Data Evaluation Report on the Acute Toxicity of SXX0665 Technical (JAU6476-desthio) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number 2004-0843 EPA MRID Number 46246011

Data Requirement:		
	PMRA DATA CODE	9.3.2
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.3.1.1
	EPA MRID	46246011
	EPA Guideline	§72-2a

Test material: SXX0665 (tech) **Purity:** 93.7%
Common name: JAU6476-desthio
Chemical name: IUPAC: 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

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Date: 8/24/2005

Reference/Submission No.: 2004-0843

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

Date Evaluation Completed:

CITATION: Heimbach, F. 1990. Acute Toxicity of SXX0665 (tech) to Waterfleas (*Daphnia magna*). Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Leverkusen, Germany. Laboratory Project Identification No. E 3200404-1. Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated February 14, 1990 and completed March 8, 1990.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of SXX0665 Technical (JAU6476-desthio) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 1.0, 1.8, 3.2, 5.6, 10, and 32 ppm a.i. Due to the solubility of the chemical, higher concentrations could not be tested. Measured zero-hour concentrations were 0.96, 1.8, 3.2, 5.5, 10, and 25 ppm a.i. The nominal 3.2 ppm a.i. test solutions was the only treatment concentration to be analytically verified at 48-hours (test termination) and had a 100% of nominal recovery.

After 48-hours of exposure, mortality was 0% in the negative control and nominal 3.2, and 5.6 ppm a.i. treatment groups; and 3, 3, 7, and 27% in the nominal 1.0, 1.8, 10, and 32 ppm a.i. treatment groups, respectively. All surviving daphnids from the negative control and nominal 1.0 through 5.6 ppm a.i. treatment levels were reported to be normal. Surviving daphnids from the 10 and 32 ppm a.i. treatment groups were reported to be lying on the bottom test vessels and /or lacking any perceivable movement. Sub-lethal effects were not quantified or reported for the main study.

This study is not scientifically sound and does not fulfill U.S. EPA guideline §72-2a because not all treatment levels were analytically verified at test termination. However, measurement of the day-4 3.2 ppm nominal treatment level showed 100% recovery. The NOAEC is based on mortality and observations of sublethal effects. This study is classified as SUPPLEMENTAL.

Results Synopsis

Test Organism Age (eg. 1st instar): 6-24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
EC_{50}: > 5.5 ppm 95% C.I.: N/A
Probit Slope:
NOAEC: 5.5 ppm
LOAEC: >5.5 ppm

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was based on procedures outlined in the OECD Guideline No. 202, (Guideline for Testing of Chemicals, "Daphnia sp., Acute Immobilization Test and Reproduction Test, Part 1- The 24 hr EC_{50} Acute Immobilization Test, (adopted 04 April 1984) except that the test duration was extended to 48 hours. Deviations from §72-2a included:

1. The biomass loading rate was not provided.

2. Test vessels (100 mL with a fill volume of 50 mL) were smaller than EPA recommended size (250 mL with a fill volume of 200 mL).

3. The hardness and the concentration of particulate matter in the dilution water were not reported.

4. The pH range of the dilution water (7.86-7.90) was higher than the EPA recommended range (7.2-7.6).

5. The health of the laboratory cultures prior to test initiation were not reported to be free of disease and/or stress.

6. It is was not reported if aeration was used or not during testing.
7. The limit-of detection and/or limit of quantitation as well as the method validation and matrix fortification percent recoveries were not reported.

8. The test solutions were analyzed at 0 hr, and only the nominal 3.2 ppm a.i. treatment group was assessed analytically after 48 hours.

The fact that not all treatment levels were assessed analytically at 48-hours (test termination) affected the validity and acceptability of this study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, Quality Assurance and Certification of Authenticity statements were provided. This study was conducted under the Principles of Good Laboratory Practice (Chemicals Law (ChemG) of July 25, 1994, Anne 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]).

A. MATERIALS:

1. Test Material SXX0665 (tech)

Description: Beige Brown Solid

Lot No./Batch No. : 17005/89

Purity: 93.7%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination of all test levels at 0 hours and in the 3.2 ppm a.i. (nominal) after 48 hours. Recoveries (all test levels) were 78-100% of nominal concentrations at 0 hours and 100% of the nominal concentration 3.2 ppm a.i. at 48 hours (Table 3, pp 7).

Storage conditions of test chemicals: Not reported

Water solubility: 53 ppm

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. All OECD requirements were not reported.

