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

1. **CHEMICAL:** JAU6476-desthio (Prothioconazole metabolite)

PC Code No.: 113961

2. **TEST MATERIAL:** SXX0665 (tech.) Purity: 97.6%

Common name: JAU6476-desthio
Chemical:
IUPAC name: 2-(1-chlorcyclopropyl)-1-(2-chlophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS name: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS No.: 120983-64-4
Synonyms: SXX0665

3. **CITATION:**

Author:	Hendel, B.
Title:	Influence of SXX0665 (tech.) on Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System
Study Completion Date:	October 19, 2000
Laboratory:	Bayer AG Crop Protection Business Group Crop Protection Development D-51368 Leverkusen, Germany
Sponsor:	Bayer CropScience 2 T.W. Alexander Drive RTP, NC 27709
Laboratory Report ID:	E 4161808-8
MRID No.:	46246132
DP Barcode:	D303488

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

Signature: **Date:** 8/27/04

APPROVED BY: Teri Myers, Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/1/04

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

Signature: **Date:**

6. **SECONDARY REVIEW BY:**

Émilie Larivière, EAD, PMRA, HC
Signature: **Date:** October 20, 2005 10-20-05

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 SXX 0665 (Prothioconazole metabolite) 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 were 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, 2, 4, 8, 16, and 32 ppm a.i. Mean-measured concentrations were not determined for each treatment level. In overlying water at the nominal 1, 8, and 32 ppm a.i. treatment levels, measured concentrations were 108, 93.3, and 40.3% of nominal levels on day 0, 35.9, 37.4, and 32.8% on day 7, and 7.3, 14.8, and 19.9% on day 28, respectively. The controls were noted to be free of test material <0.022 (<LOQ) on day 0 but were not analytically verified on days 7 and 28. In pore water at the nominal 1, 8, and 32 ppm a.i. treatment levels, measured concentrations were 0.26, 0.44, and 0.54% of nominal levels on day 0, 1.45, 1.27, and 1.37% on day 7, and 0.42, 0.74, and 0.86% on day 28, respectively. The geometric means corresponding to the nominal 1, 8, and 32 ppm a.i. treatment levels were 0.23, 2.37, and 8.93 ppm a.i. in overlying water, respectively and 0.099, 1.01, and 4.98 ppm a.i. in pore water, respectively.

There were significant effects on the total number of emerged chironomids, the mean development time, and the mean development rate at the nominal 8 ppm a.i. treatment level and higher. The corresponding geometric mean value for the nominal 8 ppm a.i. is 2.37 and 1.01 ppm a.i. for overlying water and porewater, respectively. The NOAEC for these endpoints lies between the geometric mean-measured values of 0.23 and 2.37 ppm a.i. for overlying water and 0.099 and 1.01 ppm a.i. for porewater. The EC50 for effects could not be determined due to incomplete analysis of treatment levels but it falls between the porewater geometric mean-measured 1.01 and 4.98 ppm a.i. treatment levels.

This study was designed to fulfill proposed OECD Draft Guideline 219 (February 2000), and does not fulfill any current U.S. EPA guideline. Due to the fact that some treatment concentrations were not analytically verified at test initiation and termination, the obvious instability of the test material under test conditions, and the observed treatment related effects at nominal treatment concentrations >4 ppm a.i. this study is classified as **SUPPLEMENTAL** because it is unclear what test material concentrations chironomid larvae were actually exposed to.

Results Synopsis:

Based on Nominal Concentrations in the Overlying Water
Total Emergence, Development Time (Date to emergence), Development Rate
NOAEC: between geometric mean-measured 0.099 and < 1.01 ppm a.i. in porewater (between geometric mean-measured 0.23 and < 2.37 ppm a.i. in overlying water)
LOAEC: geometric mean-measured 1.01 ppm a.i. in porewater (geometric mean-measured ≥ 2.37 ppm a.i. in overlying water)
EC_{50}: between geometric mean-measured 1.01 and 4.98 ppm a.i.
95% C.I.: N/A
Slope: N/A

Endpoints affected: Total emergence, development time, development rate

9. ADEQUACY OF THE STUDY:

A. Classification: SUPPLEMENTAL

B. Rationale: Not all treatment concentrations were analytically verified at test initiation and termination, the test material was unstable under the test conditions, and toxicity related effects were observed at treatment levels that were not analytically verified (Invalid). This study was not designed to fulfill any current U.S. EPA guideline.

