3-8-92

MRID No. 421246-02

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Technical BTS 27271.HCl (BTS 27271); Batch No. CR 19621/1; 99.1% 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 A.J. Johnson. 1991. W144 Amitraz: Technical BTS 27271.HCl: Acute Oral Toxicity LD_{50} to Bobwhite Quail. Lab. Proj. ID No. TOX 90553. Performed by Huntingdon Research Center, Huntingdon, Cambridgeshire, UK. Submitted by NOR-AM Chemical Co., Wilmington, DE. EPA MRID No. 421246-02.

5. **REVIEWED BY:**

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Signature: Mark L Mossler
Date: 1/29/92

6. **APPROVED BY:**

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Date: 3/8/92

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 71 mg/kg (based on nominal concentrations) of body weight classifies BTS 27271 as moderately toxic to bobwhite quail. The NOEL could not be determined.

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 170 to 204 g at the beginning of acclimation. 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 72 ±5.5%.

C. **Dosage:** Fourteen-day single dose oral LD_{50} test. Five nominal dosages (31, 63, 125, 250, and 500 mg/kg of body weight) and a vehicle (1% methylcellulose solution) control were initially established. Because of the high mortality observed during the study, two extra treatment groups and a set of controls were created three weeks after the first groups were dosed. The additional dosage concentrations were 16 and 8 mg/kg. 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 ten birds from the highest dose group 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 (8 and 16 mg/kg). Mortalities for the higher test dosages were as follows: 1 male on day 2 in the 31 mg/kg group, 1 female on day 2 and 2 females on day 4 in the 63 mg/kg group, 2 males on days 1 and 3 and all 5 females within three days in the 125 mg/kg group, and all birds died within three days in the highest two dose groups (Table 1, attached).

In all groups dosed with BTS 27271.HCl, temporary signs of toxicity were observed including subdued behavior, unsteadiness of gait, ruffled feathers and sitting on cage floors (Table 2, attached). Birds were generally affected for up to 3 days post-dosing.

A dose-related reduction in body weight was observed in surviving birds dosed at 31 mg/kg and above for the first week following dosing. Over the same period, food consumption of survivors was slightly reduced at 31 mg/kg, and markedly reduced at 63 mg/kg and above.

At necropsy, the intestines of all birds that died during the study were covered or filled with a clear fluid or were red in color. At termination, the only finding was one bird dosed at 125 mg/kg with a reduced amount of subcutaneous fat.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that BTS 27271.HCl was toxic to bobwhite quail since the LD_{50} value was 71 mg/kg (95% confidence interval = 52-98 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, both with and without the additional groups that were dosed after the initial groups.

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.

Two additional treatment groups and one control group were dosed 7 days after the initial test ended (See page 12 of the report). The authors combined the data from these additional groups with data from the initial groups. This is not scientifically valid and is totally

unacceptable for a toxicity test. In this case, however, the LD_{50} and slope were not altered by the addition or deletion of the later groups. Therefore, the study meets the requirements for an oral acute toxicity test. With the deletion of data from the later additional dosage groups, the study can be considered as scientifically sound.

Because of effects at all dosage levels (with and without the additional groups), the NOEL could not be determined. The LD_{50} of 71 mg/kg classifies the test substance as moderately toxic to bobwhite quail.

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.

MOSSLER AMITRAZ COLINUS VIRGINIANUS 1-9-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
500	10	10	100	9.765625E-02
250	10	10	100	9.765625E-02
125	10	9	90	1.074219
63	10	3	30	17.1875
31	10	1	10	1.074219
16	10	0	0	9.765625E-02
8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 31 AND 125 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 78.02698

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	.1144044	69.61751	46.69145	106.5706

