Data Evaluation Report on the Acute Toxicity of Tebuconazole to Fish (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 469192-04

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D332285
	OECD Data Point	{.....}
	EPA MRID	469192-04
	EPA Guideline	850.1075 (72-1)

Test material: Tebuconazole technical **Purity:** 97.5%
Common name: Tebuconazole
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Primary Reviewer: Holly Galavotti, Biologist **Date:** 11/13/07
EPA/OPP/EFED/ERB-1 Holly Galavotti

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

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

Company Code	{.....}	[For PMRA]
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Use Site Category:	{.....}	[For PMRA]
EPA PC Code	128997	

Date Evaluation Completed: 11/13/07

CITATION: Desai, Y. (2006) Acute Toxicity Study of Tebuconazole Technical in Rainbow Trout, Onchorhynchus mykiss. Project Number: 5744. Unpublished study prepared by Jai Research Foundation, Gujrat, India. 48 p. Study sponsored by Punjab Chemicals & Crop Protection LTD.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 fish. 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.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to tebuconazole technical at nominal concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 mg a.i./L, under semi-static conditions in which test solution was renewed every 24 hours. A negative control and solvent (acetone) control were also observed. 0-hr measured concentrations were 0 (negative and solvent controls), 0.46, 0.95, 1.88, 3.69, and 7.59. The 96-h LC_{50} was 2.27 mg a.i/L. The NOAEC value, based on sub-lethal effects, was 0.46 mg a.i/L. Sublethal effects (lying on the bottom and loss of equilibrium) were observed in the groups exposed to all but the lowest concentration (0.46 mg a.i./L) of tebuconazole. Based on the results of this study, tebuconazole would be classified as moderately toxic to *Oncorhynchus mykiss* in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as scientifically sound; however, it does not satisfy the guideline requirement for an acute freshwater fish toxicity study with the cold-water species, *Oncorhynchus mykiss* due to guideline deviations. This study is classified as **Supplemental** and is upgradeable pending further information about the condition of the dilution water and stability of test concentrations.

Results Synopsis

Test Organism Size/Age(mean weight or length): 5.2±0.4 – 5.4±0.5 cm (at test termination)
Test Type (Flow-through, Static, Static Renewal): Static Renewal

NOAEC: 0.46 mg a.i./L
LOAEC: 0.95 mg a.i./L
LC_{50}: 2.27 mg a.i./L 95% C.I.: 1.59 – 3.31 mg a.i./L
Probit Slope: 3.25 95% C.I.: 1.78 – 4.73

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study followed guidelines outlined in the OECD Guidelines for Testing of Chemicals No. 203, "Fish, Acute Toxicity Test," referenced as Method C.1 of Commission Directive 92/36/EEC (which constitutes Annex V of Council Directive 67/548/EEC). US CFR Title 40, Part 797, Section 1400, US EPA Pesticide Assessment Guidelines, Sub-Division E, Section 72-4 and US EPA Draft Ecological Effects Test Guideline OPPTS 850.1075. The following deviations from 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 test was performed under semi-static conditions in which the dilution water was changed every twenty-four hours. It is unclear how much water was replaced and if the test concentrations remained stable.
3. The age and weight of the fish were not reported. The length at test termination 5.2±0.4 to 5.4±0.5 cm.
4. The physiochemical properties of the test material were not reported.
5. The hardness of the dilution water (206 – 208 mg/L as $CaCO_3$) was higher than EPA recommends (40-48 mg/L as $CaCO_3$) but are within OECD guidelines. The pH values during the definitive test (7.8 – 8.28) exceeded the EPA recommended values (7.2-7.6) but are within OECD guidelines. The reported temperature of the dilution water (14.3 – 15.9°C) was warmer than EPA recommends (12.0°C) but are within OECD guidelines.
6. Total organic carbon, particulate matter, metals, and chlorine concentrations were not reported for the groundwater dilution water.