2. **Test organism**:

Species: *Daphnia magna*

Age at test initiation: 6-24 hours old

Source: Bundesgesundheitsamt in Berlin, Germany.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A pre-test was conducted at nominal treatment concentrations of 0 (negative control), 0.001, 0.01, 0.1, 1.0, 10, and 32 ppm a.i. After 48-hours of exposure, percent mortality was 0, 3, 0, 3, 13, 77, and 73% for the nominal treatment concentrations of 0 (negative control), 0.001, 0.01, 0.1, 1.0, 10, and 32 ppm a.i., respectively.

b. Definitive Study: The animals in the definitive test were exposed to a dilution water control and the nominal treatment concentrations of 1.0, 1.8, 3.2, 5.6, 10, and 32 ppm a.i.

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous laboratory cultures were maintained (at least 1 year).	
Conditions: (same as test or not)	Same as test	
Feeding:	*Daphnia* cultures were fed with a suspension of the freshwater green alga, *Selenastrum capricornutum* and occasionally received an aqueous solution of commercial fish food (TetraMin®).	*EPA requires 7 day minimum acclimation period.*
Health: (any mortality observed)	Not reported.	
Duration of the test	48 hours	*EPA requires 48 hours*

Parameter	Details	Remarks *Criteria*
Test condition - static/flow through	Static	
Type of dilution system (for flow through method)	N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Renewal rate (for static renewal)	N/A	

Parameter	Details	Remarks *Criteria*
Aeration, if any	It is was not reported if aeration was used or not during testing.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 100 mL 50 mL	Test vessels (100 mL with a fill volume of 50 mL) were smaller than EPA recommended size (250 mL with a fill volume of 200 mL). Acceptable according to the OECD guideline. *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water (M7-medium) was aerated deionized water with added mineral salts and vitamins. Constituents of the water are provided on page 4.	Analysis of dilution water for metals, pesticides, and organic contaminants were reported to be <LOD (pp. 17-24) *EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 Not reported 7.86-7.90 97-100% 20.1°C <2 mg/L Not reported <LOD <LOD Free Chlorine: <10 ug/L	The hardness of the dilution water was not reported. The pH range of the dilution water (7.86-7.90) was higher than the EPA recommended range (7.2-7.6), but acceptable according to the OECD guideline. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 10 10	The biomass loading rate was not provided. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17 °C, ≤ 0.5 g/L at > 17 °C; flow-through: ≤ 1 g/L/day.*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 3 3	
Treatment concentrations nominal: measured:	 0 (negative control), 1.0, 1.8, 3.2, 5.6, 10, and 32 ppm a.i. 0-hour: Control value not reported, 0.96, 1.8, 3.2, 5.5, 10, and 25 ppm a.i.	Measured values were only reported for 0-hour. After 48 hours, only the nominal 3.2 ppm a.i. treatment group was assessed analytically and had a recovery of 100% of nominal. The recovery in that group was 100% therefore the study author did not measure the concentrations at the other treatment levels. The control was not reported to be free of test material by test termination. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	N/A	 *EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark	Light intensity was not reported. *EPA requires 16 hours light, 8 hours dark.*

Parameter	Details	Remarks *Criteria*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	The stability of the test material in the dilution water during the course of the study was demonstrated by analytical determination of all test levels at 0 hours and in the 3.2 ppm a.i. (nominal) after 48 hours. Recoveries (all test levels) were 78-100% of nominal concentrations at 0 hours and 100% of the nominal concentration 3.2 ppm a.i. at 48 hours (Table 3, pp 7).	See Table 3, pp 7.
Recovery of chemical Level of Quantitation Level of Detection	Not reported Not reported 0.005 ppm (p. 16)	Based on matrix spikes (ranging from 0.005 to 10 ppm) analyzed concurrently with the test water samples (page 10). Actual recoveries from method validation were not reported.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Mortality (Immobility) and clinical signs of toxicity or abnormal behavior	
Observation intervals	After 24, and 48 hours	
Were raw data included?	Yes, however sub-lethal effects were noted but were not quantified at test termination.	
Other observations, if any	Oxygen saturation and pH were measured at test initiation, and were measured along with temperature at test termination in one control beaker and in one test beaker of the highest and lowest test concentrations.	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 48-hours of exposure, mortality was 0% in the negative control and nominal 3.2, and 5.6 ppm a.i. treatment groups; and 3, 3, 7, and 27% in the nominal 1.0, 1.8, 10, and 32 ppm a.i. treatment groups, respectively. The 48-hour EC_{50} was reported to be >10 ppm a.i. due to the observed precipitate in the nominal 32 ppm a.i. treatment group. The NOAEC for mortality/immobility was 5.6 ppm a.i. based on the nominal treatment concentrations.