C. Repairability: None, all treatment levels should have been analytically verified at test initiation and termination for all treatment levels regardless of the fact that toxicity related effects were observed so that mean-measured treatment concentrations may be determined and used for the determination of all toxicity values. This information is necessary to determine the actual test material concentrations that chironomids were exposed to over the duration of the test.

10. GUIDELINE DEVIATIONS:

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

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 19.9-20.3°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 eight as recommended.

11. Concentrations of SXX0665 (tech.) in the sediment were not assessed. Furthermore, overlying and pore water concentrations were not analyzed at every nominal level.

11. SUBMISSION PURPOSE: This study was submitted to provide information on the toxicity of SXX 0665 (Prothioconazole metabolite) 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 19.9-20.3°C, and was measured on days -1, 6, 13, 20, 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.	8.2-8.6, 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 7.2-9.1 mg/L, and was measured on Days -1, 6, 13, 20, 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.	591 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: 6.6 (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: 49.9 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, SXX0665 (tech.; a.i. JAU 6476-Desthio) was adequately characterized (Batch no. RUX76-105/9). Description: white crystals Purity = 97.6%
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 (656 mg) was dissolved in dimethylformamide (DMF, 2.0 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. 9.5 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 1700 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 (DMF), 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 6 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, 2, 4, 8, 16, and 32 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 1.0 mg Tetra Phyll/larvae/day. Food was added to test vessels on Days -1, 0, 1, 2, 3, 6, 7, 8, 9, 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, 20, 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, 20, 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 dilution 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, 8, and 32 ppm a.i. treatment groups. on days 0, 7, and 28; the concentrations of SXX0665 (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: 12% mortality (7/60) Solvent control: 2% mortality (1/60) These values were reviewer-interpreted from emergence data (Table 10, p. 22). Larval 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 ppm a.i. = 108% 8 ppm a.i. = 93.3% 32 ppm a .i. = 40.3% at day 7: 1 ppm a.i. = 35.9% 8 ppm a.i. = 37.4% 32 ppm a .i. = 32.8% at day 28: 1 ppm a.i. = 7.3% 8 ppm a.i. = 14.8% 32 ppm a .i. = 19.9% (Table 21, p. 32). In pore water at day 0: nominal 1 ppm a.i. = 0.26% 8 ppm a.i. = 0.44% 32 ppm a.i. = 0.54% at day 7: 1 ppm a.i. = 1.45; 0% 8 ppm a.i. = 1.27% 32 ppm a.i. = 1.37% at day 28: 1 ppm a.i. = 0.42% 8 ppm a.i. = 0.74% 32 ppm a.i. = 0.86% (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. 41).
3) Limit of quantitation (LOQ)	3. LOQ = 0.022 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 combined and separate sexes 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	7	ND
Solvent Control	ND	<LOQ	<LOQ	1	ND
1	ND	0.059	0.073	3	ND
2	ND	ND	ND	2	ND
4	ND	ND	ND	13	ND
8	ND	0.835	1.18	28	ND
16	ND	ND	ND	50	ND
32	ND	4.56	6.37	60	ND

ND - Not determined.
LOQ = 0.022 ppm a.i.
* Cumulative number dead represents those Chironomid larvae that did not emerge, based on data provided in Table 10, p. 22. Actual numbers of surviving and dead larvae were not reported in the study report.

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	53	88.3	52.8	47.2	16.19 ± 0.68	0.062 ± 0.003
Solvent Control	59	98.3	45.8	54.2	16.68 ± 1.17	0.060 ± 0.004
1	57	95.0	42.1	57.9	17.00 ± 0.29	0.059 ± 0.001
2	58	96.7	50.0	50.0	16.35 ± 0.53	0.061 ± 0.002
4	47	78.3	46.8	53.2	16.86 ± 0.48	0.059 ± 0.002
8	32	53.3	53.1	46.9	19.14 ± 1.57	0.052 ± 0.004
16	10	16.7	60.0	40.0	23.90[2]	0.042[2]
32	0	0	0	0	0	0

[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).

