DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT & ORAL LC_{50} TEST
§141-1

1. CHEMICAL: Prothioconazole PC Code No.: 113961

2. TEST MATERIAL: JAU 6476 Purity: 98.6%

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

3. CITATION:

Author: Wilhelmy, H.

Title: JAU 6476 a.i. Acute Effects on the Honeybee *Apis mellifera*

Study Completion Date: November 10, 1999

Laboratory: Dr. U. Noach Laboratorium
Sarstedt, Germany

Sponsor: Bayer CropScience
2 T.W. Alexander Dr.
RTP, NC 27709

Laboratory Report ID: IBA64051

DP Barcode: D303488

MRID No.: 46246048

4. REVIEWED BY: Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: **Date:** 8/6/04

APPROVED BY: Teri Myers, Ph.D., 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, HC, PMRA, EAD
Signature: 8/22/05 **Date:** 8/22/2005
Christopher J. Salice
Signature: 7-15-05 **Date:** 7/15/05

7. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera* L.

Age or Size of Test Organism at Test Initiation: Young worker bees, age not specified.

Type of Concentrations: Nominal

Definitive Study Duration: 48 hours

8. CONCLUSIONS:

The honey bee, *Apis mellifera* L., was exposed to Prothioconazole for 48 hours in the oral and the contact tests. The oral and contact nominal concentrations were 12.5, 25, 50, 100, and 200 µg a.i./bee. The actual intake concentrations of Prothioconazole in the oral toxicity test were 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee.

By 48 hours in the oral test, 3% mortality was observed in the 31.9 and 71.0 µg a.i./bee treatment groups, compared to 7% vehicle control mortality. No mortality was observed in the negative control, 10.7, 22.0, or 47.3 µg a.i./bee treatment groups. The corrected mortalities based on the vehicle control were -4, -4, -4, -8, and -4% in the 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee treatment groups, respectively. The sublethal effect of one bee lying on their back with problems standing up was observed in the 71.0 µg a.i./bee treatment group.

By 48 hours in the contact test, there was 23, 30, 30, 13, and 27% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The corrected mortalities based on the solvent control were 17, 25, 25, 6, and 22% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The sublethal effects of bees lying on their back and/or slow motions with coordination problems were observed in all treatment groups and the solvent control during the test. **The LC_{50} value for the oral test was estimated as >71.0 µg a.i./bee, the highest concentration of intake. The**

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Signature: **Date:** 8/6/04

APPROVED BY: Teri Myers, Ph.D., 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, HC, PMRA, EAD **Date:** 8/22/2005

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	Young worker bees, age not specified.
Type of Concentrations:	Nominal
Definitive Study Duration:	48 hours

8. **CONCLUSIONS:**

The honey bee, *Apis mellifera* L., was exposed to Prothioconazole for 48 hours in the oral and the contact tests. The oral and contact nominal concentrations were 12.5, 25, 50, 100, and 200 µg a.i./bee. The actual intake concentrations of Prothioconazole in the oral toxicity test were 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee.

By 48 hours in the oral test, 3% mortality was observed in the 31.9 and 71.0 µg a.i./bee treatment groups, compared to 7% vehicle control mortality. No mortality was observed in the negative control, 10.7, 22.0, or 47.3 µg a.i./bee treatment groups. The corrected mortalities based on the vehicle control were -4, -4, -4, -8, and -4% in the 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee treatment groups, respectively. The sublethal effect of one bee lying on their back with problems standing up was observed in the 71.0 µg a.i./bee treatment group.

By 48 hours in the contact test, there was 23, 30, 30, 13, and 27% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The corrected mortalities based on the solvent control were 17, 25, 25, 6, and 22% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The sublethal effects of bees lying on their back and/or slow motions with coordination problems were observed in all treatment groups and the solvent control during the test. **The LC_{50} value for the oral test was estimated as >71.0 µg a.i./bee, the highest concentration of intake. The LD_{50} value for the contact test was >200 µg a.i./bee. As a result, Prothioconazole is categorized as relatively nontoxic to honeybees on both an acute oral and contact basis.** The NOAELs for the oral and contact tests were 71.0 and 200 µg a.i./bee, respectively.

