Data Evaluation Report on the Acute Dietary Toxicity of JAU 6476 Technical (Prothioconazole) to Northern Bobwhite Quail (*Colinus virginianus*)

PMRA Submission Number 2004-0843 EPA MRID Number 46246038

Data Requirement:	PMRA DATA CODE	9.6.2.4
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.1.1
	EPA MRID	46246038
	EPA Guideline	§71-2a

Test material: JAU 6476 Technical **Purity:** 98.4%
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

Primary Reviewer: Christie E. Padova **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 8/24/04

QC Reviewer: Teri S. Myers **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 9/20/04

Primary Reviewer: Kevin Costello, Geologist **Date:**
OPP/EFED/ERB - III

Secondary Reviewer(s): Christopher J. Salice **Date:** 7/27/2005
OPP/EFED/ERB - IV Chris J. Salice 7-27-05

Secondary Reviewer: Émilie Larivière **Date:** 9/13/2005
HC/PMRA/EAD 9/13/05

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: Barfknecht, R. 2001. JAU6476 techn.: 5-Day Dietary LC50 for Bobwhite Quail (*Colinus virginianus*). Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany. Laboratory ID No. E 2951563-1; Report No. BAR/LC005. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated May 31, 1999 and completed April 18, 2001.

EXECUTIVE SUMMARY:

The acute dietary toxicity of JAU 6476 Technical (98.4% prothioconazole) to 10-day old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 8 days. JAU 6476 Technical was administered to the birds in the diet at nominal concentrations of 0 (negative control), 313, 625, 1250, 2500, and 5000 ppm. Mean-measured toxicant concentrations were 299, 622, 1215, 2380, and 4983 ppm a.i., respectively (control results not provided).

No treatment-related effects were observed with respect to mortality, clinical signs of toxicity, body weight, or feed consumption. The 8-day acute dietary LC_{50} was >4983 ppm a.i., the highest concentration tested, which categorizes JAU 6476 Technical (prothioconazole) as practically non-toxic to the Northern Bobwhite quail on an acute dietary basis. A concentration-dependent increase in the number of birds with intestinal inflammation was observed at necropsy. The NOAEC and LOAEC based on gross necropsy findings were 622 and 1215 ppm a.i., respectively.

This toxicity study is scientifically sound and fulfills the guideline requirements for an avian dietary toxicity study using the Northern Bobwhite quail (§71-2a). This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Size/Age : 10-days old; 22.5-28.0 g

LC_{50}: >4983 ppm a.i.
NOAEC: 622 ppm a.i.
LOEC: 1215 ppm a.i.
Endpoint(s) Affected: Intestinal inflammation (necropsy findings)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series §71-2 (1982) and OECD Guideline No. 205 (1984). The following deviations from guideline §71-2 were noted:

1. The cage size (3220 cm^2 floor space) was slightly less than required (3500 cm^2).
2. Results of analysis of the control feed were not provided, and the LOD and/or LOQ were not provided.
3. The photo-period was maintained on a 12-hour light/12-hour dark schedule, which is less than the minimum required photo-period of 14 hours of light/day.
4. In homogeneity experiments, the % RSD (relative standard deviation) of treated feed prepared at 313 ppm was 8.85%, exceeding the acceptable limit of 5%.

These deviations do not affect the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality

statements were provided. This study was conducted in accordance with Chemicals Law (ChemG; 1994) and OECD (1997) GLP standards.

A. MATERIALS:

1. Test Material JAU 6476 Technical (prothioconazole)

Description: White powder

Lot No./Batch No.: Fl. 6233/0031 (mixed batch)

Purity: 98.4%

Stability of Compound Under Test Conditions: Stability of the test material was assessed prior to the definitive study in treated feed prepared at 313 and 5000 ppm and stored under actual test conditions (ambient temperature) for 24 hours or under frozen conditions for 35 days. After 24 hours of storage under actual use conditions, recoveries averaged 79.3 and 101% of initial concentrations for the 313 and 5000 ppm levels, respectively (p. 11). After 35 days of frozen storage, recoveries averaged 86 and 100% of initial values, respectively.