[2] Mean development time and rate results for the 16 ppm a.i. treatment group were based on larvae from one of the three replicates due to a lack of emergence in the other two replicates.

Other Significant Results:

The day of first emergence was delayed compared to the controls at the nominal 8 ppm a.i. treatment concentration by at least 2 days and at the 18 ppm a.i. level for 7 days (emergence only observed in one of the three replicates; p. 16). Emergence was completely inhibited at the 32 ppm a.i. treatment level.

B. Statistical Results

Method: Endpoints assessed included the number of emerged midges per sex (and combined; 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 pooled control and the remaining groups with observed emergence. Statistically significant reductions were not found for any treatment levels assessed (1, 2, 4, 8, and 16 ppm a.i.) although only one replicate from the 16 ppm a.i. treatment group was assessed and the 32 ppm a.i. treatment group was not assessed for differences due to the obvious effect (p. 24). Based on the provided statistical methods, it appears that probit analysis was used to compare all endpoints (emergence rate (pooled sexes)), development rate (pooled and separate sexes)) and that pairwise statistical comparisons were not used to determine NOAEC and LOAEC values for each endpoint (except number emerged per sex). Consequently, only EC_x values are reported for the four endpoints noted above.

Nominal Concentrations in the Overlying Water
LC_{50} (mortality): Not assessed
EC_{50} (growth): Not assessed
EC_{15} (emergence rate, combined sexes): 4.40 ppm a.i. 95% C.I.: 3.50-5.54 ppm a.i.
Probit Slope: 1.88
EC_{50} (emergence rate, combined sexes): 8.46 ppm a.i. 95% C.I.: 7.33-9.76 ppm a.i.
Probit Slope: 1.88
EC_{15} (development rate, combined sexes): 9.00 ppm a.i. 95% C.I.: 6.84-12.75 ppm a.i.
Probit Slope: 2.44
EC_{50} (development rate, combined sexes): 22.71 ppm a.i. 95% C.I.: 15.11-65.11 ppma.i.
Probit Slope: 2.44
EC_{15} (development rate, males): 9.22 ppm a.i. 95% C.I.: 6.76-13.69 ppm a.i.
Probit Slope: 2.26
EC_{50} (development rate, males): 21.47 ppm a.i. 95% C.I.: 14.24-74.83 ppma.i.
Probit Slope: 2.26
EC_{15} (development rate, females): 8.26 ppm a.i. 95% C.I.: 6.17-12.09 ppm a.i.
Probit Slope: 3.02
EC_{50} (development rate, females): 26.01 ppm a.i. 95% C.I.: 16.06-89.63 ppma.i.
Probit Slope: 3.02

NOAEC (total number emerged): 16 ppm a.i.
LOAEC: (total number emerged): 32 ppm a.i.
Endpoints affected: total number emerged, emergence rate (combined sexes), development rate (combined and separate sexes)

14. VERIFICATION OF STATISTICAL RESULTS:

Method: After confirming normality and homogeneity of variances, 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), development time (time to emergence; combined sexes), and development rate (combined sexes) using ANOVA and William's Multiple comparison tests. The solvent control group was compared to the negative control group using a Student's t-test for each endpoint and, when no difference was found, the two groups were pooled for comparison to the treatment groups. The above analyses were conducted using TOXSTAT statistical software and the nominal treatment concentrations. Effect levels were then adjusted to reflect available measured concentrations. The geometric mean was used according to OECD 2000. EC_{50} values could not be determined due to incomplete chemical analysis although a range was provided. Toxicity values could not be determined for emergence rate because replicate data were not provided.

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 8.6 (6.1 to 12) ppm a.i. >8 to <16 ppm a.i. 12 (9.2 to 16) ppm a.i.
Probit Slope: Mortality Growth Total emergence Development time Development rate	 Assessed as total number emerged Not assessed 3.40 N/A 6.13
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 4 ppm a.i. Replicate data not provided for verification 4 ppm a.i. 4 ppm a.i.

