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

PMRA Submission Number 2004-0843 EPA MRID Number 46246039

| | | |
|---|---|---|
| **Data Requirement:** | PMRA DATA CODE | 9.6.2.4 |
| | EPA DP Barcode | D303488 |
| | OECD Data Point | IIA 8.1.1 |
| | EPA MRID | 46246039 |
| | EPA Guideline | §71-2a |

**Test material:** JAU 6476 - Desthio **Purity:** 96.8%
Common name: Prothioconazole - Desthio
Chemical name: IUPAC: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1,2,4-triazole-1-yl)-propan-2-ol
CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole
CAS No.: 120983-64-4
Synonyms: SXX 0665

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

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

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

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

**Secondary Reviewer:** Émilie Larivière
HC/PMRA/EAD
**Date:** 9/14/2005 9/14/05

**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-Desthio.: 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 Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany.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 2951564-2; Report No. BAR/LC011. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated March 29, 2000 and completed April 19, 2001.

## EXECUTIVE SUMMARY:

The acute dietary toxicity of JAU 6476 - Desthio (96.8% prothioconazole - desthio) to 10-day old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 8 days. JAU 6476 - Desthio 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 321, 639, 1243, 2577, and 5215 ppm a.i., respectively (control results not provided).

Cumulative mortality was 0% in the control and ≤1243 ppm a.i. groups, 10% in the 2577 ppm a.i. group, and 70% in the 5215 ppm a.i. group. The 8-day $LC_{50}$ was 4252 ppm (3161-6501 ppm), which categorizes JAU 6476 - Desthio (prothioconazole - desthio) as slightly toxic to the Northern Bobwhite quail on an acute dietary basis.

In addition to mortality, treatment-related effects were observed in clinical effects (reduced vigilance), body weight, and food consumption parameters at the 2577 and 5215 ppm a.i. test levels. Reduced vigilance subsided from all surviving birds by Day 6. Calculated mean test substance intakes were 3.0, 5.3, 10.4, 10.7, and 15.2 mg/bird/day for the nominal 313, 625, 1250, 2500, and 5000 ppm a.i. treatment groups, respectively.

Aside from empty stomach/intestines (ultimately causing starvation) in 1/10 birds from the 2500 ppm a.i. group and 6/10 birds from the 5000 ppm a.i. group, no clear treatment-related effects were observed at necropsy.

This toxicity study is scientifically sound and fulfills the guideline requirements for an avian dietary toxicity study using JAU 6476 - Desthio and the Northern Bobwhite quail (§71-2a). This study is classified as ACCEPTABLE.

**Results Synopsis**

Test Organism Size/Age : 10-days old; 18.2-33.7 g

$LC_{50}$: 4252 ppm 95% C.I.: 3161-6501 ppm
Probit slope: 5.96 (1.80-10.13)
NOAEC: 1243 ppm a.i.
LOAEC: 2577 ppm a.i.
Sub-lethal effects:
NOAEC: 1243 ppm a.i.
LOAEC: 2577 ppm a.i.

Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weight, food consumption (same conclusions)

## 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 brooder temperature range (25-32°C) was less than recommended (about 35°C).

4. 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.

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 - Desthio (prothioconazole - desthio)

**Description:** White crystals

**Lot No./Batch No.:** RUX76-105/9 (mixed batch)

**Purity:** 96.8%

**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 98 and 99% of initial concentrations for the 313 and 5000 ppm levels, respectively (p. 11). After 35 days of frozen storage, recoveries averaged 100 and 99% 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:** 18.2-33.7 g; group mean body weights of 22.6-27.8 g