This acute contact study is classified as ACCEPTABLE. This study is scientifically

sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020). **The acute oral study is scientifically sound and is classified as Supplemental.**

Reported Statistical Results - Oral Test:

LD_{50}: >71.0 µg a.i./bee	95% C.I.: N/A
NOAEL: 71.0 µg a.i./bee	Probit Slope: N/A
LOAEL: >71.0 µg a.i./bee	

Reported Statistical Results - Contact Test:

LD_{50}: >200 µg a.i./bee	95% C.I.: N/A
NOAEL: 200 µg a.i./bee	Probit Slope: N/A
LOAEL: >200 µg a.i./bee	

9. ADEQUACY OF THE STUDY:

A. Classification: This acute contact study is classified as ACCEPTABLE. This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020). The acute oral study is scientifically sound and is classified as Supplemental.

B. Rationale: This acute oral study is scientifically sound and is classified as Supplemental because the study is a non-guideline study and does not fulfill an OPP guideline requirement.

C. Repairability: N/A

10. GUIDELINE DEVIATIONS:

No deviations noted.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the acute oral and contact toxicity of Prothioconazole, to honeybees for the purpose of chemical registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera* L.
Age at beginning of test:	Young worker bees, age not specified.
Supplier:	Laboratory colonies.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Stainless steel cages with holes punched into the bottom plate and a glass plate at front. Cage dimensions were 102 x 56 x 86 mm.
Lighting:	Continuous darkness
Temperature:	25 ± 2°C
Relative humidity:	40-80%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No range finding study was conducted.

Guideline Criteria	Reported Information
Reference toxicant test?	The reference toxicant was Parathion 520 g/L. In the parallel oral test, the concentration of Parathion was 0.3833 µg a.i./bee (actual intake 0.23 µg a.i./bee). In the parallel contact test, the applied concentration of Parathion was 0.156 µg a.i./bee. (The reference toxicant is also tested once per season to determine LD50 values.)
Method of administration:	Oral test: The test substance was mixed with Tween 85 (2 µL/bee) and 50% sucrose solution. The test solutions were offered to the bees for three hours. Contact test: The test substance was mixed with acetone. The test solution was applied to the thorax of each bee (5 µL) using a micro piston pipette.
Nominal doses:	Oral test: 12.5, 25, 50, 100, and 200 µg a.i./bee (Actual oral concentrations were 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee). Contact test: 12.5, 25, 50, 100, and 200 µg a.i./bee.
Controls: Negative control and/or diluent/solvent control	Oral test: negative and vehicle (Tween 85) control Contact test: negative and solvent (acetone) control
Number of colonies per group:	3 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Contact test: Acetone

Guideline Criteria	Reported Information
Feeding:	Oral test: Prior to test initiation, bees were starved for up to 2 hours. After treatment with test solutions, 50% sucrose solution was provided *ad libitum*. Contact test: 50% sucrose solution was provided *ad libitum*.
Observations period:	4, 24, and 48 hours.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	Oral test: 0% negative control mortality and 7% vehicle control mortality by 48 hours. Contact test: 10% negative control mortality and 7% solvent control mortality by 48 hours.
Raw data included:	Replicate data were provided.
Signs of toxicity (if any) were described?	Yes (lying on their back and slow motions/problems with coordination).

Mortality - Oral Test

Dosage µg a.i./bee (actual intake)	No. of bees	Percent Mortality (%) Hour of Study	
		24	48
Test Substance (Prothioconazole)			
Negative Control	30	0	0
Vehicle Control	30	3	7
12.5 (10.7)	30	0	0
25 (22.0)	30	0	0
50 (31.9)	30	0	3
100 (47.3)	30	0	0
200 (71.0)	30	3	3
Toxic Standard (Parathion):			
0.3833 (0.23)	30	47	60

Observations: By 48 hours, 3% mortality was observed in the 31.9 and 71.0 µg a.i./bee treatment groups, compared to 7% vehicle control mortality. No mortality was observed in the negative control, 10.7, 22.0, or 47.3 µg a.i./bee treatment groups. The corrected mortalities based on the vehicle control were -4, -4, -4, -8, and -4% in the 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee treatment groups, respectively. The sublethal effect of one bee lying on their back with problems standing up was observed in the 71.0 µg a.i./bee treatment group.

