergence rate (combined and separate sexes) - Development rate (combined and separate sexes) -Development time (date emerged; combined sexes)
Raw data included?	Yes (Emergence rate and development rate for separate sexes replicate data were not provided)

Effects Data

Toxicant Concentration				Cumulative Number Dead	Mean Dry Weight per midge (mg)
Nominal (ppm a.i.)	Measured (Day 28)				
	Sediment (ppm a.i.)	Pore Water (ppm a.i.)	Overlying Water (ppm a.i.)		
Control	ND	<LOQ	<LOQ	3	ND
Solvent Control	ND	<LOQ	<LOQ	6	ND
1.14	ND	<LOQ	<LOQ	7	ND
2.29	ND	ND	ND	8	ND
4.57	ND	ND	ND	10	ND
9.14	ND	0.098	0.023	15	ND
18.3	ND	ND	ND	8	ND
36.6	ND	ND	ND	9	ND
57.1	ND	0.763	1.11	15	ND

ND - Not determined.
LOQ = 0.011 ppm a.i.

Nominal Concentrations (mg a.i./L)	No. of emerged midges[1]	Mean Emergence Rate (%)			Mean Development Time (days)	Mean Development Rate (1/days)
		Total	Male	Female		
Control	57	95.0	68.4	31.6	16.03 ± 0.58	0.062 ± 0.002
Solvent Control	54	90.0	48.1	51.9	16.85 ± 1.08	0.060 ± 0.004
1.14	53	88.3	34.0	6.0	17.17 ± 0.54	0.058 ± 0.002
2.29	52	86.7	53.8	46.2	16.06 ± 0.33	0.062 ± 0.001
4.57	50	83.3	34.0	66.0	16.95 ± 0.36	0.059 ± 0.001
9.14	45	75.0	48.9	51.1	16.30 ± 0.74	0.061 ± 0.003
18.3	52	86.7	48.1	51.9	16.76 ± 0.60	0.060 ± 0.002
36.6	51	85.0	27.5	72.5	16.81 ± 0.74	0.060 ± 0.003
57.1	45	75.0	60.0	40.0	16.17 ± 0.82	0.060 ± 0.003

[1] Observations were made from three replicates for each control group, and three replicates for each treatment group, with 20 animals/replicate (Tables 11-12, p. 23-24).

Other Significant Results:

The day of first emergence was not delayed at any test concentration (p. 16).

B. Statistical Results

Method: Endpoints assessed included the number of emerged midges per sex (sex ratio), date of emergence (development time), emergence rate (combined sexes; replicate data not provided), and development rate (combined sexes and separate). All endpoints were analyzed statistically to determine any possible treatment related effects. Calculations were performed using "Easy Assay" statistical software; individual test vessels were considered as replicates, and nominal concentrations were used for all calculations.

The number of emerged midges per sex were evaluated using the chi-square test to determine statistical differences between the negative control and the remaining groups. Statistical significance was observed only in single replicates each of the negative control, nominal 1.14, and 36.6 ppm a.i. treatment groups, and these differences were not considered biologically significant (p. 25). Emergence rate in the pooled control was compared to those of the treatment groups via the non-parametric Bonferroni-Holmes-test, which indicated no significant differences. However, due to the observed solubility issues (precipitate in treatment levels above 9.14 ppm a.i.) in the nominal 18.3 to 57.1 ppm a.i. test solutions and the fact that the 18.3 and 36.6 ppm a.i. treatment levels were not assessed analytically, the NOAEC for emergence rate was set at 9.14 ppm a.i. despite the fact that there were no statistically significant differences observed in those treatment levels >9.14 ppm a.i. (p. 27). The NOAEC for development rate (combined sexes and separate sexes) was also assessed for treatment related effects non-parametric Bonferroni-Holmes-test, which indicated no significant differences and the NOAEC was also set at 9.14 ppm a.i. due the above solubility issues. Development rate per sex (male and female separately) replicate data was not provided for statistical verification. No significant differences between the pooled control and treatment groups were reported for date of emergence (development time) were reported, however, the statistical test used to determine this conclusion was not reported. EC_x values could not be determined for any endpoint due to a lack of treatment related effects at any level tested.