Results Based on Geometric Mean Concentrations in the Overlying Water

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

NOAEC: < 1.01 ppm a.i. in porewater (2.37 ppm a.i. in overlying water)

LOAEC: 1.01 ppm a.i. in porewater (> 2.37 ppm a.i. in overlying water)

EC_{50}: between geometric mean-measured 1.01 and 4.98 ppm a.i.

95% C.I.: N/A Slope: N/A

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. 30) that treatment concentrations of SXX 0665 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, 8, and 32 ppm a.i. treatment levels. Analysis of the stock solutions on day 0 indicated 88.9 to 94.0% of nominal recoveries. However, the study author noted that undissolved test material (precipitate) was present in the 16 and 32 ppm a.i. treatment groups at test initiation due to the low water solubility of SXX 0665 (≤51 ppm a.i.). At 1-hour (day 0), analytical recoveries from the overlying water were 108, 93.3 and 40.3% of the nominal 1, 8, and 32 ppm a.i. treatment concentrations, respectively. By day 7, analytical recoveries were 35.9, 37.4, and 32.8% of the nominal 1, 8, and 32 ppm a.i. treatment concentrations, respectively. By day 28, analytical recoveries were 7.3, 14.8, and 19.9% of the nominal 1, 8, and 32 ppm a.i. treatment concentrations, respectively. Results from analysis of the pore water at days 7 and 28 also indicated very low recoveries. By day 7, analytical recoveries from the pore water were 1.45, 1.27, and 1.37% of the nominal 1, 8, and 32 ppm a.i. treatment concentrations, respectively (Table 21, p. 32). By day 28, analytical recoveries were 0.42, 0.74, and 0.86% of nominal for the 1, 8, and 32 ppm a.i. treatment levels, respectively. These results suggest that SXX 0665 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.

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 conducting toxicity test although reported toxicity values were adjusted to reflect measured treatment levels. The study author did not statistically determine a NOAEC or LOAEC value for any endpoint in this study, with the exception of total number of emerged midges (combined sexes). EC_x values were determined and reported for emergence rate (combined sexes) and development rate (combined and separate sexes). The reviewer did not statistically verify any endpoint based on separate sexes because the number of male and female organisms was not controlled at test initiation. The reviewer determined toxicity values based on the total emergence (as an estimate of larval mortality)

and development time, these endpoints were not statistically assessed by the study author. An EC_{50} value for any parameter could not be generated since measured concentrations were not available for all treatment levels. Consequently, estimates of the EC50 could only be determined as between measured levels. The reviewer-determined NOAEC and LOAEC values for the total emergence (combined sexes) were two treatment levels lower than those of the study author, presumably due to the different statistical methods used.

Due to the fact that not all treatment concentrations were not analytically verified at test initiation and termination, the obvious instability of the test material under test conditions, and the observed treatment related effects at nominal treatment concentrations >4 ppm a.i. this study is classified as **SUPPLEMENTAL** because it is unclear what test material concentrations chironomid larvae were actually exposed to. Results from this study may have limited value for future risk assessments.

Results:

Based on Geometric Mean Concentrations in the Overlying Water
Total Emergence, Development Time (Date to emergence), Development Rate
NOAEC: < 1.01 ppm a.i. in porewater (2.37 ppm a.i. in overlying water)
LOAEC: 1.01 ppm a.i. in porewater (> 2.37 ppm a.i. in overlying water)
EC_{50}: between geometric mean-measured 1.01 and 4.98 ppm a.i.
95% C.I.: N/A Slope: N/A

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:

6132 Total Number Emerged (M + F)
File: 6132ted Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	650.952	130.190	15.021
Within (Error)	15	130.000	8.667	
Total	20	780.952		

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

6132 Total Number Emerged (M + F)
File: 6132ted 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	18.667	18.667		
2	1	19.000	19.000	-0.160	
3	2	19.333	19.333	-0.320	
4	4	15.667	15.667	1.441	
5	8	10.667	10.667	3.843	*
6	16	3.333	3.333	7.366	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