The deviations do 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° (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: Analytical verification of the test material in the dilution water was conducted at 0-hour. Measured concentrations ranged from 92-95% 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 Test Substance Control Office (TSCO).

Physicochemical properties of Tebuconazole.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Not reported

Weight at study initiation: Not reported *EPA recommends: mean 0.5 – 5g.*

Length at study initiation: 5.2±0.4 – 5.4±0.5 cm (determined at test initiation) *EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀1.0 cm for bluegill and 5.0 ∀1.0 cm for rainbow trout*

Source: Governmental Fish Farm, Bairagana (Chamoli), Department of Fisheries, Uttaranchal. *EPA recommends that all organisms be from the same source*

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 96-hour static range-finding test was conducted by exposing 10 fish to concentrations of 0 (negative control), 0 (vehicle control) 0.1, 1.0, 10, and 25 mg/L. After 96-hours, mortality was 0, 10, 100 and 100% in the 0.1, 1.0, 10, and 25 mg/L treatment levels, respectively. No mortailities were observed in the controls. These results were used to determine the nominal concentrations used in the definitive test.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 12 days Mean temperature 15.5 ± 0.1 °C, mean DO 90.7 ± 1.3% (88.2 – 92.5) as air saturation value, total hardness 204 mg/L of $CaCO_3$ Fish were fed trout starter feed. Fish were not fed 24 hours prior to, and during, the definitive test. No mortality or abnormalities 12 days prior to test initiation	 *The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test	96- hours	*The recommended test duration is 96 hours.*
Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static Renewal N/A Every 24-hours	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	No aeration was provided	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass 80 L 50 L	The size of the test vessels (80 L) was larger than recommended (19 L); however, this would reduce the biomass loading rate and reduce the potential for crowding-induced stress. *Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	Groundwater	Dechlorinated tap water is not a recommended source of dilution water; however, it supported test organisms during the acclimation period and control organisms during the definitive test. *Recommended source of dilution water is soft, reconstituted water or water from a natural 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 (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf)* *Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 206 – 208 mg/L as $CaCO_3$ 7.8 - 8.28 72.6 – 88.8 % DO saturation Not reported Not reported Not reported Not reported Not reported 14.3 – 15.9°C N/A Water parameters were measured daily before and after each renewal of media with freshly prepared solution.	The hardness of the dilution water (206 – 207 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH values during the definitive test (7.8 – 8.28) exceeded the recommended values (7.2-7.6). The reported temperature of the dilution water (14.0°C) was warmer than recommended (12.0°C). *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: 60% during first 48 hrs and 40% during second 48 hrs; flow-through: 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 °C for coldwater species, 17 or 22 °C for warmwater species, and 22 ± 1 °C C for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated:	 1 1 5	A solvent control was acetone *Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 10 10	*Number of organisms per replicate should be 10/concentration; OECD guideline recommends at least 7 fish/concentration.*
Biomass loading rate	0.26 g of fish/L	*Recommended static conditions are 0.8 g/L at 17°C and 0.5 g/L at > 17°C. Recommended flow-through conditions are 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal: measured (at 0-hr):	 0 (negative control), 0 (solvent control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg a.i./L <0.20 (<LOQ; negative and solvent controls), 0.46, 0.95, 1.88, 3.69, and 7.59 mg a.i./L	Test concentrations measured only at test initiation.
Solvent (type, percentage, if used)	Acetone at 0.1 ml/L	*The solvent 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	16 hours of light and 8 hours of darkness with using automatic timer	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the definitive test	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantitation	 Measured only at test initiation 0.2 mg a.i./L	

Parameter	Details	Remarks *Criteria*
Level of detection	0.1 mg/L	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	Observations were made at 3, 6, 24, 48, 72 and 96 hours.	*Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

By test termination mortality was 0% in the negative and solvent controls and 0, 20, 30, 70, and 100% in the 0.46, 0.95, 1.88, 3.69, and 7.59 mg a.i./L measured treatment groups. By 48-hours, 100% mortality was observed in the 7.59 mg a.i./L treatment group. The 96-hour LC_{50} and NOAEC values, based on mortality, were 2.27 and 0.46 mg a.i./L, respectively.

