Data Evaluation Report on the Acute Oral Toxicity of SXX 0665 Technical (Prothioconazole - Desthio) on Northern Bobwhite Quail (*Colinus virginianus*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246037

Data Requirement:		
	PMRA DATA CODE	9.6.2.1
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.1.1
	EPA MRID	46246037
	EPA Guideline	§71-1

Test material: SXX 0665 Technical **Purity:** 93.7%
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: JAU 6476 - Desthio; SXX0665

Primary Reviewer: Christie E. Padova **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 8/20/04

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 7-27-05

Secondary Reviewer: Émilie Lanvière **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: Grau, R. 1990. SXX 0665 (Technical Grade) Acute Oral LD50 to Bobwhite Quail. Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany. Laboratory ID No. E2920414-2; Report No. VB-009. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated April 30, 1990 and completed November 30, 1990.

EXECUTIVE SUMMARY:

The acute oral toxicity of SXX 0665 Technical (93.7% prothioconazole - desthio) to 30- to 35-week old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 14 days. SXX 0665 Technical was administered to the birds via gelatin capsule at nominal concentrations of 0 (negative control), 125, 250, 500, 1000, and 2000 mg a.i./kg bw.

Mortality was 30% (one male and two females) at the 2000 mg a.i./kg bw level. The mortality observed was delayed, with two birds found dead on Day 6, and one bird found dead on Day 12. The 14-day LD_{50} was >2000 mg a.i./kg bw, which categorizes SXX 0665 Technical (prothioconazole - desthio) as practically non-toxic to Northern Bobwhite quail on an acute oral basis.

Apathy and/or fluffed feathers were observed in birds dosed at 1000 and 2000 mg a.i./kg bw. In the 1000 mg a.i./kg bw dose group, fluffed feathers were observed in 2/10 birds on Day 0, and apathy was observed in one bird on Day 7. In the 2000 mg a.i./kg bw dose group, fluffed feathers and apathy were observed in single birds on Day 0, and apathy was observed in 6/10 birds from Days 1-3, and 6/8 surviving birds from Days 7-9. The NOAEL for sub-lethal effects was 500 mg a.i/kg bw.

Treatment-related reductions in body weight changes were observed during the exposure period (days 0-7) at the 1000 mg a.i./kg bw and higher levels for the males and at the 500 mg a.i./kg bw and higher levels for the females. On Day 7 for the 0, 125, 250, 500, 1000, and 2000 mg a.i./kg bw dose groups, body weights averaged 181.0, 184.4, 177.4, 178.8, 160.2 and 134.8 g for males and 186.6, 182.4, 182.4, 189.6, 152.0, and 133.6 g for females, respectively. The NOAEL for body weight data was 250 mg a.i/kg bw, based on reductions in weight gain for the females during the exposure period.

Feed consumption was considerably less than controls at the 500, 1000, and 2000 mg a.i./kg bw dose levels between Days 0-3 and 3-7. From Days 0-3, mean consumption was 22.8, 17.1, 14.4, 7.5, 5.8, and 3.7 g/bird/day for the control, 125, 250, 500, 1000, and 2000 mg a.i./kg bw dose groups, respectively. From Days 3-7, mean consumption was 16.6, 17.5, 17.2, 10.4, 10.3, and 2.9 g/bird/day for the control, 125, 250, 500, 1000, and 2000 mg a.i./kg bw dose groups, respectively. The NOAEL for feed consumption was 250 mg a.i./kg bw.

One surviving bird from the 2000 mg a.i./kg bw dose group had a slightly loam-colored and fragile liver, but this finding could not firmly be linked to treatment.