Storage conditions of test chemicals: Room temperature

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

2. Test organism:

Species: Northern Bobwhite quail (*Colinus virginianus*)

Age at study initiation: 10 days

Weight at study initiation: 22.5-28.0 g; group mean body weights of 24.5-26.0 g

Source: Eggs were obtained from Morris Quail Farm, Goulds, FL and hatched in the laboratory.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None reported. It was reported that the standard dosage range of 313-5000 ppm was selected for use in the definitive study since previous dietary toxicity data for Bobwhite Quail were not available (p. 10).

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	10 days Same as test Water and standard commercial quail diet [type 0719A-Extrudat (mash form), batch no. 101099/1151 from Altromin GmbH, Lage, Germany] were provided, *ad libitum*. One chick died during acclimation and was replaced with an acclimated chick from the reserve group.	Diet composition and results from analyses for food contaminants are provided in Appendices IV and V, pp. 26-27.
Pen size and construction materials	Stainless steel wire mesh battery cages, 70 x 46 x 20 cm.	The cage size (3220 cm^2 floor space) was slightly less than required (3500 cm^2) by §71-2, but is acceptable according to EPA OPPTS 850.2200. *EPA requires: about 35 x 100 x 24 cm*
Test duration	5 days with treated feed, and 3 days with"clean" feed (recovery period).	*EPA requires: 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal: measured:	 0 (negative control), 313, 625, 1250, 2500, and 5000 ppm a.i. 299, 622, 1215, 2380, and 4983 ppm a.i. (results of control analysis not reported)	Mean-measured concentrations were provided on p. 12. Recoveries were 95-100% of nominal concentrations. *Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 ppm.*

Parameter	Details	Remarks *Criteria*
Solvent/vehicle, if used type: amount:	None used	*EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.*
Diet preparation and feeding	The appropriate amount of the test substance was directly admixed to the basal diet. No vehicles were used.	*EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets?*
Feed withholding period	None	
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes	
Number of birds per replicate/group for negative control: for vehicle control: for treated:	 20 N/A 10	*EPA requires: 10 (strongly recommended)*
Number of replicates/group (if used) for negative control: for vehicle control: for treated:	 2 N/A 1	
Test conditions temperature: relative humidity(%): photoperiod:	 room: 27-30°C cages: 27.8-39.0°C 35-55% 12 hour light/12 hour dark photo-period	Light intensity was 10-15 lux. ***Brooder temperature:*** *about 35°C (95°F)* ***Room temperature:*** *22-27°C (71-81°F)* ***Relative humidity:*** *30-80%* ***Photoperiod:*** *Minimum of 14 h of light.*
Reference chemical, if used	None used.	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured (mortality/body weight/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Body weights - Feed consumption - Necropsy	
Indicate the stability and homogeneity of test chemical in the diet	Stability: Verified. Stability of the test material was assessed in treated feed prepared at 313 and 5000 ppm and stored under actual test conditions (ambient temperature) for 24 hours or under frozen conditions for 35 days. After 24 hours of storage under actual use conditions, recoveries averaged 79.3 and 101% of initial concentrations for the 313 and 5000 ppm levels, respectively (p. 11). After 35 days of frozen storage, recoveries averaged 86 and 100% of initial values, respectively. Homogeneity: Verified at the highest test level only. Homogeneity (five points from each batch) was assessed in treated feed prepared at 313 and 5000 ppm. Coefficients of variation (RSD) were 8.85 and 1.45%, respectively (p. 11).	Stability and homogeneity assessments were performed prior to the definitive test in treated feed prepared at 313 (low) and 5000 (high) ppm (pp. 10-11). The RSD of treated feed prepared at 313 ppm exceeded the acceptable limit of 5%.
Indicate if the test material was regurgitated	Regurgitation was not reported.	
Treatments on which necropsies were performed	All birds were subject to gross necropsy.	

Observation intervals	Mortality and signs of intoxication were observed twice daily on the first day of exposure, and at least once daily thereafter. Food consumption was determined daily, and body weights were recorded on Days 0, 5, and 8.	
Were raw data included?	Yes, adequate.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred during the study in any control or treatment group (Table 1, p. 18). The 8-day LC_{50} was >5000 ppm a.i.