Table 3: Effect of Tebuconazole on Mortality of Rainbow Trout.

Treatment (mg a.i./L) Nominal and (Measured at test initiation)	Fish Length (cm) Mean ± SD	No. of fish at start of study	Observation period					
			Day 1		Day 2		Day 4	
			No. Dead	Cumulative % mortality	No. Dead	Cumulative % mortality	No. Dead	Cumulative % mortality
Negative Control	5.2 ±0.4	10	0	0	0	0	0	0
Solvent Control	5.2 ±0.4	10	0	0	0	0	0	0
0.5 (0.46)	5.3 ±0.5	10	0	0	0	0	0	0
1.0 (0.95)	5.2 ±0.5	10	0	0	0	0	2	20
2.0 (1.88)	5.3 ±0.5	10	0	0	0	0	3	30
4.0 (3.69)	5.4 ±0.4	10	0	0	2	20	7	70
8.0 (7.59)	5.2 ±0.5	10	2	20	8	100	10	100
NOAEC based on mortality	0.46 mg a.i./L							
LC_{50}	2.27 (1.59 – 3.31) mg a.i./L, slope = 3.25							
Positive control, if used mortality: LC_{50}:	none							

Values shown in the table are based on the reviewer's analysis. Study authors' toxicity values were based on nominal concentrations (mg/L); reviewer based on measured concentrations at test initiation.

B. NON-LETHAL TOXICITY ENDPOINTS:

At test-termination, no sub-lethal effects were observed in the negative or solvent controls or in the measured 0.46 mg a.i./L treatment group. In the measured 0.95 mg a.i./L treatment group, surviving fish were observed lying on the bottom beginning on days 2 - 4. In the measured 1.88 mg a.i./L treatment group, surviving fish were observed lying on the bottom beginning at 6 hours and through day 4 and loss of equilibrium was observed on day 3. In the measured 3.69 mg a.i./L treatment group, surviving fish were observed lying on the bottom beginning at 3 hours and through day 4 and loss of equilibrium was observed on days 3 and 4. In the measured 7.59 mg a.i./L treatment group, surviving fish were observed lying on the bottom beginning at 3 hours and loss of equilibrium was observed on at 6 hours. All fish were dead in the 7.59 mg/L group by day 1. The NOAEC and LOAEC values based on sub-lethal effects were 0.46 and 0.95 mg a.i./L, respectively.

Table 4: Sub-lethal Effect of Tebuconazole on Rainbow Trout.

Treatment (mg a.i./L) Nominal and (Measured at test initiation)	Observation period					
	3 Hour	6 Hour	Day 1	Day 2	Day 3	Day 4
Negative Control	1	1	1	1	1	1
Solvent Control	1	1	1	1	1	1
0.5 (0.46)	1	1	1	1	1	1
1.0 (0.95)	1	1	1	1, 10	1, 10	1, 10
2.0 (1.88)	1	10	10	10	3, 10	3, 10
4.0 (3.69)	10	10	10	10	3, 10	3, 10
8.0 (7.59)	10	3, 10	10	100% Mortality	100% Mortality	100% Mortality
NOAEC	0.46 mg a.i./L based on sublethal effects					
LOAEC	0.95 mg a.i./L based on sublethal effects					
EC_{50}	Not reported for sublethal effects					
Positive control, if used % sublethal effect: EC_{50}:	N/A					

1- Normal, 3- Loss of equilibrium, 10- Lying on the bottom
Study authors' toxicity values were based on nominal concentrations (mg/L); reviewer based on measured concentrations at test initiation