6132 Total Number Emerged (M + F)
File: 6132ted 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	3	5.419	29.0	-0.333
3	2	3	5.419	29.0	-0.667
4	4	3	5.419	29.0	3.000
5	8	3	5.419	29.0	8.000
6	16	3	5.419	29.0	15.333

6132 Total Number Emerged (M + F)
File: 6132ted 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	18.667	18.667	18.917
2	1	3	19.000	19.000	18.917
3	2	3	19.333	19.333	18.917
4	**4**	**3**	**15.667**	**15.667**	**15.667**
5	8	3	10.667	10.667	10.667
6	16	3	3.333	3.333	3.333

6132 Total Number Emerged (M + F)
File: 6132ted 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	18.917				
1	18.917	0.120		1.75	k= 1, v=15
2	18.917	0.120		1.84	k= 2, v=15
4	**15.667**	**1.441**		**1.87**	**k= 3, v=15**
8	10.667	3.843	*	1.88	k= 4, v=15
16	3.333	7.366	*	1.89	k= 5, v=15

s = 2.944
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	2.8	1.1	7.4	0.20	0.38
EC10	3.6	1.6	8.1	0.17	0.45
EC25	5.5	3.1	9.7	0.12	0.56
EC50	**8.6**	**6.1**	**12.**	**0.073**	**0.70**

Slope = 3.40 Std.Err. = 1.02

Goodness of fit: p = 0.98 based on DF= 3.0 15.

6132TED : 3132 Total Number Emerged (M + F)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	18.7	18.9	-0.207	100.	0.00
1.00	3.00	19.0	18.9	0.140	99.9	0.0742
2.00	3.00	19.3	18.6	0.753	98.4	1.56
4.00	3.00	15.7	16.4	-0.782	87.1	12.9
8.00	3.00	10.7	10.3	0.392	54.4	45.6

16.0	3.00	3.33	3.42	-0.0877	18.1	81.9

6132 Mean Development Time (days to emerge)
File: 6132dtd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	16.948	4.237	5.418
Within (Error)	13	10.170	0.782	
Total	17	27.118		

Critical F value = 3.18 (0.05,4,13)
Since F > Critical F REJECT Ho:All groups equal

6132 Mean Development Time (days to emerge)
File: 6132dtd 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	16.437	16.437		
2	1	17.000	17.000	-0.901	
3	2	16.350	16.350	0.139	
4	4	16.857	16.857	-0.672	
5	8	19.140	19.140	-4.323	

Bonferroni T table value = 2.53 (1 Tailed Value, P=0.05, df=13,4)

6132 Mean Development Time (days to emerge)
File: 6132dtd 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	3	1.584	9.6	-0.563
3	2	3	1.584	9.6	0.087
4	4	3	1.584	9.6	-0.420
5	8	3	1.584	9.6	-2.703

6132 Mean Development Time (days to emerge)

File: 6132dtd 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	16.437	16.437	16.437
2	1	3	17.000	17.000	16.675
3	2	3	16.350	16.350	16.675
4	**4**	**3**	**16.857**	**16.857**	**16.857**
5	8	3	19.140	19.140	19.140

6132 Mean Development Time (days to emerge)
File: 6132dtd 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	16.437				
1	16.675	0.381		1.77	k= 1, v=13
2	16.675	0.381		1.86	k= 2, v=13
4	**16.857**	**0.672**		**1.89**	**k= 3, v=13**
8	19.140	4.322	*	1.90	k= 4, v=13

s = 0.884
Note: df used for table values are approximate when v > 20.

6132 Mean Devlopment Rate (M + F)
File: 6132drd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	0.0170	0.0043	5.375
Within (Error)	13	0.0103	0.0008	
Total	17	0.0273		

Critical F value = 3.18 (0.05,4,13)
Since F > Critical F REJECT Ho:All groups equal

6132 Mean Devlopment Rate (M + F)
File: 6132drd 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	0.608	0.608		
2	1	0.590	0.590	0.917	
3	2	0.613	0.613	-0.250	
4	4	0.593	0.593	0.750	
5	8	0.523	0.523	4.250	*

Bonferroni T table value = 2.53 (1 Tailed Value, P=0.05, df=13,4)