This toxicity study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using the Northern Bobwhite quail (§71-1). This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Size/Age: Adult, 30- to 35-weeks old, 137-212 g (combined sexes)

LD_{50}: >2000 mg a.i./kg bw
NOAEL: 250 mg a.i./kg bw
LOEL: 500 mg a.i./kg bw
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weights, and food consumption
Most sensitive endpoint: Food consumption and female body weight change

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol followed procedures of the U.S. EPA OPP Guideline No. 71-1; U.S. EPA OPPTS *draft* Guideline No. 850.2100. The following deviation from §71-1 was noted:

1. The acclimation period was 14 days, which is 1 day less than the required minimum.

This deviation does not affect the scientific validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material SXX 0665 Technical (prothioconazole - desthio)

Description: Beige-brown powder

Lot No./Batch No.: 17005/89

Purity: 93.7%

Stability of Compound Under Test Conditions: N/A

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: Adult, 30- to 35- weeks old

Weight at study initiation: 137-212 g (combined sexes)

Source: Herbert Küberich, Wiesentheid, Federal Republic of Germany

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: Results from a previously conducted range-finding study suggested tht the LD_{50} was in the range of 2000 mg/kg (p. 13). No other details were provided.

b. Definitive Study:

Table 1. Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	14 Days Same as test Water and standard commercial quail diet (LAB 50 Laying Hen Ratio, Fa. Höveler D4018 Langenfeld, FRG) were provided, *ad libitum*. <5% Mortality during acclimation	The feed was not analyzed for unwanted contaminanats. *EPA recommends that birds be pre-conditioned to the test facilities for at least 15 days.* *OECD recommends that birds be pre-conditioned to the test facilities for at least 7 days.*
Pen size and construction materials	Stainless steel wire cages, 18 x 23 x 13 cm	Birds were housed individually. *EPA requires: pens must conform to good husbandry practices and should not create crowding stress.* *OECD lists no criteria for pen construction other than stating that pens should be suitable for the captive rearing of that species.*
Test duration	14 Days	*EPA requires a day for dosing and at least 14 days observation.*
Dose preparation Indicate method of confirmation of dose	N/A N/A	The test substance was dosed as supplied.

Parameter	Details	Remarks *Criteria*
Mode of dose administration	Gelatin capsule	*Gavage or gelatin capsule.*
Dose levels nominal: measured:	0 (negative control), 125, 250, 500, 1000, and 2000 mg a.i./kg bw N/A	Doses were adjusted for purity of the test substance. *EPA requires a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2000 mg ai/kg.*
Solvent/vehicle, if used type: amount/bw:	N/A	*EPA recommends that the test material be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.*
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	10 N/A 10/level	5 males and 5 females per treatment group. *EPA recommends 10 birds per treatment group and 10 birds for each control and vehicle group.*
No. of feed withholding days before dosing	18 hours	*EPA recommends that food should be withheld for at least 15 hours prior to dosing.*
Test conditions Temperature: Relative humidity: Photo-period:	20 ± 2°C 30-90% Natural day lengths	*EPA recommends that a 10 hr light/14 hr dark photo-period.*
Reference chemical, if used name: concentrations tested:	None used.	

2. Observations:

Table 2: Observations.

Parameter	Details	Remarks/Criteria
Parameters measured		
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/others)	- Mortality - Clinical signs of toxicity - Body weights - Feed consumption - Necropsy	*EPA recommends:* *Body weight measured at test initiation, on Day 14 and at end of the test if the test is extended beyond 14 days.* *Calculation of mortality. Mortality must NOT be more than 10% in controls.* *Feed consumption may be measured as average daily food consumption.*
Indicate if the test material was regurgitated	None reported.	*Regurgitation is an indication that the dose was rejected. The test may have to be repeated if the problem persists.*
Groups on which necropsies were performed	All birds were subjected to necropsy.	*EPA recommends that gross necropsies be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*
Observation intervals	Mortality and signs of toxicity were observed 1-2 hours post-dosing and once daily (on week days) thereafter. Body weights were determined on Days 0, 7, and 14. Feed consumption was determined on Days 7 and 14.	
Were raw data included?	Yes, sufficient.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Mortality was 30% (one male and two females) at the 2000 mg a.i./kg bw level (Table 1, p. 16). The mortality observed was delayed, with two birds found dead on Day 6, and one bird found dead on Day 12 (p. 29 of Appendix C). No other mortality occurred at any treatment or control level during the 14-day study. The 14-day LD_{50} was >2000 mg a.i./kg bw.

Table 3: Effect of SXX 0665 Technical (prothioconazole - desthio) on mortality of *Colinus virginianus*.