Table 3: Effect of JAU 6476 Technical (prothioconazole) on mortality of Northern Bobwhite quail.

Treatment, ppm a.i. mean-measured (and nominal)		No. of birds per treatment	Cumulative mortality							
			Days							
			1	2	3	4	5	6	7	8
Control		20	0	0	0	0	0	0	0	0
299 (313)		10	0	0	0	0	0	0	0	0
622 (625)		10	0	0	0	0	0	0	0	0
1215 (1250)		10	0	0	0	0	0	0	0	0
2380 (2500)		10	0	0	0	0	0	0	0	0
4983 (5000)		10	0	0	0	0	0	0	0	0
NOAEC		5000 ppm a.i.								
LC_{50}		>5000 ppm a.i.								
Reference chemical:	mortality	N/A								
	LC_{50}	N/A								
	NOAEC	N/A								

B. SUB-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed (Table 2, p. 18).

No treatment-related effect on body weight changes were observed (p. 15). Although slight, statistically-significant reduction in body weight changes were observed during the exposure period (Days 0-5) at the 313, 1250, and 5000 ppm a.i. levels, these differences were slight, and did not exhibit a dose-dependent relationship (Table 3, p. 18).

No treatment-related effect on food consumption was evident (p. 15). The slight variations in feeding rates in the course of the study showed neither a dose-dependancy nor any continuity over time, and were considered as being within natural variation (Tables 4 and 5, p. 19). Calculated mean test substance intakes were 2.1, 4.2, 9.0, 18.7, and 45.2 mg/bird/day for the 313, 625, 1250, 2500, and 5000 ppm a.i. treatment groups, respectively.

The incidence of inflamed/red areas on the intestinal wall increased from 1/10 birds grossly examined at the 1250 ppm a.i. level, to 3/10 at the 2500 ppm a.i. level, and 6/10 at the 5000 ppm a.i. level (Table 6, p. 20). These findings were considered to be related to treatment, but did not cause any observable impacts on nutritional condition and behavior of the birds (p. 15).

Table 4: Sub-lethal effects of JAU 6476 Technical (prothioconazole) on Northern Bobwhite quail.

Treatment, ppm a.i. mean-measured (and nominal)		Observation				
		Mean Body Weight, g (and Body Weight Changes, %)			Food consumption (g/bird/day)	
		Day			Day	
		0	5	8	0-4	5-7
Control		25.6	42.4 (+65.6%)	47.3 (+11.4%)	8.4	12.0
299 (313)		25.7	38.9 (+51.2%)*	45.6 (+17.3%)	7.1	9.2
622 (625)		24.8	40.3 (+62.4%)	49.0 (+21.7)	6.7	8.5
1215 (1250)		25.2	39.4 (+56.1%)*	47.5 (+20.5%)	7.4	10.9
2380 (2500)		26.0	42.3 (+62.7%)	49.5 (+17.0%)	7.8	11.9
4983 (5000)		24.5	39.5 (+61.4%)*	47.8 (+21.1%)	9.1	10.5
NOAEC		5000 ppm				
EC_{50}		Not reported				
Reference chemical	NOAEC	N/A				
	EC_{50}	N/A				

* Statistically-significant reduction compared to control.

C. REPORTED STATISTICS:

The LC_{50} could not be calculated because mortality did not exceed 50% at any test level. Body weight data were compared first for equal variance using Bartlett's test. If the variances were equal (parametric), then the data were analyzed using ANOVA. If the variances were not equal (non-parametric), then the data were analyzed using the Mann-Whitney/Wilcoxon test. Analyses were performed with the aide of STATGRAPHICS-Plus statistical software. Feed consumption data were not analyzed statistically, as there were no replicate data. Nominal concentrations were reported by the study author.

LC_{50}: >5000 ppm
NOAEC: 625 ppm
LOEC: 1250 ppm
Endpoint(s) Affected: Intestinal inflammation (necropsy findings)

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not conducted because mortality did not exceed 50% at any test level and toxicity values for the remaining endpoints could be verified visually.