6132 Mean Devlopment Rate (M + F)
File: 6132drd 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	3	0.051	8.3	0.018
3	2	3	0.051	8.3	-0.005
4	4	3	0.051	8.3	0.015
5	8	3	0.051	8.3	0.085

6132 Mean Devlopment Rate (M + F)
File: 6132drd 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	0.608	0.608	0.608
2	1	3	0.590	0.590	0.602
3	2	3	0.613	0.613	0.602
4	**4**	**3**	**0.593**	**0.593**	**0.593**
5	8	3	0.523	0.523	0.523

6132 Mean Devlopment Rate (M + F)
File: 6132drd 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	0.608				
1	0.602	0.335		1.77	k= 1, v=13
2	0.602	0.335		1.86	k= 2, v=13
4	**0.593**	**0.755**		**1.89**	**k= 3, v=13**

	8	0.523	4.277	*	1.90	k= 4, v=13

s = 0.028
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	6.6	3.0	14.	0.16	0.46
EC10	7.5	3.9	15.	0.14	0.52
EC25	9.4	5.9	15.	0.097	0.63
EC50	**12.**	**9.2**	**16.**	**0.058**	**0.76**

Slope = 6.13 Std.Err. = 2.63

Goodness of fit: p = 1.0 based on DF= 3.0 15.

6132RDE : 6132 Mean Devlopment Rate (M + F)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	0.608	0.603	0.00542	100.	0.00
1.00	3.00	0.590	0.603	-0.0129	100.	1.45e-09
2.00	3.00	0.613	0.603	0.0104	100.	7.71e-05
4.00	3.00	0.593	0.602	-0.00866	99.8	0.154
8.00	3.00	0.523	0.523	0.000351	86.7	13.3
16.0	3.00	0.140	0.140	-3.30e-05	23.2	76.8

Data Evaluation Report on the Toxicity of the transformation product JAU6476-desthio to the Development and Emergence of Larvae of *Chironomus riparius* in a Water-Sediment System
PMRA Submission Number 2004-0843 EPA MRID Number 46246132

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: October 20, 2005

PMRA Submission Number: 2004-0843

Study Type: Laboratory Studies with Other Species

Hendel, B. 2000. Influence of SXX 0665 (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 43. October 19, 2000.

PMRA DATA CODE: 9.3.4
EPA DP Barcode: D303488
OECD Data Point: IIA 8.5
EPA MRID: 46246132
EPA Guideline: n/a

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

Reviewing Agency: US EPA

EAD Executive Summary:

The 28-day chronic toxicity of the transformation product SXX 0665 (JAU6476-desthio; purity 97.6%) 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, 2, 4, 8, 16, and 32

ppm a.i. Mean-measured concentrations were not determined for each treatment level. In overlying water at the nominal 1, 8, and 32 ppm a.i. treatment levels, measured concentrations were 108, 93.3, and 40.3% of nominal levels on day 0, 35.9, 37.4, and 32.8% on day 7, and 7.3, 14.8, and 19.9% on day 28, respectively. The controls were noted to be free of test material <0.022 (<LOQ) on day 0 but were not analytically verified on days 7 and 28. In pore water at the nominal 1, 8, and 32 ppm a.i. treatment levels, measured concentrations were 0.26, 0.44, and 0.54% of nominal levels on day 0, 1.45, 1.27, and 1.37% on day 7, and 0.42, 0.74, and 0.86% on day 28, respectively. The geometric means corresponding to the nominal 1, 8, and 32 ppm a.i. treatment levels were 0.23, 2.37, and 8.93 ppm a.i. in overlying water, respectively and 0.099, 1.01, and 4.98 ppm a.i. in pore water, respectively.

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 were 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 significant effects on the total number of emerged chironomids, the mean development time, and the mean development rate at the nominal 8 ppm a.i. treatment level and higher. The corresponding geometric mean value for the nominal 8 ppm a.i. is 2.37 and 1.01 ppm a.i. for overlying water and porewater, respectively. The NOEC for these endpoints lies between the geometric mean-measured values of 0.23 and 2.37 ppm a.i. for overlying water and 0.099 and 1.01 ppm a.i. for porewater. The EC_{50} for effects could not be determined due to incomplete analysis of treatment levels but it falls between the porewater geometric mean-measured 1.01 and 4.98 ppm a.i. treatment levels.