Treatment (mg a.i./kg bw)		No. of birds	Cumulative mortality							
			day 1	day 2	day 4	day 6	day 8	day 10	day 12	day 14
Control		10	0	0	0	0	0	0	0	0
125		10	0	0	0	0	0	0	0	0
250		10	0	0	0	0	0	0	0	0
500		10	0	0	0	0	0	0	0	0
1000		10	0	0	0	0	0	0	0	0
2000		10	0	0	0	2	2	2	3	3
NOAEL		1000 mg a.i./kg bw								
LD_{50}		>2000 mg a.i./kg bw								
Reference chemical	mortality	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LD_{50}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	NOAEL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. SUB-LETHAL TOXICITY ENDPOINTS:

Apathy and/or fluffed feathers were observed in birds dosed at 1000 and 2000 mg a.i./kg bw (Table 1, p. 16). In the 1000 mg a.i./kg bw dose group, fluffed feathers were observed in 2/10 birds on Day 0, and apathy was observed in one bird on Day 7 (p. 28 of Appendix C). Birds from this group appeared normal from Days 1-6, and 8-14. In the 2000 mg a.i./kg bw dose group, fluffed feathers and apathy were observed in single birds on Day 0, and apathy was observed in 6/10 birds from Days 1-3, and 6/8 surviving birds from Days 7-9. Surviving birds from the 2000 mg a.i./kg bw dose group appeared normal from Days 12-14 (p. 29 of Appendix C). Observations were not performed on Days 4, 5, 10, 11, and 13. The NOAEL for sub-lethal effects was 500 mg a.i/kg bw.

Treatment-related reductions in body weight and body weight changes were observed at the 1000 and 2000 mg a.i./kg bw levels, although it was unclear at which exact intervals the study author found statistical significance (Table 3, p. 18 and Appendix E, p. 32).

Mean Body Weight ± standard deviation (and change), g							
Treatment, mg a.i./kg bw		**Males**			**Females**		
		Day 0	**Day 7 (0-7 Days)**	**Day 14 (0-14 Days)**	**Day 0**	**Day 7 (0-7 Days)**	**Day 14 (7-14 Days)**
Control		182.4 ±18.2	181.0 ±22.3 (-1.4)	180.8 ±20.6 (-1.6)	182.0 ±25.3	186.6 ±24.5 (4.6)	191.8 ±24.5 (9.8)
125		184.6 ±20.9	184.4 ±18.6 (-0.2)5.5	185.6 ±20.7 (1.0)	183.6 ±14.2	182.4 ±18.0 (-1.2)	190.4 ±19.9 (6.8)
250		181.0 ±6.6	177.4 ±5.5 (-3.6)7.8	179.4 ±0.9 (-1.6)	183.8 ±10.3	182.4 ±13.6 (-1.4)	196.0 ±16.0 (12.2)
500		185.8 ±12.4	178.8 ±12.5 (-7.0)	186.6 ±13.1 (0.8)	194.2 ±11.9	189.6 ±15.0 (-4.6)	199.2 ±15.3 (5.0)
1000		178.2 ±9.0	160.2 ±7.8 (-18.0)	171.2 ±9.1 (-7.0)	175.4 ±11.0	152.0 ±4.1 (-23.4)	171.0 ±8.9 (-4.4)
2000		181.0 ±12.1	134.8 ±14.8 (-46.2)	146.5 ±10.8 (-34.5)	180.8 ±14.7	133.6 ±19.7 (-47.2)	158.7 ±40.3 (-22.1)
NOAEL		500 mg a.i./kg bw			500 mg a.i./kg bw		
EC_{50}		Not determined			Not determined		
Reference chemical	effect: NOAEL: LD_{50}:	N/A	N/A	N/A	N/A	N/A	N/A

Based on visual inspection of the data, the study author noted that feed consumption was considerably less than controls at the 500, 1000, and 2000 mg a.i./kg bw dose levels (p. 8 and Tables 4 and 5, p. 19). However, since body weight were unaffected at the 500 mg a.i/kg bw level, the study author dismissed the effect on food consumption at this level, and subsequently assigned the NOAEL as 500 mg a.i./kg bw.

