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

1. CHEMICAL: Prothioconazole formulation PC Code No.: 113961

2. TEST MATERIAL: JAU 6476 SC 480 Purity: 478.18 g/L

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

3. CITATION:

Author: Schmitzer, S.

Title: Effects of JAU 6476 SC 480 (Acute Contact and Oral) on Honey Bees (*Apis Mellifera* L.) in the Laboratory

Study Completion Date: February 11, 2004

Laboratory: Institut fur Biologishe Analytik
und Consulting IBACON GmbH
Arheilger Weg 17
64380 Rossdorf, Germany

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

Laboratory Report ID: 18351035

DP Barcode: D303495

MRID No.: 46246046

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

Signature: **Date:** 8/18/04

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/3/04

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

Signature: **Date:**

6. SECONDARY REVIEW BY:

Émilie Larivière, HC, PMRA, EAD
Signature: 8/22/05 **Date:** 8/22/2005
Christopher J. Salice, EPA/EFED/ERB-IV
Signature: 9-17-05 **Date:** 9/17/05

7. STUDY PARAMETERS:

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

Age or Size of Test Organism at Test Initiation: 4-6 weeks old

Type of Concentrations: Nominal

Definitive Study Duration: 48 hours

8. CONCLUSIONS:

The honey bee, *Apis mellifera* L., was exposed to Prothioconazole formulation for 48 hours in the oral and the contact test. The oral and contact nominal concentrations were 3.1, 7.1, 16.0, 38.0, 87.0, and 200.0 µg/bee. The actual intake concentrations of Prothioconazole formulation in the oral toxicity test were 3.6, 6.6, 18.1, 44.0, 97.9, and 232.0 µg/bee. By 48 hours in the oral test, 3.3% mortality was observed in the 3.6, 6.6, 44.0, and 232.0 µg a.i./bee treatment groups. No mortality was observed in the control, 18.1, or 97.9 µg/bee treatment groups, and no sublethal effects were observed in any treatment group during the test. By 48 hours in the contact test, 3.3% mortality was observed in the control, and no mortalities were observed in the treatment groups. No sublethal effects were observed in any treatment group during the test. **The LC_{50} value**

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Signature: **Date:**

6. **SECONDARY REVIEW BY:** Émilie Larivière, HC, PMRA, EAD

Signature: **Date:** 8/22/2005

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	4-6 weeks old
Type of Concentrations:	Nominal
Definitive Study Duration:	48 hours

8. **CONCLUSIONS:**

The honey bee, *Apis mellifera* L., was exposed to Prothioconazole formulation for 48 hours in the oral and the contact test. The oral and contact nominal concentrations were 3.1, 7.1, 16.0, 38.0, 87.0, and 200.0 µg/bee. The actual intake concentrations of Prothioconazole formulation in the oral toxicity test were 3.6, 6.6, 18.1, 44.0, 97.9, and 232.0 µg/bee. By 48 hours in the oral test, 3.3% mortality was observed in the 3.6, 6.6, 44.0, and 232.0 µg a.i./bee treatment groups. No mortality was observed in the control, 18.1, or 97.9 µg/bee treatment groups, and no sublethal effects were observed in any treatment group during the test. By 48 hours in the contact test, 3.3% mortality was observed in the control, and no mortalities were observed in the treatment groups. No sublethal effects were observed in any treatment group during the test. **The LC_{50} value for the oral test was estimated as >232.0 µg/bee, the highest concentration of intake. The LD_{50} value for the contact test was >200.0 µg/bee. As a result, Prothioconazole formulation is categorized as relatively nontoxic to honeybees on both an acute oral and contact basis.** The NOAELs for the oral and contact tests were 232.0 and 200.0 µg/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}: >232.0 µg/bee 95% C.I.: N/A
NOAEL: 232.0 µg/bee Probit Slope: N/A
LOAEL: >232.0 µg/bee

Reported Statistical Results - Contact Test:

LD_{50}: >200.0 µg/bee 95% C.I.: N/A
NOAEL: 200.0 µg/bee Probit Slope: N/A
LOAEL: >200.0 µg/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 formulation 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:	4-6 weeks old
Supplier:	Laboratory colonies (IBACON)
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	The cages were stainless steel with a removable glass sheet front and perforated bottom (1 mm holes). Cage dimensions were 10 x 8.5 x 5.5 cm.
Lighting:	Continuous darkness
Temperature:	25°C
Relative humidity:	50-60%