Nominal Concentrations in the Overlying Water
LC_{50} (mortality): >9.14 ppm a.i.
EC_{50} (growth): Not assessed
EC_{50} (emergence rate, combined sexes): >9.14 ppm a.i.
95% C.I.: N/A Probit Slope: N/A
EC_{50} (development rate, combined and separate sexes): >9.14 ppm a.i.
95% C.I.: N/A Probit Slope: N/A

NOAEC: 9.14 ppm a.i.
LOAEC: >9.14 ppm a.i.
Endpoints affected: None

14. VERIFICATION OF STATISTICAL RESULTS:

Method: All statistical tests were conducted using the nominal overlying water concentrations, however, results were adjusted to incorporate available measured values when possible. The NOAEC and LOAEC values were determined for total emergence (the number of male and female organisms was not controlled at test initiation, so it was not appropriate to analyze emergence separately by sex). These data did not satisfy the assumptions of ANOVA, so the NOAEC and LOAEC were determined using the non-parametric Kruskal-Wallis test. The solvent control group was compared to the negative control group using a Student's t-test and, when no difference was found, the two groups were pooled for comparison to the treatment groups. NOAEC and LOAEC values were determined for mean development time (combined sexes) and for development rate (combined sexes). After confirming normality and homogeneity of variances, the pooled control data were compared to the treatment data for each endpoint using ANOVA and William's Multiple comparison tests. The above analyses were conducted using TOXSTAT statistical software and the nominal treatment concentrations. EC_x values could not be determined using the Probit method via Nuthatch statistical software due to a lack of $\geq$50% effects for any endpoint at any treatment level by 28 days. Emergence rate replicate data were not provided, consequently this endpoint could not be statistically verified by the reviewer.

Nominal Concentrations in the Overlying Water:

PARAMETER	RESULT
Binomial Test: LC_{50} mortality (95% C.I.) EC_{50} growth (95% C.I.) EC_{50} total emergence (95% C.I.) EC_{50} development rate (95% C.I.)	N/A
Moving Average Angle Test: LC_{50} mortality (95% C.I.) EC_{50} growth (95% C.I.) EC_{50} total emergence (95% C.I.) EC_{50} development rate (95% C.I.)	N/A
Probit Test: LC_{50} mortality (95% C.I.) EC_{50} growth (95% C.I.) EC_{50} total emergence (95% C.I.) EC_{50} development time (95% C.I.) EC_{50} development rate (95% C.I.)	 Assessed as total number emerged Not assessed >57.1 ppm a.i. >57.1 ppm a.i. >57.1 ppm a.i.
Probit Slope: Mortality Growth Total emergence Development rate	N/A
NOAEC: Mortality Growth Total number emerged Emergence Rate Development time (date emerged; combined sexes) Development rate (combined sexes)	 Assessed as total number emerged Not assessed 57.1 ppm a.i. Replicate data not provided for verification 57.1 ppm a.i. 57.1 ppm a.i.

Based on Geometric Mean Concentrations in the Porewater

Total Emergence, Development Time (Date to emergence), Development Rate

NOAEC: 0.985 ppm a.i. (4.04 ppm a.i. in overlying water)

LOAEC: > 0.985 ppm a.i. (>4.04 ppm a.i. in overlying water)

EC_{50}: > 0.985 ppm a.i. (>4.04 ppm a.i. in overlying water)

15. REVIEWER'S COMMENTS:

This study was not designed to fulfill any current U.S. EPA FIFRA guideline.

This study was conducted in compliance with the Principles of GLP, Chemical Law (ChemG) of July 25, 1994, Annex 1 and OECD GLP of November 26, 1997 [C(97) 186/Final] and includes a Quality Assurance Statement.