This study was designed to fulfill proposed OECD Draft Guideline 219 (February 2000), and does not fulfill any current U.S. EPA guideline. Due to the fact that some treatment concentrations were not analytically verified at test initiation and termination, the obvious instability of the test material under test conditions, This study is of limited utility.

Results Synopsis:

Based on Nominal Concentrations in the Overlying Water

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

NOAEC: between geometric mean-measured 0.099 and < 1.01 ppm a.i. in porewater (between geometric mean-measured 0.23 and < 2.37 ppm a.i. in overlying water)

LOAEC: geometric mean-measured 1.01 ppm a.i. in porewater (geometric mean-measured 2.37 ppm a.i. in overlying water)

EC_{50}: between geometric mean-measured 1.01 and 4.98 mg JAU6476-desthio/L

95% C.I.: N/A
Slope: N/A

Endpoints affected: Total emergence, development time, development rate

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) 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 21.

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 1700 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 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 19.9-20.3°C was slightly lower than the recommended 22-24°C."

EAD comment: The temperature of 19.920.3C 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 7.2-8.3 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 79-91%, 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: Although the cover was made of plastic, it was not in contact with the water/test substance. All test vessels and apparatus with contact to the test substance were made entirely of glass or teflon (chemically inert material) (p. 8 of study report).This deviation is considered minor and does not affect the validity of the study.

- "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 eight as 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 EC_x values were determined in this study, this does not deviate from the OECD guideline and is acceptable to the PMRA.

- "Concentrations of SXX0665 (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)

Measurements in sediment would have been useful. 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 and transformation products 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. The transformation product JAU6476-desthio was measured in water and sediment. In water, JAU6476-desthio reached maximum concentrations on days 0-1 and was found to decrease continuously thereafter in one system, and increased in concentration for the first 3-7 days (as a result of prothioconazole transforming to JAU6476-desthio) and subsequently decreased to the end of the study. In sediment, concentrations of JAU6476-desthio increased until day 14 in one system and day 59 in the other, and subsequently decreased until the end of the study (day 121) in both systems.

- EAD comment on declining water concentrations:

The decline in JAU6476-desthio 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). 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. It was not possible to determine whether the unextractable residues were attributed to the parent or to transformation products.

6. The EAD reviewer agrees with the use of the geometric mean mesured concentrations to estimate exposure.

7. The EAD reviewer verified the statistical analyses for the emergence rate and the development rate and time (combined sexes). Emergence rate (number emerged/number introduced) were square-root arcsine transformed and an ANOVA was performed (Normality and Homegeneity of variance assumptions were met). Multiple comparisons were done using Bonferonni's method. The EAD reviewer has obtained similar results as the EPA reviewer. The EC_{50} for effects could not be determined due to incomplete analysis of treatment levels but it falls between the porewater geometric mean-measured 1.01 and 4.98 ppm a.i. treatment levels.

8. All the validity criteria for this study were met: 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: 8.3-8.6) 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: 79-91% saturation, reviewer-calculated); and the water temperature did not vary by more than ±1°C (range:19.9-20.3°C).

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. Due to the fact that some treatment concentrations were not analytically verified at test initiation and termination, the obvious instability of the test material under test conditions, and the observed treatment related effects at nominal treatment concentrations >4 ppm a.i. this study is classified as **SUPPLEMENTAL** by the US EPA because it is unclear what test material concentrations chironomid larvae were actually exposed to. This study is of limited utility as concentrations of JAU6476-desthio decreased in the water and as effects were observed in treatments for which the concentration was not measured. Despite the limited utility of this study, useful information may still be derived from this study and it is therefore considered acceptable to the PMRA.

References:

Brumhard, B. and M. Oli. 2001. Amended report 2002. Aerobic Degradation and Metabolism of the Active Ingredient JAU6476 in the Water/Sediment System. Performing Laboratory: Bayer AG, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. MR-395/01. December 22, 2001, amended February 27, 2002.

