DATA EVALUATION RECORD

1. **CHEMICAL:** Amitraz.
Shaughnessey No. 106201.

2. **TEST MATERIAL:** Technical BTS 27919; Batch No. CR 19620/1; 99% active ingredient; a pale pink powder.

3. **STUDY TYPE:** Avian Single Dose Oral LD_{50} Test.
Species Tested: Bobwhite quail (*Colinus virginianus*).

4. **CITATION:** Hakin, B. and M. Rodgers. 1991. W142 Amitraz: Technical BTS 27919: Acute Oral Toxicity (LD_{50}) to Bobwhite Quail. Lab. Proj. ID No. TOX/91/179-200. Performed by Huntingdon Research Center, Huntingdon, Cambridgeshire, UK. Submitted by NOR-AM Chemical Co., Wilmington, DE. EPA MRID No. 421246-03.

5. **REVIEWED BY:**

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6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an acute oral toxicity test using the bobwhite quail. The LD_{50} value of 1,827 mg/kg (based on nominal concentrations) of body weight classifies BTS 27919 as slightly toxic to bobwhite quail. The NOEL was 125 mg/kg of body weight.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:** Data submitted to support conditional registration on cotton.

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in the study were over 16-week old bobwhite quail (*Colinus virginianus*) obtained from suppliers in Cambridgeshire, UK. The birds appeared phenotypically indistinguishable from wild birds and were in good health at the start of acclimation. They were acclimated to the laboratory for 15 days prior to testing and ranged in weight from 175 to 207 g at the beginning of the acclimation period. Except for a 19-hour fasting period immediately prior to dosing, water and a game bird ration were offered *ad libitum* during acclimation and testing. No antibiotics or growth promoters were administered during the test in the feed.

B. **Test System:** All birds were housed indoors in pens constructed of polythene-coated wire mesh. Pens measured 31 x 39 x 24 cm. Lights provided 10 hours of illumination per day. The mean temperature was 19-21°C and the average relative humidity was 65 ±4.6%.

C. **Dosage:** Fourteen-day single dose oral LD_{50} test. Five nominal dosages (125, 250, 500, 1,000, and 2,000 mg/kg of body weight) and a vehicle (1% methylcellulose solution) control were used. The birds were dosed at a constant volume to body weight ratio of 10 ml to 1 kg of body weight.

D. **Design:** Ten birds (five males and five females) were assigned to each treatment and control group by body weight so that all treatment groups would have similar initial body weight means. Groups were assigned to treatment using a random allocation system. Each dosage group was assigned two pens in which birds were segregated by sex.

The test substance was suspended in a 1% methyl cellulose solution and intubated directly into each bird using a plastic catheter and disposable syringe. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight. The control birds received a corresponding volume of vehicle.

All birds were observed daily for mortality, signs of toxicity, and abnormal behavior. The birds were weighed

individually at test initiation, day 7 and 14. Group food consumption was determined for days 1-7 and 8-14. Post-mortem examinations were conducted on all birds that died during the study and on eleven birds from the highest dose groups in which there were survivors.

Samples were collected before, during, and after dosing. The samples were frozen, and sent to the study sponsor for analysis of the test material.

E. **Statistics:** The LD_{50} was determined by probit analysis with the aid of a maximum likelihood program.

12. **REPORTED RESULTS:** There were no mortalities in the two lowest test groups (125 and 250 mg/kg). Mortalities for the higher test dosages were as follows: 1 bird on day 2 in the 500 mg/kg group, 2 birds on day 1 and 1 bird on day 3 in the 1,000 mg/kg group, and 2 birds dead on day 1 and 1 bird dead on days 2, 3, and 10 in the 2,000 mg/kg dose group (Table 1, attached).

Temporary signs of toxicity were observed at doses of 250 mg/kg and greater, which included subdued behavior, unsteadiness of gait, and inability to stand (Table 2, attached).