**Source:** Eggs were obtained from Geflügelzucht H. & E. Küberich (Wiesentheid, Germany) 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<br>*Criteria* |
|---|---|---|
| Acclimation period:<br><br>Conditions (same as test or not):<br><br>Feeding:<br><br><br><br><br>Health (any mortality observed): | 10 days<br><br>Same as test<br><br>Water and standard commercial quail diet [type 0719A-Extrudat (mash form), batch no. 021200/1441 from Altromin GmbH, Lage, Germany] were provided, *ad libitum*.<br><br>No mortality was observed during acclimation. | Diet composition and results from analyses for food contaminants are provided in Appendices IV and V, pp. 27-28. |
| 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 $cm^2$).<br>*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<br>nominal:<br><br><br>measured: | <br>0 (negative control), 313, 625, 1250, 2500, and 5000 ppm a.i.<br><br>321, 639, 1243, 2577, and 5215 ppm a.i. (results of control analysis not reported) | Mean-measured concentrations were provided on p. 12. Recoveries were 99-104% of nominal concentrations.<br>*Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless $LC_{50}$ > 5000 ppm.* |

| Parameter | Details | Remarks<br>*Criteria* |
|---|---|---|
| Solvent/vehicle, if used<br>type:<br><br>amount: | None used | <br><br>*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. | <br>*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<br>for negative control:<br>for vehicle control:<br>for treated: | <br>20<br>N/A<br>10 | <br><br>*EPA requires: 10 (strongly recommended)* |
| Number of replicates/group (if used)<br>for negative control:<br>for vehicle control:<br>for treated: | <br>2<br>N/A<br>1 | |
| Test conditions<br>temperature:<br><br>relative humidity(%):<br><br>photoperiod: | <br>room: Not specified<br>cages: 25-32°C<br><br>50-75%<br><br>12 hour light/12 hour dark photo-period | Light intensity averaged 42 lux.<br><br>***Brooder temperature:***<br>*about 35°C (95°F)*<br>***Room temperature:***<br>*22-27°C (71-81°F)*<br>***Relative humidity:***<br>*30-80%*<br>***Photoperiod:***<br>*Minimum of 14 h of light.* |
| Reference chemical, if used | None used. | |

**2. Observations:**

**Table 2: Observations**

| Criteria | Details | Remarks<br>*Criteria* |
|---|---|---|
| Parameters measured (mortality/body weight/ mean feed consumption/ others) | - Mortality<br>- Clinical signs of toxicity<br>- Body weights<br>- Feed consumption<br>- 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 98 and 99% of initial concentrations for the 313 and 5000 ppm levels, respectively (p. 11). After 35 days of frozen storage, recoveries averaged 100 and 99% of initial values, respectively.<br><br>Homogeneity: Verified. Homogeneity (five points from each batch) was assessed in treated feed prepared at 313 and 5000 ppm. Coefficients of variation (RSD) were 0.99 and 0.64%, 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. 10-12). |
| 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:

Cumulative mortality was 0% in the control group, 0% in the ≤1250 ppm a.i. groups, 10% in the 2500 ppm a.i. group, and 70% in the 5000 ppm a.i. group (Table 1, p. 19). The 8-day $LC_{50}$ was 4090 ppm a.i. (confidence intervals not provided).

**Table 3: Effect of JAU 6476 - Desthio (prothioconazole - desthio) on mortality of Northern Bobwhite quail.**

| Treatment, ppm a.i. mean-measured (and nominal) | | No. of birds per treatment | Cumulative mortality | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Days | | | | | | | |
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 321 (313) | | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 639 (625) | | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1243 (1250) | | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2577 (2500) | | 10 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 5215 (5000) | | 10 | 0 | 0 | 0 | 1 | 2 | 6 | 7 | 7 |
| NOAEC | | 1250 ppm a.i. | | | | | | | | |
| $LC_{50}$ | | 4090 ppm a.i. | | | | | | | | |
| Reference chemical: | mortality | N/A | | | | | | | | |
| | $LC_{50}$ | N/A | | | | | | | | |
| | NOAEC | N/A | | | | | | | | |

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

Reduced vigilance was observed in 1 bird from the 2500 ppm a.i. group and in all birds from the 5000 ppm a.i. group (p. 15 and Table 3, p. 19). All surviving birds appeared normal by Day 6. The NOAEC for sub-lethal signs of toxicity was 1250 ppm a.i.

The body weights of birds in the 5000 ppm a.i. group decreased during the exposure period, and the body weights of birds in the 2500 ppm a.i. group were statistically-reduced compared to controls (p. 15 and Table 4, p. 20). During the recovery period, the body weights of birds from both groups increased, but remained below the values of the other groups. The NOAEC for body weight changes was 1250 ppm a.i.