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.2362754	1	.9513017

SLOPE = 4.534602

95 PERCENT CONFIDENCE LIMITS = 2.330415 AND 6.738789

LC50 = 70.79325

95 PERCENT CONFIDENCE LIMITS = 51.70761 AND 96.7354

LC10 = 37.14695

95 PERCENT CONFIDENCE LIMITS = 18.11121 AND 51.0228

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3 x 16 mg/kg groups were dosed 21 days after the control groups (7 days after end of the study)

MOSSLER AMITRAZ COLINUS VIRGINIANUS 1-9-92

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ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 78.02698

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.167754	70.79325	46.92742	97.60292

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2603989	1	.7799972

SLOPE = 4.486315
95 PERCENT CONFIDENCE LIMITS = 2.19698 AND 6.775649

LC50 = 70.64608
95 PERCENT CONFIDENCE LIMITS = 50.70658 AND 96.69957

LC10 = 36.81333
95 PERCENT CONFIDENCE LIMITS = 16.44511 AND 51.16228

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6. RESULTS

6.1. MORTALITIES

The distribution of mortalities is shown in Table 1 below.

TABLE 1

Distribution of mortalities

Group	Treatment (mg/kg)	Initial no. of birds	Day of study				Total
			1	2	3	4	
1	Control 0	5♂ 5♀					0 0
7	Control 0	5♂ 5♀					0 0
9	BTS 27271 8	5♂ 5♀					0 0
8	BTS 27271 16	5♂ 5♀					0 0
2	BTS 27271 31	5♂ 5♀		1			1 0
3	BTS 27271 63	5♂ 5♀		1		2	0 3
4	BTS 27271 125	5♂ 5♀	2 1	2	2 2		4 5
5	BTS 27271 250	5♂ 5♀	1	4 5			5 5
6	BTS 27271 500	5♂ 5♀	1 1	3 4	1		5 5

From the above mortalities, the LD_{50} value was determined by a method of probit analysis* with MLP**

LD_{50} value	:	71 mg/kg
95% confidence limits	:	52 - 98 mg/kg
Slope of dose-response line	:	4.5
Standard error of slope	:	1.12

* Finney, D.J. (1978) Statistical Method in Biological Assay, Griffin & Co., London.

** Ross, G.J.S. (1987) Maximum Likelihood Program, Rothamsted Experimental Station, Harpenden, U.K.

6.2. CLINICAL SIGNS

Detailed results are given in Appendix 1 and are summarised in Table 2.

TABLE 2

Group incidence of clinical signs

(Number of affected birds)

Group	Treatment (mg/kg)	Clinical sign	Day of study						
			1	2	3	4	5	6	7
1 + 7	Control 0	None observed							
9	BTS 27271 8	Subdued	10	10					
		Unsteady	10	1					
8	BTS 27271 16	Subdued	10	10					
		Unsteady	10	10					
		Ruffled feathers	1						
		Leaning on side of cage/sitting on cage floor	1						
2	BTS 27271 31	Subdued	10	10					
		Unsteady	10	10					
		Ruffled feathers	10	10					
		Sitting on cage floor	10	10					
3	BTS 27271 63	Subdued	10	10	9				
		Unsteady	10	10	2				
		Ruffled feathers	10	10	7				
		Sitting on cage floor	10	10	9				
4	BTS 27271 125	Subdued	10	9	1	1			
		Unsteady	10	9	1	1			
		Ruffled feathers	10	9	1				
		Sitting on cage floor	10	9	1				
		Unbalanced						1	
		Limping							1
5	BTS 27271 250	Subdued	10	9					
		Unsteady	10	9					
		Ruffled feathers	10	9					
		Sitting on cage floor	9	9					
6	BTS 27271 500	Subdued	10	9	1				
		Unsteady	10	9	1				
		Ruffled feathers	10	9	1				
		Sitting on cage floor	9	9	1				

Clinical signs of toxicity were seen at all dose levels of BTS 27271 and included subdued behaviour, unsteadiness of gait, ruffled feathers and sitting on the cage floor. In all groups, the signs were mainly temporary affecting birds for up to 3 days post-dosing.

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