The study author noted (p. 34) that treatment concentrations of JAU6476 in the test solution overlying and pore water were analytically determined three times during the 28-day exposure period (days 0, 7, and 28) at the nominal 1.14, 9.14, and 57.1 ppm a.i. treatment levels. Analysis of the stock solutions on day-0 indicated 95.9 to 101.0% of nominal recoveries. However, the study author noted that undissolved test material (precipitate) was present in the 18.3, 36.6, and 57.1 ppm a.i. throughout the duration of the exposure period due to the low water solubility of JAU6476 (7.0 ppm a.i.). Analysis of the highest treatment concentration (57.1 ppm a.i.) in the overlying water approximately one hour after it was introduced into the test vessels indicated a 9.84% of nominal recovery (Table 25, p. 36). For unknown reasons, the analysis of the lowest test concentration approx. one hour after introduction into the test system indicated a 216.67% of nominal recovery (p. 34). Analysis of the nominal 9.14 ppm a.i. treatment level, also after approx. one hour indicated 101.71% of nominal recovery. By 7-days, analytical recoveries were 1.05, 10.08, and 17.17% of the nominal for the 1.14, 9.14, and 57.14 ppm a.i. treatment concentrations, respectively. By 28-days, analytical recoveries were <LOQ, 0.25%, and 1.94% of the nominal 1.14, 9.14, and 57.14 ppm a.i. treatment concentrations, respectively. Results from analysis of the pore water at 7- and 28-days also indicated very low recoveries. By 7-days, analytical recoveries from the pore water were <LOQ, 0.17%, and 0.20% of the nominal 1.14, 9.14, and 57.14 ppm a.i. treatment concentrations, respectively (Table 25, p. 36). By 28-days, analytical recoveries were <LOQ, 0.08%, and 0.09% of nominal for the 1.14, 9.14, and 57.14 ppm a.i. treatment levels, respectively. These results suggest that JAU6476 degraded continuously during the study, and only a small portion absorbed to the sediment as indicated by the pore water analyses. The reviewer only partially agrees with this conclusion because it is unclear if the test material absorbed to the actual sediment, which was not directly analyzed for test material at any time during the study.

The results of the reviewer's statistical verification were identical to those of the study author, no treatment related effects were identified for total emergence, development time (time to emergence), and development rate. The reviewer was unable to statistically verify

the toxicity values determined for emergence rate because the replicate data were not provided. Since overlying and pore water concentrations were not analyzed at every nominal level during the study (see comment below), nominal concentrations (overlying water) were used in analyses. However, results were adjusted to account for measured treatment levels. The reviewer determined NOAEC was 0.985 ppm a.i. in porewater, which corresponds to an overlying water concentration of 4.04 ppm a.i.

Given the uncertainties associated with exposure concentrations and the fact that the chemical was added to overlying water and not mixed in sediment, this study is classified as SUPPLEMENTAL and has limited value for future risk assessments.

Based on Geometric Mean Concentrations in the Overlying Water
Total Emergence, Development Time (Date to emergence), Development Rate
NOAEC: 0.985 ppm a.i. (4.04 ppm a.i. in overlying water)
LOAEC: > 0.985 ppm a.i. (>4.04 ppm a.i. in overlying water)
EC_{50}: > 0.985 ppm a.i. (>4.04 ppm a.i. in overlying water)

16. REFERENCES:

OECD 2000. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental, Health and Safety Publications, Series on Testing and Assessment No. 23.

OECD 2004. Test Guideline 219. Sediment-Water Chironomid Toxicity Test Using Spiked Water.

U.S. EPA. 1996. Ecological Effects Test Guidelines, OPPTS 850.1735 (Public Draft), EPA-712-C-96-354. April 1996.

U.S. EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates. Office of Research and Development and Office of Water, Washington, D.C. EPA/600/R-99/064. March 2000.

17. RESULTS OF REVIEWER'S STATISTICAL VERIFICATION:

6131 Total number emerged (M + F)
File: 6131tes 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	18.500	18.500	118.000
2	1.14	17.667	17.667	49.000
3	2.29	17.333	17.333	45.000
4	4.57	16.667	16.667	36.000
5	9.14	15.000	15.000	24.500
6	18.3	17.333	17.333	48.500
7	36.6	17.000	17.000	40.500
8	**57.1**	**15.000**	**15.000**	**16.500**

Calculated H Value = 9.194 Critical H Value Table = 14.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

6131 Total number emerged (M + F)
File: 6131tes 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 0 0 5 8 4 7 3 6 2 1
5	9.14	15.000	15.000	\
8	**57.1**	**15.000**	**15.000**	. \
4	4.57	16.667	16.667	. . \
7	36.6	17.000	17.000	. . . \
3	2.29	17.333	17.333	. . . . \
6	18.3	17.333	17.333	. . . . . \
2	1.14	17.667	17.667	. . . . . \
1	GRPS 1&2 POOLED	18.500	18.500	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,8) = 3.124 Unequal reps - multiple SE values