Food consumption was adversely affected at the 2500 and 5000 ppm a.i. test levels (p. 15 and Tables 5 and 6, pp. 20-21). During the recovery period, surviving birds from these groups consumed similar or greater amounts of feed. Calculated mean test substance intakes were 3.0, 5.3, 10.4, 10.7, and 15.2 mg/bird/day for the 313, 625, 1250, 2500, and 5000 ppm a.i. treatment groups, respectively. The NOAEC for food consumption was 1250 ppm a.i.

Aside from empty stomach/intestines (ultimately causing starvation, p. 15) in 1/10 birds from the 2500 ppm a.i. group and 6/10 birds from the 5000 ppm a.i. group, no clear treatment-related effects were observed at necropsy (Table 7, p. 21). An enlarged gall bladder was observed in 1/10 birds from the 5000 ppm a.i. group. No other gross abnormalities were observed.

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

| Treatment, ppm a.i. mean-measured (and nominal) | Observation | | | | |
|---|---|---|---|---|---|
| | Mean Body Weight ± standard deviation, g (and Body Weight Changes, %) | | | Food consumption (g/bird/day) | |
| | Day | | | Day | |
| | 0 | 5 | 8 | 0-4 | 5-7 |
| Control | 26.0±2.6 | 35.2±4.2 (+35.0%) | 49.1±5.8 (+39.6%) | 6.3 | 10.2 |
| 321 (313) | 24.1±2.0 | 37.8±3.6 (+57.1%) | 51.5±4.2 (+36.1%) | 9.8 | 9.7 |
| 639 (625) | 26.8±2.1 | 36.8±3.2 (+37.1%) | 51.8±4.4 (+40.9) | 8.3 | 13.4 |
| 1243 (1250) | 23.3±2.2 | 30.2±5.4 (+29.7%) | 43.5±6.1 (+44.2%) | 6.4 | 8.5 |
| 2577 (2500) | 23.9±2.3 | 28.1±4.0 (+17.7%)* | 40.2±5.2 (+43.0%) | 4.1 | 7.9 |
| 5215 (5000) | 22.6±2.2 | 20.6±3.5 (-9.1%)* | 34.1±3.2 (+65.9%) | 2.9 | 11.8 |
| NOAEC | 1250 ppm a.i. | | | 1250 ppm a.i. | |
| $EC_{50}$ | Not reported | | | | |
| Reference chemical NOAEC | N/A | | | | |
| Reference chemical $EC_{50}$ | N/A | | | | |

* Statistically-significant reduction compared to control.

## C. REPORTED STATISTICS:

The $LC_{50}$ was calculated by Probit analysis via Toxcalc statistical software. 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 used in all calculations.

$LC_{50}$: 4090 ppm 95% C.I.: Not reported
Probit slope: 6.1
NOAEC: 1250 ppm
LOAEC: 2500 ppm
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weight, food consumption (same conclusions)

## D. VERIFICATION OF STATISTICAL RESULTS:

The $LC_{50}$ was determined using the Probit method via TOXANAL statistical software. The percent change body weight over the exposure period was calculated using EXCEL. These data passed the normality test and the homegeneity of variance test and were compared using ANOVA via TOXSTAT statistical software. Feed consumption data were not analyzed statistically, as there were no replicate data.

$LC_{50}$: 4252 ppm 95% C.I.: 3161-6501 ppm
Probit slope: 5.96 (1.80-10.13)
NOAEC (body weight): 1243 ppm a.i.
LOAEC (body weight): 2577 ppm a.i.
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weight, food consumption

## E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA Guideline §71-2 that affected the validity or acceptability of this study.

## F. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the NOAEC and LOAEC were similar to the study author's. These were reported based on mean-measured exposure levels. The reviewer's $LC_{50}$ value is reported because it also had an associated 95% confidence interval and slope value.

## 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 - Desthio (prothioconazole - desthio) is categorized as slightly toxic to Northern Bobwhite quail on an acute dietary basis.