A dose-related reduction in body weight was observed in birds dosed at 500 mg/kg and above for the first week following dosing. Over the same period, food consumption of survivors was reduced at the two highest dosages (1,000 and 2,000 mg/kg).

At necropsy, one bird at 2,000 mg/kg was found to be very thin. No other abnormalities were observed.

The authors stated that the NOEL was 125 mg/kg for clinical signs of toxicity (Table 2).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that BTS 27919 was of low acute oral toxicity to bobwhite quail since the LD_{50} value was 1,827 mg/kg (95% confidence interval = 1,155-7,894 mg/kg).

Quality Assurance and Good Laboratory Practice Statements were included in the report indicating compliance with the regulations under the Federal Insecticide, Fungicide and Rodenticide Act (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 and SEP guidelines with the following exceptions:

The report did not state if the birds were from the same hatch.

The birds were not randomly assigned to groups.

B. **Statistical Analysis:** The LD_{50} and 95% confidence interval were determined using EPA's Toxanal program. The reviewer obtained the same results as the authors with a slightly narrower confidence interval (1,153-7,497 mg/kg).

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 and meets the requirements for an acute oral toxicity test using the bobwhite quail. The LD_{50} value of 1,827 mg/kg of body weight classifies BTS 27919 as slightly toxic to bobwhite quail. The NOEL was 125 mg/kg of body weight.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 1-9-92..

RESULTS

CLINICAL SIGNS AND MORTALITIES

Mortalities in the main study are shown in Table 1.

TABLE 1

Mortalities

Group	Treatment (mg/kg)		No. of birds	Day of study					Total
				1	2	3	4	10	
1	Control	0	10						0
2	BTS 27919	125	10						0
3	BTS 27919	250	10						0
4	BTS 27919	500	10		1				1
5	BTS 27919	1000	10	2	1				3
6	BTS 27919	2000	10	2	1	1		1	5

From the mortalities shown above, the LD_{50} value was determined using a method of probit analysis[1] using MLP[2] as follows:

LD_{50} value:	1827 mg/kg
95% confidence limits:	1155 - 7894 mg/kg[3]
Slope of the line:	2.48
Standard error of slope:	0.87

The heterogeneity factor was not significant.

1 Finney, D.J. (1978) Statistical Method in Biological Assay, Griffin and Company, London
2 Ross, G.J.S. (1987) Maximum Likelihood Program, Rothamsted Experimental Station, Harpenden, UK
3 The 95% confidence limits quoted must be regarded as approximate values only

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A summary of clinical observations following dosing is as follows:

TABLE 2

Group incidence of clinical signs (number of affected birds)

Dose level (mg/kg)	Clinical signs	Days										
		1	2	3	4	5	6	7	8	9	10	14
250	Unsteadiness	1										
	Subdued	10										
500	Unsteadiness	10										
	Subdued	10										
1000	Ruffled feathers	3	1									
	Unable to stand	3										
	Unsteadiness	7	7	1								
	Subdued	7		1								
2000	Ruffled feathers	1										
	Unsteadiness	8	7	4	4	3	6					
	Subdued	8	7	4	4	6	6	6	6			

125 mg/kg was the no-effect level for clinical signs of toxicity.

Detailed clinical observations are shown in Appendix 1.

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MOSSLER AMITRAZ COLINUS VIRGINIANUS 1-9-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2000	10	5	50	62.30469
1000	10	3	30	17.1875
500	10	1	10	1.074219
250	10	0	0	9.765625E-02
125	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2000

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	5.230068	1999.998	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.4536326	1	.9438721

SLOPE = 2.481816
95 PERCENT CONFIDENCE LIMITS = .810257 AND 4.153374

LC50 = 1827.241
95 PERCENT CONFIDENCE LIMITS = 1153.124 AND 7496.566

LC10 = 562.4366
95 PERCENT CONFIDENCE LIMITS = 129.4195 AND 894.4554

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