One surviving bird from the 2000 mg a.i./kg bw dose group had a slightly loam-colored and fragile liver, but the study author reported that this effect can also be observed in untreated birds, and was not necessarily related to treatment (p. 8 and Table 2, p. 17).

Mean Feed Consumption, g/bird/day				
Treatment, mg a.i./kg bw		**Days 0-3**	**Days 3-7**	**Days 7-14**
Control		22.8	16.6	16.2
125		17.1	17.5	17.1
250		14.4	17.2	17.5
500		7.5	10.4	19.1
1000		5.8	10.3	19.7
2000		3.7	2.9	16.5
NOAEL		500 mg a.i./kg bw		
EC_{50}		Not determined		
Reference chemical	effect NOAEL LD_{50}	N/A		

C. REPORTED STATISTICS:

The LD_{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 followed by Tukeys test. If the variances were not equal (non-parametric), then the data were analyzed using the Kruskal-Wallis followed by Nemenyis tests. Analyses were performed with the aide of STATGRAPHICS statistical software. Feed consumption data were not analyzed statistically, as there were no replicate data.

LD_{50}: >2000 mg a.i./kg bw
NOAEL: 500 mg a.i./kg bw
LOEL: 1000 mg a.i./kg bw
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weights, and food consumption
Most sensitive endpoint: Clinical signs of toxicity, body weights, and food consumption

D. VERIFICATION OF STATISTICAL RESULTS:

The LD_{50} could not be calculated because mortality did not exceed 50% at any test level. The percent change body weight data were calculated and compared using ANOVA and William's multiple comparison test during the exposure period and using the non-parametric Kruskal-Wallis test during the recovery period. Analyses were performed using Excel to calculate percent change data and TOXSTAT statistical software to determine the NOAEC and LOAEC. Feed consumption data were not analyzed statistically, as there were no replicate data.

LD_{50}: >2000 mg a.i./kg bw

NOAEL: 250 mg a.i./kg bw
LOAEL: 500 mg a.i./kg bw
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weights, and food consumption
Most sensitive endpoint: Food consumption and female body weight change

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA Guideline §71-1 that affected the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from the study author's; the study author dismissed the significant reduction in food consumption at the 500 mg a.i./kg bw level because they did not detect a similar reduction in body weight. However, the reviewer's analysis did detect a significant reduction in female body weight change during the exposure period at the 500 mg a.i./kg bw treatment level. Based on this evidence (female body weight change and food consumption), the reported NOAEC for the study is 250 mg a.i./kg bw.

G. CONCLUSIONS:

This toxicity study is scientifically sound and fulfills the guideline requirements for an acute toxicity study using the Bobwhite quail (§71-1). The 14-day acute oral toxicity LD_{50} was >2000 mg a.i./kg bw, which categorizes SXX 0665 Technical (prothioconazole - desthio) as practically non-toxic to the Bobwhite quail. Based on treatment-related effects on body weight and food consumption, the NOAEL was 250 mg a.i./kg bw. This study is classified as ACCEPTABLE.

LD_{50}: >2000 mg a.i./kg bw
NOAEL: 250 mg a.i./kg bw
LOAEL: 500 mg a.i./kg bw
Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weights, and food consumption
Most sensitive endpoint: Food consumption and female body weight change

III. REFERENCES:

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

Stephen, 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 65-84.

Statgraphics Users Guide. 1989. Version 4.0, Statistical Graphics Corporation, Rockville, MD, USA.

