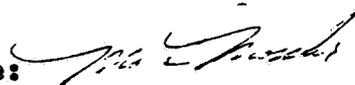


US EPA ARCHIVE DOCUMENT

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Amitraz.  
Shaughnessey No. 106201.
2. **TEST MATERIAL:** Technical BTS 27919; Batch No. CR 19620/1;  
99% active ingredient; a fine white powder.
3. **STUDY TYPE:** Avian Dietary LC<sub>50</sub> Test. Species Tested:  
Bobwhite quail (*Colinus virginianus*).
4. **CITATION:** Hakin, B. and M. Rodgers. 1991. W141 Amitraz:  
Technical BTS 27919: Subacute Dietary Toxicity (LC<sub>50</sub>) to  
Bobwhite Quail. Lab. Proj. ID No. TOX/91/179-199.  
Performed by Huntingdon Research Center, Huntingdon,  
Cambridgeshire, UK. Submitted by NOR-AM Chemical Co.,  
Wilmington, DE. EPA MRID No. 421246-05.
5. **REVIEWED BY:**  

Mark A. Mossler, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature:  Date: 1/30/92
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6. **APPROVED BY:**  

Michael Whitten, M.S. Wildlife Toxicologist KBN Engineering and Applied Sciences, Inc.	Signature:  Date: 1/30/92
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature:  Date: 2/9/92
7. **CONCLUSIONS:** This study is scientifically sound but does  
not meet the guideline requirements for a dietary avian  
acute test. The results of the homogeneity, stability, and  
concentration verification tests were not included in the  
report. The LC<sub>50</sub> was >5,200 ppm (based on nominal  
concentrations), which classifies BTS 27919 as practically  
non-toxic to bobwhite quail. The NOEC was 325 ppm.
8. **RECOMMENDATIONS:** See Section 14 D-3.
9. **BACKGROUND:** Data submitted to support conditional registration on  
cotton.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.11. MATERIALS AND METHODS:

A. Test Animals: Bobwhite quail (*Colinus virginianus*) were obtained from a supplier in Cambridgeshire, UK. The birds were from the same hatch and were one-day old when received. All birds were acclimated to the caging and facilities for 3 days. The birds weighed between 10.5 and 11.5 g at the beginning of the acclimation period and were 10 days of age at test initiation. The birds were phenotypically indistinguishable from wild birds and were in apparent good health at the start of acclimation.

B. Test System: The birds were housed indoors in wooden boxes measuring 80 x 50 x 60 cm. Lids were constructed of wire mesh. During the test, the mean daily temperature in the building was 23-26°C. A 300 watt infra-red lamp was suspended above each cage to provide additional heat. The average relative humidity was 58 ±6.1%. A continuous photoperiod was used throughout the study.

The test diets were prepared by adding the test substance into the diet (standard chick diet) to form a pre-mix from which the final diets were prepared. The diets were prepared immediately prior to use and the remainder of the premix was frozen until needed.

The birds were offered water and feed *ad libitum* throughout the study. A list of the ingredients in the feed was given in the report and it appeared to be free of unfamiliar ingredients and medications.

C. Dosage: Acute dietary LC<sub>50</sub> test. Dosage levels selected for the study were 163, 325, 650, 1,300, 2,600, and 5,200 ppm.

D. Design: Ten quail were used per test level and in each of two controls. Birds were assigned to treatment groups by body weight so that all treatment groups would have similar initial body weight means. Groups were assigned to treatments using a random allocation system. Signs of toxicity, abnormal behavior, and mortality were assessed daily. Group body weights were measured at initiation and day 5 and 8 of the test. Average feed consumption was determined by group for days 1, 2, 3, 4,

and 5 (the exposure period) and 6-8 (the observation period).

Samples of the test diet were taken from a trial mix (163 and 5,200 ppm) to determine homogeneity of the test material. Samples were taken from actual test diets (all concentrations) for determination of test substance concentration. Stability samples were taken from the 163, 650, and 5,200 ppm concentration diets.

A post-mortem examination was conducted on ten birds in the highest test group and five control birds.

E. **Statistics:** Due to the pattern of mortality, the  $LC_{50}$  was estimated by visual assessment.

12. **REPORTED RESULTS:** There were no mortalities or clinical signs of toxicity and all birds remained in good health during the study.

There were reductions in body weight gain observed in the two highest test concentration groups (2,600 and 5,200 ppm) during the treatment period. During the recovery period, the body weights were similar to the controls (Table 1, attached). Food consumption was reduced at 2,600 and 5,200 ppm during the treatment period (Table 2, attached).

Reductions in the amount of subcutaneous fat were observed in three birds from the highest concentration test group. No other abnormalities were detected in any of the birds examined by post-mortem necropsy.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**  
The authors concluded that BTS 27919 was of low subacute dietary toxicity to the bobwhite quail since the  $LC_{50}$  was in excess of 5,200 ppm.

Good Laboratory Practice and Quality Assurance Unit Statements were included in the report indicating that the study conformed with Good Laboratory Practice standards published by the U.S. Environmental Protection Agency (40 CFR Part 160).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, ASTM, and SEP guidelines with the following exceptions:

Body weights were measured by group. Individual body weights should have been measured.

The results from the analyses conducted to verify the stability, homogeneity, and concentration of test substance were not included in the report.

The birds were not randomly assigned to groups.