6131 Mean development time (date emerged; combined sexes)
File: 6131dtd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	3.376	0.482	0.982
Within (Error)	19	9.328	0.491	
Total	26	12.703		

Critical F value = 2.54 (0.05,7,19)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

6131 Mean development time (date emerged)
File: 6131dtd 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	16.443	16.443		
2	1.14	17.170	17.170	-1.467	
3	2.29	16.060	16.060	0.774	
4	4.57	16.950	16.950	-1.023	
5	9.14	16.303	16.303	0.283	
6	18.3	16.763	16.763	-0.646	
7	36.6	16.810	16.810	-0.740	
8	57.1	16.167	16.167	0.558	

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

6131 Mean development time (date emerged)
File: 6131dtd 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	6			
2	1.14	3	1.336	8.1	-0.727
3	2.29	3	1.336	8.1	0.383
4	4.57	3	1.336	8.1	-0.507
5	9.14	3	1.336	8.1	0.140
6	18.3	3	1.336	8.1	-0.320
7	36.6	3	1.336	8.1	-0.367
8	57.1	3	1.336	8.1	0.277

6131 Mean development time (date emerged)
File: 6131dtd 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	6	16.443	16.443	16.686
2	1.14	3	17.170	17.170	16.686
3	2.29	3	16.060	16.060	16.577
4	4.57	3	16.950	16.950	16.577

5	9.14	3	16.303	16.303	16.577
6	18.3	3	16.763	16.763	16.577
7	36.6	3	16.810	16.810	16.577
8	**57.1**	**3**	**16.167**	**16.167**	**16.167**

6131 Mean development time (date emerged)
File: 6131dtd 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	16.686				
1.14	16.686	0.489		1.73	k= 1, v=19
2.29	16.577	0.270		1.81	k= 2, v=19
4.57	16.577	0.270		1.84	k= 3, v=19
9.14	16.577	0.270		1.85	k= 4, v=19
18.3	16.577	0.270		1.86	k= 5, v=19
36.6	16.577	0.270		1.87	k= 6, v=19
57.1	**16.167**	**0.558**		**1.87**	**k= 7, v=19**

s = 0.701
Note: df used for table values are approximate when v > 20.

6131 mean development rate (combined sexes)
File: 6131drd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	0.0057	0.0008	1.143
Within (Error)	19	0.0137	0.0007	
Total	26	0.0195		

Critical F value = 2.54 (0.05,7,19)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

6131 mean development rate
File: 6131drd 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	0.610	0.610		
2	1.14	0.580	0.580	1.604	
3	2.29	0.623	0.623	-0.713	

4	4.57	0.590	0.590	1.069
5	9.14	0.617	0.617	-0.356
6	18.3	0.597	0.597	0.713
7	36.6	0.593	0.593	0.891
8	57.1	0.623	0.623	-0.713

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

6131 mean development rate
File: 6131drd 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	6			
2	1.14	3	0.050	8.3	0.030
3	2.29	3	0.050	8.3	-0.013
4	4.57	3	0.050	8.3	0.020
5	9.14	3	0.050	8.3	-0.007
6	18.3	3	0.050	8.3	0.013
7	36.6	3	0.050	8.3	0.017
8	57.1	3	0.050	8.3	-0.013

6131 mean development rate
File: 6131drd 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	6	0.610	0.610	0.600
2	1.14	3	0.580	0.580	0.600
3	2.29	3	0.623	0.623	0.604
4	4.57	3	0.590	0.590	0.604
5	9.14	3	0.617	0.617	0.604
6	18.3	3	0.597	0.597	0.604
7	36.6	3	0.593	0.593	0.604
8	**57.1**	**3**	**0.623**	**0.623**	**0.623**

6131 mean development rate
File: 6131drd 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	0.600				
1.14	0.600	0.527		1.73	k= 1, v=19

2.29	0.604	0.316	1.81	k= 2, v=19
4.57	0.604	0.316	1.84	k= 3, v=19
9.14	0.604	0.316	1.85	k= 4, v=19
18.3	0.604	0.316	1.86	k= 5, v=19
36.6	0.604	0.316	1.87	k= 6, v=19
57.1	**0.623**	**0.703**	**1.87**	**k= 7, v=19**

s = 0.027
Note: df used for table values are approximate when v > 20.