C. REPORTED STATISTICS:

The 96-hour LC_{50} value and associated 95% C.I. was calculated as 2.67 mg/L and the 95% fiducial limits were found to be between 1.59 and 4.46 mg/L. The regression equation established [Probit mortality (Y) vs log concentration (mg/L) of tebuconazole technical (χ) was $Y - 4.01 + 2.33\ \chi$.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The 96-hour LC_{50} (and 95% C.I.) was determined using the Probit method via Toxanal Statistical Software. The NOAEC value was determined visually based on no mortality or sublethal effects in the measured 0.46 mg a.i./L treatment group. The LOAEC was determined visually based on 20% mortality observations of fish lying at the bottom in the 0.95 mg/L treatment group. All toxicity values were determined using the mean-measured concentrations at test initiation. The EC_{50} value based on sub-lethal effects was not analyzed as this is a subjective observation and not a quantitative measurement.

NOAEC: 0.46 mg a.i./L
LOAEC: 0.95 mg a.i./L
LC_{50}: 2.27 mg a.i./L 95% C.I.: 1.59 – 3.31 mg a.i./L
Probit Slope: 3.25 95% C.I.: 1.78 – 4.73

E. STUDY DEFICIENCIES:

The three main study deficiencies are as follows:

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 test was performed under semi-static conditions in which the dilution water was changed every twenty-four hours. It is unclear how much water was replaced and if the test concentrations remained stable.
3. Total organic carbon, particulate matter, metals, and chlorine concentrations were not reported for the groundwater dilution water.

F. REVIEWER'S COMMENTS:

There are uncertainties with this study concerning the test concentrations during the main test. 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) and were 92 – 95% of nominal. It is unclear if the test substance concentrations remained constant during the entire test duration. The dilution water was changed every twenty-four hours and it is unclear how much water was replaced and if the test concentrations remained stable.

During the range finding study, test concentrations were measured at test initiation and after 24-hours. At test initiation, the test concentrations were 97 – 100% of nominal. After 24 hours, test concentrations were 90 – 102% of the initial test concentrations. It is unclear if test samples at 24-hours were taken before or after media renewal.

In addition, there are uncertainties about the condition of the dilution water. The dilution water was natural groundwater; however the total organic carbon, particulate matter, metals, and chlorine concentrations were not reported. High levels of organic matter could bind to the test material and therefore reduce the toxicity to fish.

Toxicity endpoints for fish could be underestimated if high concentrations of organic matter were present. It is requested that the laboratory provide further information about the levels of total organic carbon, particulate matter, metals, and chlorine concentrations in the groundwater used in this test.

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.

The EC_{50} based on sub-lethal effects was not determined as this is a subjective observation and not a quantitative measurement.

The criteria for death were the absence of respiratory movement and response to physical stimulation.

G. CONCLUSIONS:

This toxicity study is classified as scientifically sound; however, it does not satisfy the guideline requirement for an acute freshwater fish toxicity study with the cold-water species, *Oncorhynchus mykiss* due to guideline deviations. This study is classified as Supplemental and is upgradeable pending further information about the condition of the dilution water and stability of test concentrations.

NOAEC: 0.46 mg a.i./L
LOAEC: 0.95 mg a.i./L
LC_{50}: 2.27 mg a.i./L 95% C.I.: 1.59 – 3.31 mg a.i./L
Probit Slope: 3.25 95% C.I.: 1.78 – 4.73

III. REFERENCES:

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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.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS     G              H            GOODNESS OF FIT PROBABILITY
 4              .2068663      1             .5794149

SLOPE     =      3.254555
95 PERCENT CONFIDENCE LIMITS  = 1.7743      AND     4.734811

LC50 =           2.273559
95 PERCENT CONFIDENCE LIMITS = 1.587305 AND  3.307283

LC10 =           .9257291
95 PERCENT CONFIDENCE LIMITS = .3975607 AND  1.370021
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