

US EPA ARCHIVE DOCUMENT

**Data Evaluation Report on the Acute Toxicity of Tebuconazole to Freshwater Invertebrates  
– *Daphnia magna***

PMRA Submission Number {.....}

EPA MRID Number 469192-05

<b>Data Requirement:</b>	PMRA Data Code	{.....}
	EPA DP Barcode	D332285
	OECD Data Point	{.....}
	EPA MRID	469192-05
	EPA Guideline	850.1010 (72-2)

<b>Test material:</b>	Tebuconazole technical	<b>Purity:</b> 97.5%
<b>Common name:</b>	Tebuconazole	
<b>Chemical name:</b>	IUPAC: Not reported	
	CAS name: Not reported	
	CAS No.: Not reported	
	Synonyms: Not reported	

<b>Primary Reviewer:</b>	Holly Galavotti, Biologist	<b>Date:</b> 11/13/07
<b>EPA/OPP/EFED/ERB-1</b>	<i>Holly Galavotti</i>	

<b>Secondary Reviewer(s):</b>	Paige Doelling Brown	<b>Date:</b> 11/13/07
<b>EPA/OPP/EFED/ERB-1</b>	<i>Paige Doelling Brown</i>	

Reference/Submission No.: {.....}

<b>Company Code</b>	{.....}	[For PMRA]
<b>Active Code</b>	{.....}	[For PMRA]
<b>Use Site Category:</b>	{.....}	[For PMRA]
<b>EPA PC Code</b>	128997	

**Date Evaluation Completed:** 11/13/07

**CITATION:** Desai, Y. (2006) Acute Immobilization Study of Tebuconazole Technical in *Daphnia magna*. Project Number: 5742. Unpublished study prepared by Jai Research Foundation, Gujrat, India. 55 p. Study sponsored by Punjab Chemicals & Crop Protection LTD. New Link Road, Andheri, Mumbai, India. Study completed January 31, 2006.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.



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## EXECUTIVE SUMMARY:

The 48-hour acute toxicity of tebuconazole to *Daphnia magna* was studied under static conditions. Daphnids were exposed to initial measured concentrations of <0.20(<LOQ; negative and solvent controls), 0.49, 1.22, 2.66, 6.0 and 13.13 mg a.i./L. Immobility was observed at 24- and 48-hours. The 48-hour EC<sub>50</sub> was 2.88 mg a.i./L. The 48-hr NOAEC based on immobility was 0.49 mg a.i./L.

Based on the results of this study, tebuconazole would be classified as moderately toxic to *Daphnia magna* in accordance with the classification system of the U.S. EPA.

This study is classified as scientifically sound and satisfies guideline requirements for an acute toxicity study with freshwater invertebrates for tebuconazole.

### Results Synopsis

Test Organism Age (e.g., 1<sup>st</sup> instar): <24-hours

Test Type (Flow-through, Static, Static Renewal): Static

EC<sub>50</sub>: 2.88 mg a.i./L 95% C.I.: 2.1 – 3.94 mg a.i./L

NOAEC: 0.49 mg a.i./L

Probit Slope: 2.38 95% C.I.: 1.61 – 3.14

Endpoint(s) Affected: Immobility

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## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study was conducted following the guidelines outlined in the OECD Guideline for Testing of Chemicals (2004) No 202 "*Daphnia* sp., Acute Immobilization Test." The following deviations from US EPA Draft Ecological Effects Test Guideline OPPTS 850.1075 were noted:

1. Analytical verification of the stability of the test material was not performed for the duration of the main testing period. Measured concentrations were only taken at test initiation (0 hr). It is unclear if the test substance concentrations remained stable during the entire test duration.
2. The physiochemical properties of the test material were not reported.
3. The hardness of the dilution water (196 mg/L as CaCO<sub>3</sub>) was higher than EPA recommends (40-48 mg/L as CaCO<sub>3</sub>) but is within OECD guidelines. The pH values during the definitive test (7.68 – 7.93) exceeded the EPA recommended values (7.2-7.6) but are within OECD guidelines.
4. Total organic carbon, particulate matter, metals, and chlorine concentrations were not reported for the dilution water.

The deviations do not impact the validity of the study.

**COMPLIANCE:** Signed and dated No Data Confidentiality Claims, Statement of GLP Compliance, and Statement of Quality Assurance Unit were provided. This study was conducted in accordance with GLP Principles as published by the OECD in 1998, N° 1 (ENV/MC/CHEM(98)17) which are considered by the U.S EPA to be compatible with the U.S GLP Standards, 40 CFR Part 160.