Mortality - Contact Test

Dosage (µg a.i./bee)	No. of bees	Percent Mortality (%) Hour of Study	
		24	48
Test Substance (Prothioconazole):			
Negative Control	30	0	10
Solvent Control	30	7	7
12.5	30	20	23
25	30	20	30
50	30	30	30
100	30	13	13
200	30	27	27
Toxic Standard (Parathion):			
0.156	30	93	93

Observations: By 48 hours, there was 23, 30, 30, 13, and 27% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The corrected mortalities based on the solvent control were 17, 25, 25, 6, and 22% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The sublethal effects of bees lying on their back and/or slow motions with coordination problems were observed in all treatment groups and the solvent control during the test.

Statistical method: The LD_{50} values in the oral and contact toxicity tests were not calculated due to less than 50% mortality in all treatment groups. The NOAEL and LOAEL were determined based on mortalities and behavioral abnormalities. The mortalities were corrected based on the formula found on page 15.

Reported Statistical Results - Oral Test:

LD_{50}: >71.0 µg a.i./bee
NOAEL: 71.0 µg a.i./bee
LOAEL: >71.0 µg a.i./bee

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

Reported Statistical Results - Contact Test:

LD_{50}: >200 µg a.i./bee	95% C.I.: N/A
NOAEL: 200 µg a.i./bee	Probit Slope: N/A
LOAEL: >200 µg a.i./bee	

14. VERIFICATION OF STATISTICAL RESULTS:

For the oral toxicity test, statistical analyses were not necessary, as it could visually be determined that there was not significant mortality. The LC_{50}/LD_{50} and NOAEL estimates could be determined visually. For the contact toxicity test, the survival data was analyzed using ANOVA. The LC_{50}/LD_{50} was determined visually, as mortality did not exceed 50%.

Results - Oral Test:

LD_{50}: >71.0 µg a.i./bee	95% C.I.: N/A
NOAEL: 71.0 µg a.i./bee	Probit Slope: N/A
LOAEL: >71.0 µg a.i./bee	

Results - Contact Test:

LD_{50}: >200 µg a.i./bee	95% C.I.: N/A
NOAEL: 200 µg a.i./bee	Probit Slope: N/A
LOAEL: >200 µg a.i./bee	

15. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study authors.

The LC_{50} value for the oral test was estimated as >71.0 µg a.i./bee, the highest concentration of intake. The LD_{50} value for the contact test was >200 µg a.i./bee. As a result, Prothioconazole is categorized as relatively nontoxic to honeybees on both an acute oral and contact basis.

The test was conducted in compliance with the OECD Principles of Good Laboratory Practice.

16. REFERENCES:

Schneider-Orelli, O. (1947): Entomologisches Praktikum, Einfuhrung in die land -und forstwirtschaftliche Insektenkunde. Verlag H.R. Sauerlander & Co. Aarau.

Anonymous (1993): Decision-making schemes for the environmental risk assessment for plant protection products. Chapter 10: Honeybees. Bulletin OEPP EPPO Bulletin 23 (1) 151-165

European and Mediterranean Plant Protection Organisation (EPPO): Guideline on test methods for evaluating the side-effects of plant protection products on honey bees. Bulletin OEPP EPPO Bulletin 22, 203-215 (1992) Guideline No. 170.

Felton, J.C., Oomen, P.A., Stevenson, J.H. (1986): Toxicity and hazards to honeybees: Harmonization of test methods. Bee World 67, 114-124

SETAC (1995): Procedures for assessing the environmental fate and ecotoxicity of pesticides. Ed. By M.R. Lynch. Brussels 1995: SETAC. Section Honeybees pp. 43-46.

Weiss, K. (1990): Bienenpathologie. Second Edition. Munich, Ehrenwirth Publishers.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

survival
File: 6048s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1383.333	276.667	1.158
Within (Error)	12	2866.667	238.889	
Total	17	4250.000		

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

survival
File: 6048s Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	93.333	93.333		
2	12.5	76.667	76.667	1.321	
3	25	70.000	70.000	1.849	
4	50	70.000	70.000	1.849	
5	100	86.667	86.667	0.528	
6	200	73.333	73.333	1.585	

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

survival
File: 6048s Transform: NO TRANSFORMATION

DUNNETTS 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	control	3			
2	12.5	3	31.549	33.8	16.667
3	25	3	31.549	33.8	23.333
4	50	3	31.549	33.8	23.333
5	100	3	31.549	33.8	6.667
6	200	3	31.549	33.8	20.000

survival
File: 6048s Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	93.333	93.333	93.333
2	12.5	3	76.667	76.667	76.667
3	25	3	70.000	70.000	75.556
4	50	3	70.000	70.000	75.556
5	100	3	86.667	86.667	75.556
6	200	3	73.333	73.333	73.333

survival
File: 6048s 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
control	93.333				
12.5	76.667	1.321		1.78	k= 1, v=12
25	75.556	1.409		1.87	k= 2, v=12
50	75.556	1.409		1.90	k= 3, v=12
100	75.556	1.409		1.92	k= 4, v=12
200	73.333	1.585		1.93	k= 5, v=12

s = 15.456
Note: df used for table values are approximate when v > 20.