Data Evaluation Report on the Toxicity of JAU6476 Technical (Prothioconazole) to the Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System
PMRA Submission Number 2004-0843 EPA MRID Number 46246131

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: October 19, 2005

PMRA Submission Number: 2004-0843

Study Type: Laboratory Studies with Other Species

Hendel, B. 2000. Influence of JAU6476 (tech.) on Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. HDB/Ch 42. September 14, 2000.

PMRA DATA CODE: 9.3.4
EPA DP Barcode: D303488
OECD Data Point: IIA 8.5
EPA MRID: 46246131
EPA Guideline: n/a

Company Code: BCZ
Active Code: PRB
Use Site Category: 7, 13, 14
EPA PC Code: 113961

Reviewing Agency: US EPA

The 28-day chronic toxicity of JAU6476 (Prothioconazole) to the midge, *Chironomus riparius*, was studied under static conditions in water-spiked exposures. This study was conducted following the proposal BBA (1995) and proposal for a new OECD Guideline 219 (02/2000), and was in compliance with German and OECD Principles of Good Laboratory Practice. The nominal test concentrations were 0 (negative and solvent controls), 1.14, 2.29, 4.57, 9.14, 18.3, 36.6, and 57.1 mg a.i./L. Mean measured concentrations were not determined for all treatment levels. In overlying water at the nominal 1.14, 9.14, and 57.1 mg a.i./L treatment levels, measured concentrations were 216.67, 101.71, and 9.84% of nominal levels on Day 0, 1.05, 10.08, 17.17% on Day 7, and <LOQ (<0.011 mg a.i./L), 0.25%, and 1.94% on Day 28,

respectively. In pore water at 1.14, 9.14, and 57.1 mg a.i./L, measured concentrations were 0.17, 0.33, and 0.03% of nominal levels on Day 0, <LOQ, 0.17%, and 0.20% on Day 7, and <LOQ, 0.08%, and 0.09% on Day 28, respectively. These measured concentrations suggest that prothioconazole transformed continuously during the study and was adsorbing to the sediment (as supported by results of an aerobic aquatic biotransformation study). Given the large drop in measured concentrations, the geometric mean was used for toxicity endpoints. For the nominal 1.14, 9.14, and 57.1 mg a.i./L treatment levels the geometric mean concentrations were 0.017, 0.308, and 4.04 mg a.i./L and 0.007, 0.0162, and 0.985 mg a.i./L for the overlying water and pore water, respectively.

Endpoints that were assessed by the study author and verified by the reviewers included total emergence, development time (days to emergence), and development rate (all endpoints assessed with combined sexes because the number of male and female organisms were not controlled at test initiation, so it was not appropriate to analyze emergence separately by sex). Survival (actual larval survival) and ash-free dry weights were not assessed in this study. There were no treatment-related effects at any of the treatment concentrations by the end of the 28-days study. The NOEC, LOEC and EC_{50} based on the geometric mean measured overlying water concentrations were set at 4.04, >4.04 and >4.04 mg a.i./L, respectively, and were 0.985, >0.985 and >0.985 mg a.i./L, respectively when based on mean measured pore water concentrations.

This study was designed to fulfill proposed OECD Draft Guideline 219 (February 2000) and does not fulfill any current U.S. EPA guideline. There were no treatment-related effects at any of the nominal overlying water treatment concentrations by 28-days. However, it is difficult to determine actual exposure concentrations since concentrations dropped significantly through the course of the experiment. In addition, JAU 6476 (technical) was added to overlying water, not sediment. This study is scientifically sound and is considered acceptable to the PMRA eventhough it does not fulfill US EPA requirements for a sediment toxicity test and is therefore classified as SUPPLEMENTAL by the US EPA.

Results Synopsis:

Based on Geometric Mean Concentrations in the Porewater
Total Emergence, Development Time (Date to emergence), Development Rate
NOEC: 0.985 mg a.i./L (4.04 mg a.i./L in overlying water)
LOEC: > 0.985 mg a.i./L (>4.04 mg a.i./L in overlying water)
EC_{50}: > 0.985 mg a.i./L (>4.04 mg a.i./L in overlying water)

Endpoints affected: None

Evaluator Comments:

1. The appropriate PMRA information (PMRA Submission Number, PMRA Data Code, PMRA company code, PMRA active ingredient code, PMRA use site category, OECD data point) did not appear in the EPA-DER but was added to the PMRA review portion of the DER. The PMRA Submission Number was added to the Header of the DER. Information on the chemical name (IUPAC name, CAS name and synonym) available from the PMRA Chemistry review was added at the beginning of the DER. The name of the EAD secondary reviewer was added to the front portion of the DER and the sections were renumbered to account for the addition.