### A. MATERIALS:

**1. Test material** Tebuconazole technical

**Description:** off white-powder

**Lot No./Batch No. :** 051109 (Batch No.)

**Purity:** 97.5%

**Stability of compound under test conditions:** The stability of the test material was verified by analytical determination at 0- hour (test initiation). Mean recoveries were 82 - 94% of nominal.  
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

**Storage conditions of test chemicals:** Stored at room temperature in the Test Substance Control Office (TSCO)

#### Physicochemical properties of Tebuconazole.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	

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Parameter	Values	Comments
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

## 2. Test organism:

**Species:** *Daphnia magna*; (EPA preferred species is *Daphnia magna*; OECD preferred species is *Daphnia magna* or any other suitable *Daphnia* species)

**Age at test initiation:** 1<sup>st</sup> instar (<24-hours); (EPA recommends that Daphnids are in their first instar (<24 hrs old) and that all organisms are approximately the same size and age; OECD requires age <24 hrs old)

**Source:** In house culture; The masture culture of *Daphnia magna* was originally procured from the Department of Zoology, University of Pune, India and regularly subculture at Ecotoxicity Laboratory of Jai Research Foundation; (EPA requires that all organisms are from the same source. Daphnids from ehippia-producing cultures should not be used; Daphnids should be from the fourth or later brood of a given parent)

## B. STUDY DESIGN:

### 1. Experimental Conditions

a. **Range-finding study:** A 48-hour static range-finding test was conducted at nominal concentrations of 0 (negative control), 0 (acetone control), 0.1, 1.0, 10.0, and 20 mg/L, with 5 daphnids exposed to each treatment level and the negative and solvent controls.

During the range finding test, concentrations were measured at test initiation and test termination (48-hr). The mean measured concentration of three replicates at test initiation were 0.10, 9.69, and 19.37 mg a.i./L. The mean measured concentration of three replicates at test termination were 0.10, 9.65, and 19.32 mg a.i./L.

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## b. Definitive Study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u> Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous Mean temperature $21.6 \pm 0.0$ °C, mean DO $9.09 \pm 0.02$ mg/L as air saturation value, total hardness 196 mg/L of CaCO <sub>3</sub> , pH $7.93 \pm 0.01$ Cultures were fed daily with a suspension of algae ( <i>Pseudokirchneriella subcapitata</i> ) Not reported	The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.
Duration of the test	48-hours	EPA requires 96 hours, except daphnids which are 48 hours.
<u>Test condition</u> Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	Static N/A N/A	The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.
Aeration, if any	None	
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass 600 mL 500 mL	EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill

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Parameter	Details	Remarks
		Criteria
Source of dilution water	<p>Reconstituted water</p> <p>11.76 g/L <math>\text{CaCl}_2 \cdot 2\text{H}_2\text{O}</math>  4.93 g/L <math>\text{MgSO}_4 \cdot 7\text{H}_2\text{O}</math>  2.59 g/L <math>\text{NaHCO}_3</math>  0.23 g/L <math>\text{NaHCO}_3</math>  Added to double distilled water.  Media was aerated thoroughly for two days</p>	<p>Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (<a href="http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf">http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf</a>). Dilution water should be intensely aerated before the study.</p>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	196 mg/L as CaCO <sub>3</sub> 7.68-7.93 8.52 – 9.11mg O <sub>2</sub> /L 18.2 – 19.4°C Not reported Not reported Not reported Not reported Not reported	<p>The reported hardness (196 mg/L as CaCO<sub>3</sub>) was higher than recommended (40-48 mg/L as CaCO<sub>3</sub>). The reported pH values (7.68-7.93) were slightly higher than those values in the recommended range (7.2-7.8).</p> <hr/> <p><u>Hardness:</u>  EPA recommends 40 - 48 mg/L as CaCO<sub>3</sub> (OECD recommends 140 - 250 mg/L)</p> <p><u>pH:</u>  EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations</p> <p><u>Temperature:</u>  EPA recommends: 20°C for <i>Daphnia</i> (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary &gt; 1°C;  OECD recommends range of 18-22EC (±1EC)</p> <p><u>Dissolved oxygen:</u>  EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.  Static: 60-100% during 1<sup>st</sup> 48 hr and 40-100% during 2<sup>nd</sup> 48 hr  Flow-through: 60-100% at all times</p>
<u>Number of replicates</u> Negative Control: Solvent Control: Treatments:	4 4 5 treatments with 4 replicates each treatment	<hr/> <p>EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels</p> <p>OECD recommends 4 groups of 5 animals for each test concentration and the controls</p>