Table 3: Effects of SXX0665 Technical (Prothioconazole) on Mortality/Immobilization of *Daphnia magna.*

Treatment, ppm a.i. 0-Hour Measured and (Nominal) Concn.	Observation Period			
	24 Hours		48 Hours	
	No. Dead	% Affected	No. Dead	% Affected
Dilution water Control	0	0	0	0
0.96 (1.0)	0	0	0	0
1.8 (1.8)	1	3	1	3
3.2 (3.2)	0	0	0	0
5.5 (5.6)	0	0	0	0
10 (10)	0	0	2	7
25 (32)	1	3	8	27
NOAEC, ppm a.i.	5.6			
LOAEC, ppm a.i.	10			
EC_{50} (with 95% C.I.), ppm a.i.	>10			

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 48-hours of exposure, all surviving daphnids from the negative control and nominal 1.0 through 5.6 ppm a.i. treatment levels were reported to be normal. Surviving daphnids from the 10 and 32 ppm a.i. treatment groups were reported to be lying on the bottom test vessels and /or lacking any perceivable movement. Sub-lethal effects were not quantified in the study report.

C. REPORTED STATISTICS:

All toxicity values were determined empirically based on the nominal treatment concentrations.

48-Hour
EC_{50}: >10 ppm a.i. 95% C.I.: NA
Probit Slope: N/A
NOAEC: 5.6 ppm a.i.
LOAEC: 10 ppm a.i.

D. VERIFICATION OF STATISTICAL RESULTS:

Due to the fact that no treatment level tested elicited a 50% or greater effect and due to the fact that precipitate was reported in the nominal 32 ppm a.i. test solutions (the highest level tested), the EC_{50} was determined visually. The 48-hour LOAEC was also visually determined as the treatment level which indicated a $\geq$10%,

mortality due to a lack of mortality in the control, the non-linear distribution of the mortality/immobility data, and lacked sub-lethal effects. All toxicity values were estimated in terms of the 0-hour treatment concentrations because not all treatment levels were analytically verified at test termination (48-hours).

48-Hour
EC_{50}: >5.5 ppm a.i. 95% C.I.: NA
Probit Slope: N/A
NOAEC: 5.5 ppm a.i.
LOAEC: 10 ppm a.i.

E. STUDY DEFICIENCIES:

The test solutions were analyzed at 0 hr, and only the nominal 3.2 ppm a.i. treatment concentration was analytically verified at 48 hours. The study author reported that the one concentration analyzed after 48 hours (3.2 ppm a.i.) was 100% of nominal and therefore assumed that the other treatment levels had not degraded over time. The nominal 32 ppm a.i. treatment group (the highest level tested) was reported to have test material precipitate at test termination due to the low water solubility associated with the test material (p. 7). The deficiencies affected the validity and acceptability of this study because it is unclear what concentrations daphnids were actually exposed. In addition, it is not clear exactly how many daphnids were affected as at least some of the observed individuals showed sub-lethal effects. Consequently, the study is classified as INVALID and the toxicity values are not reported in the Executive Summary or Conclusion section of this DER.

Minor deficiencies included the fact that the test vessels (100 mL with a fill volume of 50 mL) were smaller than EPA recommended sizes (250 mL with a fill volume of 200 mL), the hardness of the dilution water was not reported, the pH range of the dilution water (7.86-7.90) was higher than the EPA recommended range (7.2-7.6), and the biomass loading rate was not provided. These deficiencies did not affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification was nearly identical to those of the study author. The reviewer's NOAEC values (5.5 ppm a.i.) was sightly lower than that of the study author's due to the fact that the study author used the nominal treatment concentrations to estimate the toxicity values rather than the 0-hour measured treatment concentrations (the only complete set of analytical measurements reported).

G. CONCLUSIONS:

This study is not scientifically sound and does not fulfill U.S. EPA guideline §72-2a because not all treatment levels were analytically verified at test termination. However, measurement of the day-4 nominal 3.2 ppm treatment level showed 100% recovery. The NOAEC was based on observations of mortality and sublethal effects. This study is classified as SUPPLEMENTAL.

48-Hour
EC_{50}: > 5.5 ppm 95% C.I.: N/A
Probit Slope:
NOAEC: 5.5 ppm
LOAEC: 10 ppm

III. REFERENCES:

No References Cited.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 24, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to *Daphnia* sp.

Heimbach, F. 1990. Acute Toxicity of SXX0665 (tech) to Waterfleas (*Daphnia magna*). Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Leverkusen, Germany. Laboratory Project Identification No. E 3200404-1. Study submitted by Bayer CropScience, Research Triangle Park, NC. Report No. HBF/Dm 95. Study initiated February 14, 1990 and completed March 8, 1990.

PMRA DATA CODE: 9.3.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.3.1.1
EPA MRID: 46246011
EPA Guideline: §72-2a

Reviewing Agency: US EPA

EAD Executive Summary:

The 48-hour acute toxicity of the transformation product SXX0665 Technical (JAU6476-desthio; purity 93.7%) to the water flea, *Daphnia magna*, was studied under static conditions. The study followed OECD Guideline No. 202, part 1 (1984), except that the test duration was extended to 48 hours, and was conducted under OECD and German principles of GLP. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 1.0, 1.8, 3.2, 5.6, 10, and 32 mg JAU6476-desthio/L. Measured zero-hour concentrations were <0.005 (<LOQ), 0.96, 1.8, 3.2, 5.5, 10, and 25 mg JAU6476-desthio/L. The nominal 3.2 mg JAU6476-desthio/L test solutions was analytically verified at 48 hours (test termination) and had a 100% of nominal recovery. There is no reason to believe the stability of the chemical was different in the other treatment vessels.