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 dimethoate, as Perfekthion EC (401.2 g/L). In the oral test, the applied concentrations of dimethoate were 0.04, 0.08, and 0.15 µg/bee (measured: 0.04, 0.09, and 0.17 µg/bee). In the contact test, the applied concentrations of dimethoate were 0.10, 0.15, and 0.20 µg/bee.
Method of administration:	Oral test: The JAU 6476 SC 480 test solution and 50% syrup (sugar) solution were mixed in a 1:1 ratio. The food was offered in syringes for 1 hour of uptake. Contact test: 5 µL of test substance in solvent (tap water and 1% Adhasit) was applied to the ventral thorax of each bee using a Burkard-Applicator.
Nominal doses:	Oral test: 3.1, 7.1, 16.0, 38.0, 87.0, and 200.0 µg/bee (Actual oral concentrations were 3.6, 6.6, 18.1, 44.0, 97.9, and 232.0 µg/bee). Contact test: 3.1, 7.1, 16.0, 38.0, 87.0, and 200.0 µg/bee.
Controls: Negative control and/or diluent/solvent control	Oral test: negative control Contact test: solvent control (CO_2/tap water + Adhasit treated control, p. 15)
Number of colonies per group:	Oral test: 3 replicates; 10 bees/replicate Contact test: 3 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Contact test: Tap water and 1% Adhasit

Guideline Criteria	Reported Information
Feeding:	Oral test: Prior to test initiation, bees were starved for 30 minutes. After treatment with test solutions, bees were supplied with commercial syrup (Apiinvert), *ad libitum*. Contact test: Commercial syrup (Apiinvert) was provided *ad libitum*.
Observations period:	Oral: 4, 24, and 48 hours. Contact: 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: 0.0% negative control mortality by 48 hours. Contact test: 3.3% solvent control mortality by 48 hours.
Raw data included:	Mean replicate data were provided.
Signs of toxicity (if any) were described?	No abnormal behavior was observed.

Mortality - Oral Test

Dosage µg/bee (actual intake)	No. of bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance (Prothioconazole formulation)			
Negative Control	30	0.0	0.0
3.1 (3.6)	30	3.3	3.3
7.1 (6.6)	30	0.0	3.3
16.0 (18.1)	30	0.0	0.0
38.0 (44.0)	30	3.3	3.3
87.0 (97.9)	30	0.0	0.0
200.0 (232.0)	30	3.3	3.3
Toxic Standard (Dimethoate):			
0.04 (0.04)	30	0.0	3.3
0.08 (0.09)	30	20.0	26.7
0.15 (0.17)	30	90.0	100.0

Observations: By 48 hours, 3.3% mortality was observed in the 3.6, 6.6, 44.0, and 232.0 µg/bee treatment groups. No mortality was observed in the control, 18.1, or 97.9 µg/bee treatment groups, and no sublethal effects were observed in any treatment group during the test.

Mortality - Contact Test

Dosage (µg/bee)	No. of bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance (Prothioconazole formulation):			
Solvent control	30	3.3	3.3
3.1	30	0.0	0.0
7.1	30	0.0	0.0
16.0	30	0.0	0.0
38.0	30	0.0	0.0
87.0	30	0.0	0.0
200.0	30	0.0	0.0
Toxic Standard (Dimethoate):			
0.10	30	16.7	40.0
0.15	30	90.0	93.3
0.20	30	93.3	96.7

Observations: By 48 hours, 3.3% mortality was observed in the control, and no mortalities were observed in the treatment groups. No sublethal effects were observed in any treatment group 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 (p. 18). The NOAEL and LOAEL were determined based on mortalities and behavioral abnormalities.

Reported Statistical Results - Oral Test:

LD_{50}: >232.0 µg/bee 95% C.I.: N/A
NOAEL: 232.0 µg/bee Probit Slope: N/A

LOAEL: >232.0 µg/bee

Reported Statistical Results - Contact Test:

LD_{50}: >200.0 µg/bee 95% C.I.: N/A
NOAEL: 200.0 µg/bee Probit Slope: N/A
LOAEL: >200.0 µg/bee

14. VERIFICATION OF STATISTICAL RESULTS:

For the oral and contact toxicity tests, statistical analyses were not necessary, as there was not significant mortality. The LC_{50}/LD_{50} and NOEC estimates could be determined visually.

Results - Oral Test:

LD_{50}: >232.0 µg/bee 95% C.I.: N/A
NOAEL: 232.0 µg/bee Probit Slope: N/A
LOAEL: >232.0 µg/bee

Results - Contact Test:

LD_{50}: >200.0 µg/bee 95% C.I.: N/A
NOAEL: 200.0 µg/bee Probit Slope: N/A
LOAEL: >200.0 µg/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 >232.0 µg/bee, the highest concentration of intake. The LD_{50} value for the contact test was >200.0 µg/bee. As a result, Prothioconazole is categorized as relatively nontoxic to honeybees on both an acute oral and contact basis.

16. REFERENCES:

Chemikaliengsetz der Bunderrepublik Deutschland (ChemG), Anhang 1, in der Fassung der

Bekanntmachung vom 20. Juni 2002 (BGB1. IS. 2090).