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Parameter	Details	Remarks
		Criteria
<u>Number of organisms per replicate</u> Negative control: Solvent control: Treatments:	5 daphnia in each of 4 replicates 5 daphnia in each of 4 replicates 5 daphnia in each of 4 replicates for each treatment (5 treatments)	<p>A negative control was used in lieu of a solvent control.</p> <hr/> <p><i>EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively</i></p> <p><i>EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the <math>LC_{50}/EC_{50}</math> is <math>&gt;100</math> mg/L by exposing <math>\geq 30</math> organisms to <math>\geq 100</math> mg/L or greater. Biomass loading rate for static <math>\leq 0.8</math> g/L at <math>\leq 17^{\circ}\text{C}</math> and <math>\#0.5</math> g/L at <math>&gt; 17^{\circ}\text{C}</math>; flow-through: <math>\#10</math> g/L at <math>\leq 17^{\circ}\text{C}</math> and <math>\leq 5</math> g/L at <math>&gt; 17^{\circ}\text{C}</math>.</i></p> <p><i>OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.</i></p>

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Parameter	Details	Remarks
		Criteria
<u>Treatment concentrations</u> Nominal:  Measured:	0 (negative control), 0 (solvent control) 0.6, 1.3, 2.9, 6.4 and 14.1 mg a.i./L  Treatment concentrations were only measured at test initiation during main test.  0.2 (<LOQ, negative control), 0.2 (<LOQ, solvent control), 0.49, 1.22, 2.66, 6.0, and 13.13 mg a.i./L	Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.  OECD recommends that the highest test concentration should result in 100% immobilization and not be $\geq 1$ g/L, while the lowest concentration should have no observable effect.
Solvent (type, percentage, if used)	Acetone (0.5 mL/L)	Solvents should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. OECD recommends that the solvent not exceed 100 mg/L.
Lighting	Photoperiod of 16 hours of light and 8 hours of dark	EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD: optional light-dark cycle or complete darkness.
Stability of chemical in the test system	Treatment concentrations were only measured at test initiation during main test. Mean recoveries were 82 - 94% of nominal at 0-hours  In the range finding test, Mean recoveries were 97 - 100% of nominal at 0-hours and 48-hours.	

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Parameter	Details	Remarks
		Criteria
<u>Recovery of chemical</u> Level of Quantitation Level of Detection	0.20 mg a.i./L 0.10 mg a.i./L	
Positive control {if used, indicate the chemical and concentrations}	Potassium dichromate (0.31, 0.63, 1.25, 2.50, 5.0 mg a.i./L)	
Other parameters, if any	None	

**2. Observations:**

**Table 2: Observations**

Criteria	Details	Remarks
Parameters measured including the sublethal effects	Immobility	
Observation intervals	24- and 48-hours	
Were raw data included?	Yes	
Other observations, if any	None	

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## **II. RESULTS AND DISCUSSION**

### **A. MORTALITY:**

By test termination (48-hours), no mortality was observed in the controls and mean-measured 0.49 mg a.i./L treatment level. Mortality was 30, 45, 65 and 100% in the mean-measured 1.22, 2.66, 6.0 and 13.3 mg a.i./L treatment levels, respectively, at test termination. The 48-hour EC<sub>50</sub> and NOAEC values were 2.88 and 0.49 mg a.i./L, respectively.

**Table 3: Effect of Tebuconazole on Mortality of *Daphnia magna* .**

Treatment (mg a.i./L) Measured at test initiation (Nominal) Concentrations	No. of organisms	Observation period				
		Day 1			Day 2	
		No. Dead		% mortality	Cumulative No. Dead	Cumulative % mortality
Negative Control	20	0		0	0	0
Solvent Control	20	0		0	0	0
0.49 (0.60)	20	0		0	0	0
1.22 (1.3)	20	3		15	6	30
2.66 (2.9)	20	5		25	9	45
6.0 (6.4)	20	8		40	13	65
13.13(14.1)	20	17		85	20	100
NOAEC <sup>a</sup>	0.49 mg a.i./L based on immobility					
LC <sub>50</sub> <sup>a</sup>	2.88 (2.1 – 3.94) mg a.i./L, slope = 2.38 (1.61 – 3.14)					

<sup>a</sup> Values based on 0-hr measured concentrations. Study authors' toxicity values are based on the nominal concentrations.

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**Table 3: Effect of Positive Control (potassium dichromate) on Mortality of *Daphnia magna* .**

Treatment (mg a.i./L) Measured at test initiation (Nominal) Concentrations	No. of organisms	Observation period			
		Day 1		Day 2	
		No. Dead	% mortality	Cumulative No. Dead	Cumulative % mortality
Negative Control	20	0	0	0	0
0.31	20	0	0	0	0
0.63	20	3	15	6	30
1.25	20	8	40	12	60
2.50	20	10	50	18	90
5.0	20	14	70	20	100
NOAEC	0.31 mg a.i./L based on immobility				
LC <sub>50</sub>	0.97 (0.73 – 1.30) mg a.i./L				

## **B. SUB-LETHAL TOXICITY ENDPOINTS:**

At 24 hours, symptoms of lethargy were observed in three replicates in the 13.3 mg a.i./L treatment group. In the 6.0 mg a.i./L treatment group, daphnia were observed on the surface and bottom of test vessel and lethargic. In the 2.66 mg a.i./L treatment group, daphnia were observed on the surface of the test vessel. In the 1.22 and 0.49 mg a.i./L treatment groups and controls no behavioral symptoms were observed.