Sachs, L. 1978. Angewandte Statistik, Springer Verlag, p. 420-422.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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male exposure weight change
File: 6037me        Transform: NO TRANSFORMATION
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2359.036	471.807	31.326
Within (Error)	24	361.474	15.061	
Total	29	2720.510		

Critical F value = 2.62 (0.05,5,24)
Since F > Critical F REJECT Ho:All groups equal

male exposure weight change
File: 6037me 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	-0.958	-0.958		
2	125	0.056	0.056	-0.413	
3	250	-1.962	-1.962	0.409	
4	500	-3.780	-3.780	1.150	
5	1000	-10.036	-10.036	3.699	*
6	2000	-25.454	-25.454	9.980	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

male exposure weight change
File: 6037me 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	5			
2	125	5	5.793	-604.6	-1.014
3	250	5	5.793	-604.6	1.004
4	500	5	5.793	-604.6	2.822
5	1000	5	5.793	-604.6	9.078
6	2000	5	5.793	-604.6	24.496

male exposure weight change
File: 6037me Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	control	5	-0.958	-0.958	-0.451
2	125	5	0.056	0.056	-0.451
3	250	5	-1.962	-1.962	-1.962
4	500	5	-3.780	-3.780	-3.780
5	1000	5	-10.036	-10.036	-10.036
6	2000	5	-25.454	-25.454	-25.454

male exposure weight change
File: 6037me 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	-0.451				
125	-0.451	0.207		1.71	k= 1, v=24
250	-1.962	0.409		1.79	k= 2, v=24
500	-3.780	1.150		1.82	k= 3, v=24
1000	-10.036	3.699	*	1.83	k= 4, v=24
2000	-25.454	9.980	*	1.84	k= 5, v=24

s = 3.881
Note: df used for table values are approximate when v > 20.

male recovery weight change
File: 6037mr Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	0.014	0.014	47.000
2	125	0.570	0.570	42.000
3	250	1.200	1.200	48.500
4	500	4.388	4.388	89.500
5	1000	6.858	6.858	116.000
6	2000	10.938	10.938	92.000

Calculated H Value = 15.892 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

male recovery weight change
File: 6037mr Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 1	2	3	4	5	6
1	control	0.014	0.014	\					
2	125	0.570	0.570	.	\				
3	250	1.200	1.200	.	.	\			

4	500	4.388	4.388	. . . \
5	1000	6.858	6.858	. . . . \
6	2000	10.938	10.938	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

female exposure weight change
File: 6037fe Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3020.482	604.096	31.953
Within (Error)	24	453.756	18.906	
Total	29	3474.238		

Critical F value = 2.62 (0.05,5,24)
Since F > Critical F REJECT Ho:All groups equal

female exposure weight change
File: 6037fe 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	2.750	2.750		
2	125	-0.774	-0.774	1.281	
3	250	-0.832	-0.832	1.303	
4	500	-2.430	-2.430	1.884	
5	1000	-13.042	-13.042	5.743	*
6	2000	-26.368	-26.368	10.588	*

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

female exposure weight change
File: 6037fe 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	5			
2	125	5	6.490	236.0	3.524
3	250	5	6.490	236.0	3.582
4	500	5	6.490	236.0	5.180
5	1000	5	6.490	236.0	15.792
6	2000	5	6.490	236.0	29.118

female exposure weight change
File: 6037fe Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	5	2.750	2.750	2.750
2	125	5	-0.774	-0.774	-0.774
3	250	5	-0.832	-0.832	-0.832
4	500	5	-2.430	-2.430	-2.430
5	1000	5	-13.042	-13.042	-13.042
6	2000	5	-26.368	-26.368	-26.368

female exposure weight change
File: 6037fe 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	2.750				
125	-0.774	1.281		1.71	k= 1, v=24
250	-0.832	1.303		1.79	k= 2, v=24
500	-2.430	1.884	*	1.82	k= 3, v=24
1000	-13.042	5.743	*	1.83	k= 4, v=24
2000	-26.368	10.588	*	1.84	k= 5, v=24

s = 4.348
Note: df used for table values are approximate when v > 20.

female recovery weight change
File: 6037fr Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	2.836	2.836	41.000
2	125	4.364	4.364	58.000
3	250	7.450	7.450	83.000
4	500	5.116	5.116	63.000
5	1000	12.584	12.584	107.000
6	2000	7.487	7.487	54.000

Calculated H Value = 8.210 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

female recovery weight change
File: 6037fr Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 1	0 2	0 4	0 3	0 6	0 5
1	control	2.836	2.836	\					
2	125	4.364	4.364	.	\				
4	500	5.116	5.116	.	.	\			
3	250	7.450	7.450	.	.	.	\		
6	2000	7.487	7.487	.	.	.	.	\	
5	1000	12.584	12.584	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

EAD Assessment of USEPA DER

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

PMRA Submission Number: 2004-0843

Study Type: Acute Oral Toxicity to Bobwhite Quail

Grau, R. 1990. SXX 0665 (Technical Grade) Acute Oral LD50 to Bobwhite Quail. Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany. Laboratory ID No. E2920414-2; Report No. VB-009. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated April 30, 1990 and completed November 30, 1990.