- B. Statistical Analysis:** Upon review of the results, the reviewer concurs that the  $LC_{50}$  was greater than 5,200 ppm. However, the reviewer noted that there appeared to be reductions in feed consumption at test concentrations less than 2,600 ppm. Dunnett's test was used to compare reductions in feed consumption in comparison to the control using daily mean food consumption values for days 1-5. Based on the results from Dunnett's test (attached), the NOEC was 325 ppm.
- C. Discussion/Results:** The birds were assigned to groups on the basis of body weight, after which the groups were randomly assigned to a particular treatment. Although this method of assignment probably did not affect the results of the test, it is not the same as random assignment to pens. A fundamental requirement of statistical analysis is that sampling of individuals be at random. The risk of non-random sampling is that the results may be biased in some way. For this reason, ASTM and the SEP guidelines specify that birds be randomly assigned to pens. The SEP actually states that birds "must be" randomly assigned to pens. The report stated that body weights were used to make assignments to groups in order to achieve similar initial bodyweight means in all groups. However, if birds were of the same age and from the same hatch, random assignment should produce similar initial body weights among groups. Although the method of assignment probably did not affect the results of the test, the registrant should enact procedures in future tests that provide random assignments to groups.

This study is scientifically sound but does not meet the guideline requirements for a dietary avian acute test since the results of the homogeneity, stability, and concentration verification tests were not included in the report. The  $LC_{50}$  was  $>5,200$  ppm, which classifies BTS 27919 as practically non-toxic to bobwhite quail. The NOEC was 325 ppm.

**D. Adequacy of the Study:**

- (1) **Classification:** Supplemental.
- (2) **Rationale:** The results of the homogeneity, stability, and concentration verification tests were not included in the report.
- (3) **Repairability:** This study can be upgraded to "core" upon satisfactory submission of dietary analyses.

**15. COMPLETION OF ONE-LINER: Yes, 1-10-92.**

**RESULTS**

**CLINICAL OBSERVATIONS AND MORTALITIES**

All birds remained in good health throughout the study and no clinical signs of toxicity were observed. Excreta remained normal throughout the study.

There were no mortalities, therefore it was not possible to calculate the dietary LC<sub>50</sub> of Technical BTS 27919 to the Bobwhite quail. This value must be in excess of 5200 ppm, the maximum dose level used.

**BODYWEIGHT**

Group mean bodyweights and bodyweight increases are given in Table 1.

**TABLE 1**

**Group mean bodyweights and bodyweight increases (g/bird)**

Group	Treatment (ppm)	Days of study							
		Bodyweight				Bodyweight increase			
		-3	0	5	8	-3 to 0	0 to 5	5 to 8	
1	Control 0	10.8	15.5	23.2	28.8	4.7	7.7	5.6	
2	Control 0	11.5	17.1	22.2	26.3	5.6	5.1	4.1	
3	BTS 27919 163	10.5	16.0	26.1	34.0	5.5	10.1	7.9	
4	BTS 27919 325	10.6	16.1	25.0	29.9	5.5	8.9	4.9	
5	BTS 27919 650	10.8	15.3	24.5	29.8	4.5	9.2	5.3	
6	BTS 27919 1300	10.5	15.1	21.9	26.8	4.6	6.8	4.9	
7	BTS 27919 2600	10.9	15.4	19.2	25.3	4.5	3.8	6.1	
8	BTS 27919 5200	11.1	17.3	17.9	25.2	6.2	0.6	7.3	

Reductions in bodyweight gain compared with control values were observed in Groups 7 and 8 (2600 and 5200 ppm BTS 27919) during the treatment period. Post-treatment bodyweight gains were comparable in all groups.

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**FOOD CONSUMPTION**

Group mean food consumption data are given in Table 2.

**TABLE 2**

**Group mean food consumption (g/bird/day)**

Group	Treatment (ppm)	Days of study							
		-3 to -1	1	2	3	4	5	1 to 5	6 to 8
1	Control 0	3.3	4.3	4.2	4.8	4.7	5.0	4.6	5.0
2	Control 0	4.0	4.5	4.2	3.8	5.0	4.9	4.5	4.6
3	BTS 27919 163	3.5	4.7	3.9	4.9	4.4	4.6	4.5	5.0
4	BTS 27919 325	3.5	4.0	4.0	4.4	4.8	4.7	4.4	4.7
5	BTS 27919 650	3.3	3.3	3.5	4.0	4.2	3.9	3.8	4.7
6	BTS 27919 1300	3.4	3.6	3.0	3.1	3.1	4.0	3.4	4.5
7	BTS 27919 2600	3.2	3.0	2.6	3.0	3.4	3.4	3.1	4.8
8	BTS 27919 5200	3.8	2.3	1.0	2.9	2.3	3.8	2.5	4.6

Slight reductions in food consumption during the treatment period were observed in Groups 7 and 8 (2600 and 5200 ppm BTS 27919) when compared with control values. There were no other treatment-related effects.

**MACROSCOPIC POST-MORTEM EXAMINATION**

Reductions in the amount of subcutaneous fat were noted in the birds 73J, 74J and 80J from Group 8 (5200 ppm BTS 27919) when compared with birds from the control group. No other abnormalities were detected.

bobwhite feed consumption

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	4.5400	.4061	8.9
2 16.3	5	4.5000	.3808	8.5
3 325	5	4.3800	.3768	8.6
4* 650	5	3.7800	.3701	9.8
5* 1300	5	3.3600	.4278	12.7
6* 2600	5	3.0800	.3347	10.9
7* 5200	5	2.4600	1.0213	41.5

*NiFC = 325 ppm  
 Raw data from  
 Table 2, attached.*

\*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

Minimum detectable difference for t-tests with Bonferroni adjustment = -.573655  
 This difference corresponds to -12.64 percent of control

\*\*\*\*\*  
 \*  
 \* Note - the above value for the minimum  
 \* detectable difference is approximate as  
 \* the sample sizes are not the same for all of  
 \* the groups.  
 \*  
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Between groups sum of squares = 22.112000 with 6 degrees of freedom.  
 Error mean square = .258545 with 33 degrees of freedom.  
 Bartlett's test p-value for equality of variances = .140