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA Guideline §71-2 that affected the validity or acceptability of this study. Although a slightly higher than acceptable level of variance was observed in homogeneity assessment of feed prepared at 313 ppm a.i., the difference was only slightly higher than acceptable, and feed prepared at 5000 ppm a.i. was homogeneously mixed.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's.

Although the mean-measured concentration was actually less than the limit concentration of 5000 ppm, it was obvious from that data that an accurate Toxicity Category could be assigned with the data obtained with a toxicant level of 4983 ppm a.i.

G. CONCLUSIONS:

This toxicity study is scientifically sound, fulfills the guideline requirements for an avian dietary study using the Northern Bobwhite quail (§71-2a), and is classified as ACCEPTABLE. Based on the results of this study, JAU 6476 Technical (prothioconazole) is categorized as practically non-toxic to Northern Bobwhite quail on an acute dietary basis.

LC_{50}: >4983 ppm a.i.
NOAEC: 622 ppm a.i.
LOEC: 1215 ppm a.i.
Endpoint(s) Affected: Intestinal inflammation (necropsy findings)

III. REFERENCES:

Statgraphics Plus for Windows. 1994-1996. Version 2.1, Serial No. 3869060. Statistical Graphics Corporation, Rockville, MD 20852, USA.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: September 13, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Dietary Toxicity to Bobwhite Quail

Barfknecht, R. 2001. JAU6476 techn.: 5-Day Dietary LC50 for Bobwhite Quail (*Colinus virginianus*). Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany. Laboratory ID No. E 2951563-1; Report No. BAR/LC005. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated May 31, 1999 and completed April 18, 2001.

PMRA DATA CODE: 9.6.2.4
EPA DP Barcode: D303488
OECD Data Point: IA 8.1.1
EPA MRID: 46246038
EPA Guideline: §71-2a

Reviewing Agency: US EPA

EAD Executive Summary:

The acute dietary toxicity of JAU 6476 Technical (98.4% prothioconazole) to 10-day old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 8 days. The study was conducted following U.S. EPA Pesticide Assessment Guidelines, Series §71-2 (1982) and OECD Guideline No. 205 (1984) and was in compliance with German and OECD Principles of GLP. JAU 6476 Technical was administered to the birds in the diet at nominal concentrations of 0 (negative control), 313, 625, 1250, 2500, and 5000 mg/kg food. Mean measured toxicant concentrations were 299, 622, 1215, 2380, and 4983 mg a.i./kg food, respectively (control results not provided).

No treatment-related effects were observed with respect to mortality, clinical signs of toxicity, body weight, or feed consumption. The NOEC based on these endpoints is 622 mg a.i./kg food. The 8-day acute dietary LC_{50} was >4983 mg a.i./kg food, the highest concentration tested, which categorizes prothioconazole as practically non-toxic to the Northern Bobwhite quail on an acute dietary basis, according to the classification scheme of the U.S. EPA. A concentration-dependent increase in the number of birds with intestinal inflammation was observed at necropsy. The NOEC and LOEC based on gross necropsy findings were 622 and 1215 mg a.i./kg food,

respectively.

Results Synopsis

Test Organism Size/Age : 10-days old; 22.5-28.0 g

LC_{50}: >4983 mg a.i./kg food
NOEC (mortality): 4983 mg a.i./kg food
LOEC (mortality): >4983 mg a.i./kg food
NOEC: 622 mg a.i./kg food
LOEC: 1215 mg a.i./kg food
Endpoint(s) Affected: Intestinal inflammation (necropsy findings)

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 and synonym) available from the PMRA Chemistry review.

2. OECD Guideline 205 recommends having 12 to 16 hours of light per day, therefore, the lighting used in this study is acceptable..

3. U.S. EPA OPPTS 850.2200 and OECD Guideline 205 require a minimum floor area of 300 cm^2/bird for bobwhite quail. The size of the pens used in this study is therefore acceptable.

4. The EAD reviewer agrees with the conclusions of the study author and the EPA reviewer.

Study Acceptability: This toxicity study is scientifically sound and fulfills the guideline requirements for an avian dietary toxicity study using the Northern Bobwhite quail. This study is classified as ACCEPTABLE.