OECD Guideline 211. *Daphnia magna* Reproduction Test. OECD Guidelines for the Testing of Chemicals. Adopted 21 September 1998.

OECD Guideline 219. Sediment-Water Chironomid Toxicity Test Using Spiked Water. OECD Guidelines for the Testing of Chemicals. Adopted 13 April 2004.

Verification of statistical analyses performed by EAD reviewer

Emergence rate (number emerged/number introduced, combined sexes)
Data were arcsin square root transformed

One Way Analysis of Variance Wednesday, October 19, 2005, 15:41:48

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.107)

Group Name	N	Missing	Mean	Std Dev	SEM
controls	6	0	79.621	12.211	4.985
1 mg/L	3	0	79.548	9.462	5.463
2 mg/L	3	0	81.386	7.460	4.307
4 mg/L	3	0	67.102	21.303	12.299
8 mg/L	3	0	46.923	4.396	2.538
16 mg/L	3	0	15.000	25.981	15.000
32 mg/L	3	0	0.000	0.000	0.000

Source of Variation	DF	SS	MS	F	P
Between Groups	6	22012.034	3668.672	18.716	<0.001
Residual	17	3332.239	196.014		
Total	23	25344.274			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: Col 12

Comparison	Diff of Means	t	P	P<0.050
controls vs. 32 mg/L	79.621	8.043	<0.001	Yes
controls vs. 16 mg/L	64.621	6.527	<0.001	Yes
controls vs. 8 mg/L	32.698	3.303	0.025	Yes
controls vs. 4 mg/L	12.520	1.265	1.000	No
controls vs. 2 mg/L	1.765	0.178	1.000	Do Not Test
controls vs. 1 mg/L	0.0732	0.00740	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between

means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Mean Development rate (1/d)

One Way Analysis of Variance Wednesday, October 19, 2005, 16:51:03

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.218)

Group Name	N	Missing	Mean	Std Dev	SEM
controls	6	0	0.0608	0.00325	0.00133
1 mg/L	3	0	0.0590	0.00100	0.000577
2 mg/L	3	0	0.0613	0.00208	0.00120
4 mg/L	3	0	0.0593	0.00153	0.000882
8 mg/L	3	0	0.0523	0.00416	0.00240
16 mg/L	1	0	0.0420	0.000	0.000

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.000170	0.0000425	5.376	0.009
Residual	13	0.000103	0.00000791		
Total	17	0.000273			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.009).

Power of performed test with alpha = 0.050: 0.813

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatment

Comparison	Diff of Means	t	P	P<0.050
controls vs. 16 mg/L	0.0188	6.200	<0.001	Yes
controls vs. 8 mg/L	0.00850	4.274	0.005	Yes
controls vs. 1 mg/L	0.00183	0.922	1.000	No
controls vs. 4 mg/L	0.00150	0.754	1.000	Do Not Test
controls vs. 2 mg/L	0.000500	0.251	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between

means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

Mean Development time (d)

One Way Analysis of Variance Wednesday, October 19, 2005, 16:53:19

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.047)

Group Name	N	Missing	Mean	Std Dev	SEM
controls	6	0	16.437	0.898	0.366
1 mg/L	3	0	17.000	0.288	0.166
2 mg/L	3	0	16.350	0.536	0.309
4 mg/L	3	0	16.857	0.481	0.278
8 mg/L	3	0	19.140	1.571	0.907
16 mg/L	1	0	23.900	0.000	0.000

Source of Variation	DF	SS	MS	F	P
Between Groups	4	16.948	4.237	5.416	0.009
Residual	13	10.170	0.782		
Total	17	27.118			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.009).

Power of performed test with alpha = 0.050: 0.817

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatment

Comparison	Diff of Means	t	P	P<0.050
controls vs. 16 mg/L	7.463	7.812	<0.001	Yes
controls vs. 8 mg/L	2.703	4.322	0.004	Yes
controls vs. 1 mg/L	0.563	0.901	1.000	No
controls vs. 4 mg/L	0.420	0.672	1.000	Do Not Test
controls vs. 2 mg/L	0.0867	0.139	1.000	Do Not Test

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that

enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.