$LC_{50}$: 4252 ppm 95% C.I.: 3161-6501 ppm
Probit slope: 5.96 (1.80-10.13)
NOAEC: 1243 ppm a.i.
LOAEC: 2577 ppm a.i.
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weight, food consumption (same conclusions)

## III. REFERENCES:

Toxcalc V. 1.0 by Stephan, C.E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, MN. Personal Communication to Dr. Lowell Bahner, Chairman, ASTM Task Group on Calculating LC50.

Stephan, C.E. 1977. Methods for Calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F.L. Mayer and J.L. Hamelink, eds. American Society for Testing and Materials, Philadelphia, PA p. 65-84.

Box, G.E.P., W.G. Hunter, and J.S. Hunter. 1987. *Statistics for Experimenters.* New York: Wiley.

Neter, J., W. Wassermann, and M. Kutner. 1985. *Applied Linear Statistical Methods.* Homewood, IL: Richard E. Irwin, Inc.

Hollander, J., and D.A. Wolfe. 1973. Nonparametric Statistical Methods. New York: Wiley.

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

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

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4184.797

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 1 | .5098017 | 4184.792 | 3193.894 | 7221.137 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 12 | .4879053 | 1 | .9998319 |

SLOPE = 5.963409
95 PERCENT CONFIDENCE LIMITS = 1.797955 AND 10.12886

LC50 = 4251.58
95 PERCENT CONFIDENCE LIMITS = 3161.316 AND 6501.45

LC10 = 2603.665
95 PERCENT CONFIDENCE LIMITS = 886.7872 AND 3414.407

prothio desthio bobwhite change in body weight
File: dbobbw2.dat Transform: NO TRANSFORMATION

ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 5 | 13787.815 | 2757.563 | 28.757 |
| Within (Error) | 57 | 5465.835 | 95.892 | |
| Total | 62 | 19253.650 | | |

Critical F value = 2.45 (0.05,5,40)
Since F > Critical F REJECT Ho: All equal

prothio desthio bobwhite change in body weight
File: dbobbw2.dat Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | control | 35.920 | 35.920 | | |
| 2 | 313 | 57.250 | 57.250 | -5.624 | |
| 3 | 625 | 37.060 | 37.060 | -0.301 | |
| 4 | 1250 | 29.250 | 29.250 | 1.759 | |
| 5 | 2500 | 17.589 | 17.589 | 4.664 | * |
| 6 | 5000 | -3.400 | -3.400 | 7.331 | * |

Bonferroni t table value = 2.40 (1 Tailed Value, P=0.05, df=50,5)

prothio desthio bobwhite change in body weight
File: dbobbw2.dat Transform: NO TRANSFORMATION

BONFERRONI t-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 | 20 | | | |
| 2 | 313 | 10 | 9.115 | 25.4 | -21.330 |
| 3 | 625 | 10 | 9.115 | 25.4 | -1.140 |
| 4 | 1250 | 10 | 9.115 | 25.4 | 6.670 |
| 5 | 2500 | 9 | 9.446 | 26.3 | 18.331 |
| 6 | 5000 | 4 | 12.890 | 35.9 | 39.320 |

prothio desthio bobwhite change in body weight
File: dbobbw2.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | control | 20 | 35.920 | 35.920 | 43.030 |
| 2 | 313 | 10 | 57.250 | 57.250 | 43.030 |
| 3 | 625 | 10 | 37.060 | 37.060 | 37.060 |
| 4 | 1250 | 10 | 29.250 | 29.250 | 29.250 |
| 5 | 2500 | 9 | 17.589 | 17.589 | 17.589 |
| 6 | 5000 | 4 | -3.400 | -3.400 | -3.400 |

prothio desthio bobwhite change in body weight
File: dbobbw2.dat 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 | 43.030 | | | | |
| 313 | 43.030 | 1.875 | * | 1.68 | k= 1, v=57 |
| 625 | 37.060 | 0.301 | | 1.76 | k= 2, v=57 |
| 1250 | 29.250 | 1.759 | | 1.79 | k= 3, v=57 |
| 2500 | 17.589 | 4.664 | * | 1.80 | k= 4, v=57 |
| 5000 | -3.400 | 7.331 | * | 1.80 | k= 5, v=57 |

s = 9.792
Note: df used for table values are approximate when v > 20.