At 48 hours, complete immobilization was observed in three replicates in the 13.3 mg a.i./L treatment group. In the 6.0 mg a.i./L treatment group, daphnia were observed on the surface and bottom of test vessel and completely immobilized. In the 2.66 and 1.22 mg a.i./L treatment groups, daphnia were observed on the surface of the test vessel. In the 0.49 mg a.i./L treatment group and controls no behavioral symptoms were observed.

## **C. REPORTED STATISTICS:**

The 24- and 48-hour EC<sub>50</sub> values (and associated 95% C.I.) based on immobilization were determined by the maximum-likelihood probit method (Finney, 1971) using in-house computer program based on "Microsoft Excel 2000." The NOAEC value was determined visually based on % immobilization. All toxicity values were determined using the nominal mg/L concentrations.

EC<sub>50</sub>: 3.38 mg a.i./L 95% C.I.: 1.89 – 6.03 mg a.i./L

NOAEC: 0.49 mg a.i./L

## **D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method(s): The 48-hour LC<sub>50</sub> value (and 95% C.I.) based on immobility was determined using the probit method via Toxanal Statistical Software. The 48-hour NOAEC value was determined visually based on the percent immobilization. All toxicity values were determined using the 0-hr measured concentrations.

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EC<sub>50</sub>: 2.88 mg a.i./L 95% C.I.: 2.1 – 3.94 mg a.i./L  
NOAEC: 0.49 mg a.i./L  
Probit Slope: 2.38 95% C.I.: 1.61 – 3.14

## E. STUDY DEFICIENCIES:

There were no study deficiencies that affected the classification of this study.

## F. REVIEWER'S COMMENTS:

Analytical verification of the stability of the test material was not performed at test termination. Measured concentrations were only taken at test initiation (0 hr) and were 82 – 94% of nominal. It is unclear if the test substance concentrations remained constant during the entire test duration. However, during the range finding study, test concentrations were measured at test initiation and after 24-hours and were 97– 100% of nominal. Therefore, it is likely that test concentrations were stable in main test also.

The reviewer's toxicity values were based on the measured concentrations at test initiation, whereas the study authors' values were based on nominal concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

## G. CONCLUSIONS:

The study is scientifically sound and is thus acceptable. Acceptability classification can be used.

EC<sub>50</sub>: 2.88 mg a.i./L 95% C.I.: 2.1 – 3.94 mg a.i./L  
NOAEC: 0.49 mg a.i./L  
Probit Slope: 2.38 95% C.I.: 1.61 – 3.14

## III. REFERENCES:

BIS, 1983: Methods of Sampling and Test (physical and chemical) for "Water and Wastewater". Part 21 Total Hardness, First Revision. IS: 3025 (Part 21), Bureau of Indian Standard, adopted December 30, 1983.

Finney D. J. 1971 Probit Analysis: 3<sup>rd</sup> Edition Cambridge, The University Press. P 333.

OECD 1998: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1 "OECD Principles on Good Laboratory Practice" ENV/MC/CIEM(98)17 (as revised in 1997).

OECD, 2004: OECD N° 202, *Daphnia* sp, Acute Immobilization Test." The Organisation for Economic Co-operation and Development (OECD) guidelines for the Testing of Chemicals, adopted by the Council on April 13, 2004.

Patel, A.H., 2004. "Validation of analytical method for a.i. analysis of tebuconazole technical by HPLC". JRF Study N° 4807, May 01, 2004. Jai research Foundation, Valvada-396 108, Gujarat, India, Un-published confidential report of JRF.

Shivakumar, R., 2005. "Acute Immobilisation Study of Potassium Dichromate in *Daphnia magna*". JRF Study N° 5499, August 31, 2005 Jai Research Foundation, Valvada—396 108, Gujarat, India, unpublished confidential report of JRF.

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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	5.110812E-02		2.819859	2.195157
3.642273				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1033433	1	.1424775

SLOPE = 2.375623

95 PERCENT CONFIDENCE LIMITS = 1.61193 AND 3.139315

LC50 = 2.878214

95 PERCENT CONFIDENCE LIMITS = 2.104735 AND 3.940299

LC10 = .8404779

95 PERCENT CONFIDENCE LIMITS = .4252588 AND 1.252135

\*\*\*\*\*

DO YOU WISH TO RUN ANOTHER DATA SET?

ENTER Y OR N.

?