Commission Directive 96/12/EC, amending Council Directive 91/414/EEC, 1996. Official Journal of the European Communities NO. L 65: 20-37.

Commission Directive 96/11/EC of 8 March 1999 amending Council Directive 87/18/EEC, Official Journal of the European Communities No. L 77: 8-21.

ICPBR (2000) Hazards of pesticides to bees, 7th International Symposium of the ICPBR Bee Protection Group, Avignon (France), 07-09 September 1999; Les Colloques d'INRA.

Japanese Ministry of Agriculture, Forestry and Fisheries, Guidance Document on the Good Laboratory Practice Standards for Toxicological Studies on Agricultural Chemicals, 59 NohSan Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

OECD Principles of Good Laboratory Practice , adopted by Council on 26th November 1997 [C(97)186/Final], Environment Dictorate, Organization for Economic Co-operation and Development, ENV/MC/CHEM(98)17, Paris 1998.

OECD Guideline 213 for Testing of Chemicals on Honeybees, Acute Oral Toxicity Test, adopted on 21st September 1998.

OECD Guideline 214 for Testing of Chemicals on Honeybees, Acute Contact Toxicity Test, adopted on 21st September 1998.

Thompson W.R. & Weil C.S. 1952: On the construction of tables for moving average interpolation. Biometrics 8: 51-54.

United States Environmental Protection Agency, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Title 40 Code of Federal Regulations, Part 160, Federal Register, 29 November 1983 and subsequent Amendment Federal Register 17 August 1989.

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

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EAD Assessment of USEPA DER

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

PMRA Submission Number: 2004-0844

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

Schmitzer, S. 2004. Effects of JAU 6476 SC 480 (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory. Study performed by: Institut für Bilogische Analytik und Consulting IBACON GmbH, Germany. Report no. 18351035 Submitted by: Bayer CropScience, RTP, North Carolina. February 11, 2004.

PMRA DATA CODE: 9.2.8
EPA DP Barcode: D303488
OECD Data Point: IIIA 10.4.2.1; IIIA 10.4.2.2
EPA MRID: 46246046
EPA Guideline: §141-1

Reviewing Agency: US EPA

EAD Executive Summary:

The honey bee, *Apis mellifera* L., was exposed to prothioconazole formulation (JAU6476 SC 480; 478.18 g JAU6476/L) for 48 hours in an oral and a contact toxicity test. The study was conducted according to OECD Guidelines 213 and 214 and was in compliance with principles of GLP. The nominal concentrations for the oral and contact tests were 3.1, 7.1, 16.0, 38.0, 87.0, and 200.0 µg/bee. The actual intake concentrations of Prothioconazole formulation in the oral toxicity test were 3.6, 6.6, 18.1, 44.0, 97.9, and 232.0 µg/bee. By 48 hours in the oral test, 3.3% mortality was observed in the 3.6, 6.6, 44.0, and 232.0 µg a.i./bee treatment groups. No mortality was observed in the control, 18.1, or 97.9 µg/bee treatment groups, and no sublethal effects were observed in any treatment group during the test. By 48 hours in the contact test, 3.3% mortality was observed in the control, and no mortalities were observed in the treatment groups. No sublethal effects were observed in any treatment group during the test. **The LC_{50} value for the oral test was estimated as >232.0 µg/bee, the highest concentration of intake. The LD_{50} value for the contact test was >200.0 µg/bee. As a result, Prothioconazole formulation is categorized as relatively nontoxic to honeybees on both an acute oral and**

contact basis, according to the classification scheme of Atkins *et al.* (1981). The NOELs for the oral and contact tests were 232.0 and 200.0 µg JAU6476 SC 480/bee, respectively.

Results Synopsis:

Oral Test:

LD_{50}: >232.0 µg JAU6476 SC 480/bee
NOEL: 232.0 µg JAU6476 SC 480/bee
LOEL: >232.0 µg JAU6476 SC 480/bee

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

Reported Statistical Results - Contact Test:

LD_{50}: >200.0 µg JAU6476 SC 480/bee
NOEL: 200.0 µg JAU6476 SC 480/bee
LOEL: >200.0 µg JAU6476 SC 480/bee

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

Reviewer 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) 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) available from the study report, the PMRA Chemistry review at the beginning of the DER. The name of the EAD secondary reviewer was added to the front portion of the DER and sections were renumbered accordingly.

2. This study was conducted following OECD Guidelines 213 and 214 and recommendations of ICPBR group in Avignon (1999), and was in compliance with OECD-GLP (1997), Chemikaliengesetz (Chemicals Act, Annex 1 (2002), Commission Directive 1999/11 EC of 08 March 1999 (Official Journal N° L 77/8 and was also consistent with US EPA FIFRA (40 CFR Part 160) and Japan MAFF (1984).

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.