After 48 hours of exposure, mortality was 0% in the negative control and zero-hour measured 3.2, and 5.5 mg JAU6476-desthio/L treatment groups; and 3, 3, 7, and 27% in the zero-hour measured 0.96, 1.8, 10, and 25 mg JAU6476-desthio/L treatment groups, respectively. All surviving daphnids from the negative control and zero-hour measured 0.96 through 5.5 mg JAU6476-desthio/L treatment levels were reported to be normal. Surviving daphnids from the 10 and 25 mg JAU6476-desthio/L treatment groups exhibited sub-lethal effects (lying on the

bottom test vessels and /or exhibiting hardly any perceivable movement) considered by the PMRA reviewer to be equivalent to immobilization. Therefore, the 48-hour EC_{50} and NOEC values based on mortality/immobilization are set at >5.5 and 5.5 mg JAU6476-desthio/L, respectively.

Results Synopsis

Test Organism Age (eg. 1st instar): 6-24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
EC_{50}: >5.5 mg JAU6476-desthio/L (measured at time 0) 95% C.I.: n/a
Probit Slope: n/a
NOEC: 5.5 mg JAU6476-desthio/L (measured at time 0)
LOEC: 10 mg JAU6476-desthio/L (measured at time 0)

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, name of PMRA secondary reviewer) was added to the EPA-DER as well as information on the chemical name (IUPAC name, CAS name and synonym) available from the PMRA Chemistry review.

2. The name Prothioconazole was removed from the title of the DER and the Executive Summary and replace with the name JAU6476-desthio because the study was conducted with the transformation product JAU6476-desthio (SXX 0665) and not the parent compound prothioconazole.

3. JAU6476-desthio could not be detected in the control and the lowest concentration analyzed in water was 0.005 mg JAU6476-desthio/L (p. 16).

4. The OECD Guideline does not specify a vessel size or test volume, but recommends a minimum of 2 mL of test solution per animal. The test vessels consisted of 100 mL beakers filled to 50 mL, each containing 10 daphnids (5 mL of solution per daphnid). The vessels and fill volumes used in this study are therefore acceptable to the PMRA. A comment as to the acceptability of the test vessel and fill volume was added to the 'Remarks' about the Test vessel in Table 1.

5. The pH range of the dilution water (7.86-7.90) is within the range recommended by the OECD

guideline 202 (pH 6-9) and is considered acceptable to the PMRA. A comment as to the acceptability of this parameter was added to the 'Remarks' on water parameters in Table 1.

6. The OECD guideline 202 (adopted 13 April 2004) requires that: "the concentration be measured as a minimum, at the highest and lowest test concentration, at the beginning and end of the test." The concentration of the 3.2 mg JAU6476-desthio/L was measured at the end of the study and was shown to be 100% of the nominal concentration. There is no reason to believe the stability of the chemical is any different in the other treatment vessels. This deviation is therefore considered minor and measured time 0 concentrations are considered acceptable.

7. The study author measured mortality (animals were checked under a stereomicroscope for signs of movement), yet an EC_{50} was reported instead of an LC_{50}. As sub-lethal effects that could be considered as fitting the criteria for immobilization were observed, the endpoint will be reported as EC_{50} for mortality/immobilization.

8. According to the OECD guideline the definition of immobilization is the following: "Those animals that are not able to swim within 15 seconds, after gentle agitation of the test vessel are considered to be immobilized (even if they can still move their antennae)." The surviving animals of the 10 and 25 mg JAU646-desthio/L treatment levels were noted to lying on the bottom and have hardly any movements perceivable. These sub-lethal effects are considered by the PMRA reviewer to fit the criteria for immobilization. Therefore, the 48-hour EC_{50} for mortality/immobilization is determined to be >5.5 mg JAU6476-desthio/L.

Study Acceptability: This study provides limited information. The sub-lethal effects (lying on the bottom and hardly any movements perceivable) observed in the surviving organisms of the 10 and 32 mg JAU6476-desthio/L treatment levels are considered equivalent to immobilization. The 48-hour EC_{50} and NOEC values based on mortality/immobilization for JAU6476-desthio are >5.5 and 5.5 mg JAU6476-desthio/L, respectively (concentrations measured at time 0).

References:

OECD Guideline 202. OECD Guideline for Testing of Chemicals. *Daphnia* sp., Acute Immobilisation Test. Adopted 13 April 2004.