**EAD Assessment of USEPA DER**

Reviewer: Émilie Larivière (#1269); PMRA Date: September 14, 2005

**PMRA Submission Number:** 2004-0843

**Study Type:** Acute Dietary Toxicity to Bobwhite Quail

Barfknecht, R. 2001. JAU6476-Desthio.: 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 2951564-2; Report No. BAR/LC011. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated March 29, 2000 and completed April 19, 2001.

PMRA DATA CODE: 9.6.2.4
EPA DP Barcode: D303488
OECD Data Point: IIA 8.1.1
EPA MRID: 46246039
EPA Guideline: §71-2a

**Reviewing Agency:** US EPA

**EAD Executive Summary:**

The acute dietary toxicity of the transformation product JAU 6476-desthio (purity 96.8%) to 10-day old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 8 days. JAU 6476-desthio was administered to the birds in the diet at nominal concentrations of 0 (negative control), 313, 625, 1250, 2500, and 5000 mg/kg. Mean measured toxicant concentrations were 321, 639, 1243, 2577, and 5215 mg JAU6476-desthio/kg food, respectively (control results not provided). 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.

Cumulative mortality was 0% in the control and ≤1243 mg JAU6476-desthio/kg food groups, 10% in the 2577 mg JAU6476-desthio/kg food group, and 70% in the 5215 mg JAU6476-desthio/kg food group. The 8-day $LC_{50}$ was 4252 mg JAU6476-desthio/kg food (3161-6501 mg JAU6476-desthio/kg food), which categorizes JAU 6476-desthio as slightly toxic to the Northern Bobwhite quail on an acute dietary basis, according to the classification scheme of the U.S. EPA (1985).

In addition to mortality, treatment-related effects were observed in clinical effects (reduced vigilance), body weight, and food consumption parameters at the 2577 and 5215 mg JAU6476-desthio/kg food test levels. Reduced vigilance subsided from all surviving birds by Day 6. Calculated mean test substance intakes were 3.0, 5.3, 10.4, 10.7, and 15.2 mg/bird/day for the 321, 639, 1243, 2577, and 5215 mg JAU6476-desthio/kg food treatment groups, respectively.

Aside from empty stomach/intestines (ultimately causing starvation) in 1/10 birds from the 2577 mg JAU6476-desthio/kg food group and 6/10 birds from the 5215 mg JAU6476-desthio/kg food group, no clear treatment-related effects were observed at necropsy.

**Results Synopsis**

Test Organism Size/Age : 10-days old; 18.2-33.7 g

$LC_{50}$: 4252 mg JAU6476-desthio/kg food 95% C.I.: 3161-6501 mg JAU6476-desthio/kg food

Probit slope: 5.96 (1.80-10.13)
NOEC: 1243 mg JAU6476-desthio/kg food
LOEC: 2577 mg JAU6476-desthio/kg food
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weight, food consumption (same conclusions)

**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 (CAS number).

2. The standard deviation was added to the Table with body weight data, to include information on the variation within treatments.

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

4. 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 pens is therefore acceptable.

4. U.S. EPA OPPTS 850.2200 requires a temperature gradient in the pen of approximately 38 to

approximately 22 °C in order to allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. OECD Guideline 205 recommends temperature ranges for bobwhite quail which span from 25 to 38°C, depending on the age of the birds. The temperature in the test is therefore acceptable.

5. The EAD reviewer calculated the percent weight change for exposure and verified the differences between treatments and control by doing an ANOVA. Assumptions of equal variance and normality were met, allowing for parametric analyses. A significant difference was observed between the control and the 321, 2577, and 5215 mg JAU6476-desthio/kg food treatment levels. The difference observed at the 321 mg/kg diet group is dismissed, as the effect is in the opposite direction as that expected (there is an greater weight increase compared to the control). The NOEC is therefore 1243 mg JAU6476-desthio/kg diet, identical to that reported by the study author.

6. The EAD verified the $LC_{50}$ using probit analysis and obtained identical results to those reported by 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.