PMRA DATA CODE: 9.6.2.1
EPA DP Barcode: D303488
OECD Data Point: IA 8.1.1
EPA MRID: 46246037
EPA Guideline: §71-1

Reviewing Agency: US EPA

EAD Executive Summary:

The acute oral toxicity of the transformation product SXX 0665 Technical (93.7% JAU6476-desthio) to 30- to 35-week old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 14 days. JAU6476-desthio was administered to the birds via gelatin capsule at nominal concentrations of 0 (negative control), 125, 250, 500, 1000, and 2000 mg JAU6476-desthio/kg bw. The study was conducted following U.S. EPA OPP Guideline No. 71-1 and U.S. EPA OPPTS *draft* Guideline No. 850.2100 and was in compliance with U.S. EPA, 40 CFR Part 160 as was OECD Principles of GLP.

Mortality was 30% (one male and two females) at the 2000 mg JAU6476-desthio/kg bw level. The mortality observed was delayed, with two birds found dead on Day 6, and one bird found dead on Day 12. The 14-day LD_{50} was >2000 mg JAU6476-desthio/kg bw, which categorizes JAU6476-desthio as practically non-toxic to Northern Bobwhite quail on an acute oral basis, according to the classification scheme of the U.S. EPA (1985). The NOEL based on mortality is 1000 mg JAU6476-desthio/kg bw.

Apathy and/or fluffed feathers were observed in birds dosed at 1000 and 2000 mg JAU6476-desthio/kg bw. In the 1000 mg JAU6476-desthio/kg bw dose group, fluffed feathers were

observed in 2/10 birds on Day 0, and apathy was observed in one bird on Day 7. In the 2000 mg JAU6476-desthio/kg bw dose group, fluffed feathers and apathy were observed in single birds on Day 0, and apathy was observed in 6/10 birds from Days 1-3, and 6/8 surviving birds from Days 7-9. The NOEL for sub-lethal effects was 500 mg JAU6476-desthio/kg bw.

Treatment-related reductions in body weight changes were observed during the exposure period (days 0-7) at the 1000 mg JAU6476-desthio/kg bw and higher levels. On Day 7 for the 0, 125, 250, 500, 1000, and 2000 mg JAU6476-desthio/kg bw dose groups, body weights averaged 181.0, 184.4, 177.4, 178.8, 160.2 and 134.8 g for males and 186.6, 182.4, 182.4, 189.6, 152.0, and 133.6 g for females, respectively. The NOEL for body weight data was 500 mg JAU6476-desthio/kg bw, based on reductions in weight gain during the exposure period.

Based on visual observation, feed consumption was considerably less than controls at the 500, 1000, and 2000 mg JAU6476-desthio/kg bw dose levels between Days 0-3 and 3-7. From Days 0-3, mean consumption was 22.8, 17.1, 14.4, 7.5, 5.8, and 3.7 g/bird/day for the control, 125, 250, 500, 1000, and 2000 mg JAU6476-desthio/kg bw dose groups, respectively. From Days 3-7, mean consumption was 16.6, 17.5, 17.2, 10.4, 10.3, and 2.9 g/bird/day for the control, 125, 250, 500, 1000, and 2000 mg JAU6476-desthio/kg bw dose groups, respectively. As no statistically significant reduction in weight was observed at the 500 mg JAU6476-desthio/kg bw treatment level, the difference in food consumption at 500 mg JAU6476-desthio/kg bw was dismissed and the NOEL for feed consumption was set at 500 mg JAU6476-desthio/kg bw.

One surviving bird from the 2000 mg JAU6476-desthio/kg bw dose group had a slightly loam-colored and fragile liver, but this finding could not firmly be linked to treatment.