2. Emergence in the controls occurred between days 14 and 22, and was between days 14 and 23 in the other treatments.

3. It was not clear whether replicates used strictly for chemical analyses purposes contained organisms. According to OECD guideline 219, the presence of organisms is required in additional vessels used for analyses. This deviation is considered minor and does not affect the validity of the study.

4. The light intensity of 1500 lux was higher than the recommended 500-1000 lux. As control organisms did not seem affected, this deviation is minor and does not affect the validity of the study.

5. The EAD reviewer agrees with the results of the statistical analyses. Upon visual inspection of the data and the statistical results, the EAD reviewer feels the results are acceptable and that a verification of the statistical analyses was not warranted and would not have produced different conclusions.

6. The study was conducted according to OECD Guideline 219 as opposed to a US EPA guideline (non-existant for this study). As a result, many of the guideline deviations reported by the EPA reviewer are not deviations according to OECD Guideline 219, and are therefore acceptable to the PMRA. The EAD reviewer has the following comments on the deviations noted by the EPA reviewer:

- "The study was initiated with <2-3-day old larvae (first instar), whereas 10-day old larvae (second to third instar) are recommended."

EAD comment: According to OECD Guideline 219, the study requires the use of first instar larvae (par. 24, p.6). Therefore, the first instar larvae used in this study are acceptable to the PMRA.

- "Pre-test mortality of the larvae were not reported."

EAD comment: This is not a requirement of the OECD guideline. The guideline only states that first instar larvae of freshly laid egg masses should be used in the test (par. 24, p.6).

- "Initial measurements of length and weight should have been provided for a sub-set, and terminal ash-free dry weights should have been determined at study termination."

EAD comment: These measurements are not a requirement of the guideline. If data on 10-day larval and survival and growth are to be provided, additional vessels should be included at the state, so that they may be used subsequently. The ash-free dry weight of the surviving larvae per test vessel is determined and the mean individual dry weight per vessel calculated (par. 37, p. 8). The guideline does not require measurement of the dry weight of emerged midges.

- "The water temperature of 19.5-20.0°C was slightly lower than the recommended 22-24°C."

EAD comment: The temperature of 19.5-20.0°C is within the required temperature of 20±2°C according to OECD Guideline 219 (par. 33, p. 7).

- "The dissolved oxygen content was not provided in terms of percent saturation."

EAD comment: This deviation is considered to be minor. The dissolved oxygen content in the overlying water at the end of the experiment ranged from 7.1-8.1 mg/L. Assuming that the solubility of oxygen at 20.0°C is 9.1 mg/L (USGS National Field Manual (http://water.usgs.gov/owq/FieldManual/Chapter6/table6.2 6.html), the oxygen content ranged from 78-91%, which satisfies the validity criteria of >60% saturation at the end of the study required by OECD Guideline 219 (par. 10, p. 2).

- "Sediments were not analyzed for total volatile sulfides, which is a required analysis. In addition, sediments were not analyzed for BOD, COD, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, oil and grease, and petroleum hydrocarbons; these analyses are suggested in the guidance documents."

EAD comment: The study was conducted using formulated sediment as opposed to natural sediment. The sediment was formulated according to the specifications outlined in OECD Guideline 219. The above-mentioned analyses are not required by the Guideline.

- "The test chemical was mixed into stock solutions and added to the overlying water instead of the sediment as recommended."

EAD comment: OECD Guideline 219 requires spiking of overlying water to simulate a pesticide spray drift event and covers the inital peak of concentrations in pore water (par.2, p.1). The spiking of the water column is acceptable to the PMRA.

- "The test vessels were covered by clear plastic plates instead of glass covers as recommended for static tests."

EAD comment: This deviation is considered minor and does not affect the validity of the study, as the plate does not come into contact with the water.

- "The overlying water was not renewed during testing."