## Statistical Verification of the EAD Reviewer:

### Percent weight change during exposure

One Way Analysis of Variance Wednesday, September 14, 2005, 11:25:32

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.095)

Equal Variance Test: Passed (P = 0.062)

| Group Name | N | Missing | Mean | Std Dev | SEM |
|---|---|---|---|---|---|
| control | 20 | 0 | 35.922 | 6.014 | 1.345 |
| 313 mg/kg diet | 10 | 0 | 57.244 | 9.370 | 2.963 |
| 625 mg/kg diet | 10 | 0 | 37.058 | 2.095 | 0.663 |
| 1250 mg/kg diet | 10 | 0 | 29.255 | 16.857 | 5.331 |
| 2500 mg/kg diet | 10 | 1 | 17.589 | 13.053 | 4.351 |
| 5000 mg/kg diet | 10 | 6 | -14.279 | 13.164 | 6.582 |

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Between Groups | 5 | 17422.872 | 3484.574 | 33.342 | <0.001 |
| Residual | 57 | 5957.135 | 104.511 | | |
| Total | 62 | 23380.007 | | | |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Multiple Comparisons versus Control Group (Bonferroni t-test):

Comparisons for factor: treatments

| Comparison | Diff of Means | t | P | P<0.050 |
|---|---|---|---|---|
| control vs. 5000 mg/kg diet | 50.201 | 8.965 | <0.001 | Yes |
| control vs. 313 mg/kg diet | 21.321 | 5.385 | <0.001 | Yes |
| control vs. 2500 mg/kg diet | 18.333 | 4.468 | <0.001 | Yes |
| control vs. 1250 mg/kg diet | 6.668 | 1.684 | 0.488 | No |
| control vs. 625 mg/kg diet | 1.136 | 0.287 | 1.000 | Do Not Test |

A result of "Do Not Test" occurs for a comparison when no significant difference is found between two means that enclose that comparison. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed means is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the means, even though one may appear to exist.

EPA PROBIT ANALYSIS PROGRAM
USED FOR CALCULATING LC/EC VALUES
Version 1.5

avian dietary desthio

| Conc. | Number Exposed | Observed Number Resp. | Proportion Responding | Predicted Proportion Adjusted for Controls | Proportion Responding |
|---|---|---|---|---|---|
| 321.0000 | 10 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 639.0000 | 10 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 1243.0000 | 10 | 0 | 0.0000 | 0.0000 | 0.0007 |
| 2577.0000 | 10 | 1 | 0.1000 | 0.1000 | 0.0974 |
| 5215.0000 | 10 | 7 | 0.7000 | 0.7000 | 0.7016 |

Chi - Square for Heterogeneity (calculated) = 0.008
Chi - Square for Heterogeneity
(tabular value at 0.05 level) = 7.815

Mu = 3.628554
Sigma = 0.167713

| Parameter | Estimate | Std. Err. | 95% Confidence Limits |
|---|---|---|---|
| Intercept | -16.635509 | 7.654904 | ( -31.639122, -1.631897) |
| Slope | 5.962571 | 2.124824 | ( 1.797915, 10.127227) |

Theoretical Spontaneous Response Rate = 0.0000

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Estimated LC/EC Values and Confidence Limits

| Point | Exposure Conc. | 95% Confidence Limits Lower | Upper |
|---|---|---|---|
| LC/EC 1.00 | 1731.425 | 239.746 | 2567.815 |
| LC/EC 5.00 | 2252.595 | 561.043 | 3066.167 |

| | | | |
|---|---|---|---|
| LC/EC 10.00 | 2591.869 | 874.500 | 3402.308 |
| LC/EC 15.00 | 2849.287 | 1170.896 | 3677.961 |
| LC/EC 50.00 | 4251.614 | 3161.340 | 6501.857 |
| LC/EC 85.00 | 6344.122 | 4788.181 | 20489.037 |
| LC/EC 90.00 | 6974.208 | 5131.729 | 27670.748 |
| LC/EC 95.00 | 8024.628 | 5652.606 | 43448.688 |
| LC/EC 99.00 | 10440.094 | 6706.868 | 102325.531 |