Data Evaluation Report on the Acute Oral and Contact Toxicity of JAU 6476 (Prothioconazole) to the Honey Bee

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 22, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Contact and Acute Oral Toxicity to the Honey Bee

Wilhelmy, H. 1999. JAU 6476 a.i. Acute Effects on the Honeybee *Apis mellifera*. Study Performed by Dr. U. Noach-Laboratorium, Für Angewandte Biologie, Sarstedt. Submitted by Bayer CropScience, RTP, North Carolina. Unpublished. Laboratory ID IBA64051. November 10, 1999.

PMRA DATA CODE: 9.2.4.1/9.2.4.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.7.1; IIA 8.7.2
EPA MRID: 46246048
EPA Guideline: §141-1

Reviewing Agency: US EPA

EAD Executive Summary:

The honey bee, *Apis mellifera* L., was exposed to prothioconazole (JAU6476; purity 98.6%) for 48 hours in oral and contact toxicity tests. These studies were conducted following the EPPO-Guideline 170 and SETAC (1995) and was in compliance with OECD Principles of GLP. The nominal concentrations for the oral and contact tests were 12.5, 25, 50, 100, and 200 µg a.i./bee. The actual intake concentrations of prothioconazole in the oral toxicity test were 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee.

By 48 hours in the oral test, 3% mortality was observed in the 31.9 and 71.0 µg a.i./bee treatment groups, compared to 7% vehicle control (Tween 85) mortality. No mortality was observed in the negative control, 10.7, 22.0, or 47.3 µg a.i./bee treatment groups. The corrected mortalities based on the vehicle control were -4, -4, -4, -8, and -4% in the 10.7, 22.0, 31.9, 47.3, and 71.0 µg a.i./bee treatment groups, respectively. The sublethal effect of one bee lying on its back with problems standing up was observed in the 71.0 µg a.i./bee treatment group after 4 hours. No sub-lethal effects were observed in any treatment after 24 and 48 hours.

By 48 hours in the contact test, there was 23, 30, 30, 13, and 27% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The corrected mortalities based on the solvent control (acetone) were 17, 25, 25, 6, and 22% mortality in the 12.5, 25, 50, 100, and 200 µg a.i./bee treatment groups, respectively. The sublethal effects of bees lying on their back and/or slow motions with coordination problems were observed in all treatment groups and the solvent control 4 hours after treatment, but no sublethal effects were observed in any treatment after 48 hours. **The LC_{50} value for the oral test was estimated as >71.0 µg a.i./bee, the highest concentration of intake. The LD_{50} value for the contact test was >200 µg a.i./bee. As a result, prothioconazole is categorized as relatively nontoxic to honeybees on both an acute oral and contact basis, according to the scheme of Atkins *et al.* (1981).** The NOELs for the oral and contact tests were 71.0 and 200 µg a.i./bee, respectively.

Results Synopsis:

Oral Toxicity Test:

LD_{50}: >71.0 µg a.i./bee
NOEL: 71.0 µg a.i./bee
LOEL: >71.0 µg a.i./bee

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

Contact Toxicity Test:

LD_{50}: >200 µg a.i./bee
NOEL: 200 µg a.i./bee
LOEL: >200 µg a.i./bee

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

Evaluator Comments:

1. The PMRA Submission Number was added to the Header of the DER. 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, CAS number and synonym) available from the PMRA Chemistry review. 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. The EAD has verified the NOEL for survival in the contact toxicity test using ANOVA. Assumptions of normality and homogeneity of variances were met. The EAD reviewer obtained identical results to those of the EPA reviewer.

3. The EAD reviewer agrees with the conclusions of the EPA reviewer.

Study Acceptability: The acute contact study and the acute oral study are scientifically sound and satisgy the guideline requirements for an acute oral and an acute contact toxicity test with the honey bee. The acute contact and acute oral studies are classified as ACCEPTABLE to the PMRA.