EAD comment: The OECD guideline requires a static system. The guideline states that: "Static are used. Semi-static or flow-through systems with intermittent or continuous renewal of overlying water might be used in exceptional cases as for instance if water quality specifications become inappropriate for the test organisms or affect chemical equilibrium (e.g. dissolved oxygen levels fall too low, the concentration of excretory products rises to high or minerals leach from sediment and affect pH and/or water hardness). However, other methods for ameliorating the quality of overlying water, such as aeration, will normally suffice and be preferable." (par. 30, p. 6). The static system is acceptable to the PMRA.

- "Only three replicate vessels were used to collect biological data, instead of the eight recommended."

EAD comment: The OECD guideline requires five concentrations with at least three replicate vessels per treatment for the determination of EC_x values and five concentrations with at least four replicate vessels per treatment if the LOEC/NOEC are to be estimated (par. 19 and 20, p. 5). As no effects were observed at any treatment level, this deviation is considered minor and does not affect the validity of the results.

- "Concentrations of JAU6476 (tech.) in the sediment were not assessed. Furthermore, overlying and pore water concentrations were not analyzed at every nominal level."

EAD comment: The OECD guideline states that: "As a minimum, samples of the overlying water, the pore water and the sediment must be analysed at the start (preferably one hour after application of test substance) and at the end of the test, at the highest concentration and a lower one. These determinations of test substance concentration inform on the behaviour/partitioning of the tested chemical in the water-sediment system. Sampling of sediment at the start of the test may influence the test system (e.g. removing test larvae), thus additional test vessels should be used to perform analytical determinations at the start and during the test if appropriate (see paragraph 39). Measurements in sediment might not be necessary if the partitioning of the test

substance between water and sediment has been clearly determined in a water/sediment study under comparable conditions (e.g. sediment to water ratio, type of application, organic carbon content of sediment)." (par. 38, p. 8)

An aerobic aquatic biotransformation study in two systems was submitted for prothioconazole (Brumhard and Oli, 2001. Report No MR-395/01; MRID 46246515) in which the fate and behaviour of prothioconazole was characterized. The type of application was the same as the chironomid study (spiking of the water) and the organic carbon content of the sediment in the two systems was 1.37-4.8%. The sediment to water ratio was 1:10 as opposed to the 1:4 ratio used in this study. This deviation is deemed minor and does not affect the validity of the study, as chemical bound to the sediment would not be bioavailable to the chironomids, as they do not ingest the sediment.

- EAD comment on declining water concentrations:

The decline in prothioconazole concentrations in the water over time is to be expected, as the chemical transforms and/or binds to the sediment. Similar results were observed in the aerobic aquatic biotransformation study submitted for prothioconazole (Brumhard and Oli, 2001. Report No MR-395/01; MRID 46246515). In the study, prothioconazole concentrations in water declined rapidly (via transformation and partitioning and binding to the sediment) and were approximately 18-43% of the applied radioactivity after 1 day, depending on the system. Concentrations of prothioconazole were less than 2% of the applied radioactivity in the water column after 29 days. Unextractable residues in the sediment increased throughout the study. Depending on the system, from 16 to > 45% of the applied radioactivity was unextractable (bound to sediment) after 29 days.

7. All the validity criteria for this study were met, according to OECD Guideline 219: emergence in controls were >70% at the end of the test; emergence occured between 12 and 23 days after insertion of larvae into the vessels; the pH in the overlying water in each vessel at the end of the test (range: 8.4-8.7) was within the required range of 6-9; the dissolved oxygen in each vessel was at least 60 percent of the air saturation value at the temperature used (range: 78-91% saturation, reviewer-calculated); and the water temperature did not vary by more than ±1°C (range:19.5-20°C).

8. The EAD reviewer agrees with the use of the geometric mean measured concentrations to estimate exposure.

Study Acceptability: This study was designed to fulfill proposed OECD Draft Guideline 219 (February 2000) and does not fulfill any current U.S. EPA guideline. There were no treatment-related effects at any of the nominal overlying water treatment concentrations by 28-days.

However, it is difficult to determine actual exposure concentrations since concentrations dropped significantly through the course of the experiment. In addition, JAU 6476 (technical) was added to overlying water, not sediment. This study is scientifically sound and is considered acceptable to the PMRA eventhough it does not fulfill US EPA requirements for a sediment toxicity test and is therefore classified as SUPPLEMENTAL by the US EPA.