Results Synopsis

Test Organism Size/Age: Adult, 30- to 35-weeks old, 137-212 g (combined sexes)

LD_{50}: >2000 mg JAU6476-desthio/kg bw
NOEL (mortality): 1000 mg JAU6476-desthio/kg bw
LOEL (mortality): 2000 mg JAU6476-desthio/kg bw
NOEL (body weight, feed consumption and clinical signs of toxicity): 500 mg JAU6476-desthio/kg bw
LOEL (body weight, feed consumption and clinical signs of toxicity): 1000 mg JAU6476-desthio/kg bw

Endpoint(s) Affected: Mortality, clinical signs of toxicity, body weights, and food consumption
Most sensitive endpoint: Clinical signs of toxicity, food consumption and body weight change

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 and synonym) available from the PMRA Chemistry review.

2. The acclimation period is 14 days, according to US EPA draft OPPTS 850.2100.

3. The standard deviation was added in the table of mean body weight, to provide information on the variation within treatments.

4. The EAD evaluator verified the statistical analyses for body weight using ANOVA and Bonferonni and Dunnett's multiple comparisons and obtained similar results as the study author. The tests did not find the 2.5% decrease in the weight of females from days 0-7 to be statistically significant at the 500 mg JAU6476-desthio/kg bw level, contrary to results reported by the EPA reviewer. The EAD reviewer could not reproduce the results of the EPA reviewer. The difference in food consumption at the 500 mg JAU6476-desthio/kg bw treatment level is dismissed as no difference in weight was observed at that level. The EAD therefore sets the NOEL for food consumption and weight change at 500 mg JAU6476-desthio/kg bw.

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

Verification of statistics by EAD reviewer:

Male % weight change 0-7 days

One Way Analysis of Variance Monday, September 12, 2005, 15:10:29

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.088)

Equal Variance Test: Passed (P = 0.369)

Group Name	N	Missing	Mean	Std Dev	SEM
control	5	0	-0.959	2.520	1.127
125 mg/kg	5	0	0.0539	3.679	1.645
250 mg/kg	5	0	-1.965	1.509	0.675
500 mg/kg	5	0	-3.779	1.059	0.474
1000 mg/kg	5	0	-10.035	3.689	1.650
2000 mg/kg	5	0	-25.454	7.315	3.271

Source of Variation	DF	SS	MS	F	P
Between Groups	5	2358.729	471.746	31.311	<0.001
Residual	24	361.592	15.066		
Total	29	2720.321			

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: Col 1

Comparison	Diff of Means	t	P	P<0.050
control vs. 2000 mg/kg	24.496	9.978	<0.001	Yes
control vs. 1000 mg/kg	9.077	3.697	0.006	Yes
control vs. 500 mg/kg	2.821	1.149	1.000	No
control vs. 125 mg/kg	1.012	0.412	1.000	Do Not Test
control vs. 250 mg/kg	1.006	0.410	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.

Female % weight change day 0-7

One Way Analysis of Variance Monday, September 12, 2005, 15:12:30

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Passed (P = 0.296)

Group Name	N	Missing	Mean	Std Dev	SEM
control	5	0	2.749	4.547	2.033
125 mg/kg	5	0	-0.774	2.540	1.136
250 mg/kg	5	0	-0.832	2.121	0.949
500 mg/kg	5	0	-2.431	2.479	1.109
1000 mg/kg	5	0	-13.041	6.394	2.859
2000 mg/kg	5	0	-26.369	5.899	2.638

Source of Variation	DF	SS	MS	F	P
Between Groups	5	3020.631	604.126	31.954	<0.001
Residual	24	453.746	18.906		
Total	29	3474.377			

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: Col 1

Comparison	Diff of Means	t	P	P<0.050
control vs. 2000 mg/kg	29.119	10.589	<0.001	Yes
control vs. 1000 mg/kg	15.790	5.742	<0.001	Yes
control vs. 500 mg/kg	5.180	1.884	0.359	No
control vs. 250 mg/kg	3.581	1.302	1.000	Do Not Test
control vs. 125 mg/kg	3.523	1.281	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.