3-8-92

MRID No. 421246-04

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 Dietary LC_{50} Test. Species Tested: Bobwhite quail (*Colinus virginianus*).

4. **CITATION:** Hakin, B. and A.J. Johnson. 1991. W137 Amitraz: Technical BTS 27271.HCl: Subacute Dietary Toxicity (LC_{50}) to Bobwhite Quail. Lab. Proj. ID No. TOX 90554. Performed by Huntingdon Research Center, Huntingdon, Cambridgeshire, UK. Submitted by NOR-AM Chemical Co., Wilmington, DE. EPA MRID No. 421246-04.

5. **REVIEWED BY:**

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Date: 1/29/92

6. **APPROVED BY:**

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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_{50} was 1,276 ppm (based on nominal concentrations) which classifies BTS 27271.HCl as slightly toxic to bobwhite quail. The NOEC could not be determined.

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 one-day old when received. All birds were acclimated to the caging and facilities for 4 days. The birds weighed between 14 and 14.4 g at the beginning of the acclimation period and were 14 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. Each box contained a drinker and feeding tray covered with wire mesh to minimize spillage of the diet. During the test, the mean daily temperature in the building was 21-24°C. A 300 watt infra-red lamp was suspended above each cage to provide additional heat. The average relative humidity was 44 ±2.5%. 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_{50} test. Dosage levels selected for the study were 81, 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 at least daily. Group body weights were measured at initiation, day 5, 8, and 9 of the test. Average feed consumption was determined by group for

days 1, 2, 3, 4, and 5 (the exposure period) and 6-9 (the observation period).

Homogeneity of mixing was tested by analyzing samples taken (prior to study initiation) from trial mixes of 81 and 5,200 ppm. Samples were taken from all actual test concentrations for concentration verification, and from the 81, 650, 5,200 ppm concentrations for stability verification.

A post-mortem examination was conducted on ten surviving birds in the highest test groups, five control birds, and on all birds that died during the study.

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

12. **REPORTED RESULTS:** One mortality occurred in the highest concentration test group one day before test initiation. It was replaced with a spare quail.

There were three mortalities (1 each on days 3, 4, and 5) in the 325-ppm concentration group. Mortalities for the higher test concentrations were as follows: 1 bird on day 4 in the 650-ppm group, 1 bird each on days 4 and 5 in the 1,300-ppm group, 1 bird each on days 2 and 3, 2 birds on day 4, and 4 birds on day 5 in the 2,600-ppm group, and 4 birds each on days 2 and 3 and 2 birds on day 4 for the 5,200-ppm group (Table 1, attached).

Clinical signs of toxicity including subdued behavior and unsteadiness of gait were observed at 325 ppm and above.

There were treatment-related reductions in body weight gain observed in all groups treated with BTS 27271.HCl during the exposure period. During the recovery period, the body weights were similar to the controls at concentrations below 2,600 ppm. Food consumption was reduced at 325 ppm and above (Tables 2 & 3, attached).

No abnormalities were detected in any of the birds examined at post-mortem necropsy.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors concluded that BTS 27271.HCl was of fairly low subacute dietary toxicity to the bobwhite quail since the LC_{50} was 1,362 ppm, with a 95% confidence interval from 904 to 2,247 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 pens.

B. **Statistical Analysis:** The reviewer analyzed the mortality data using EPA's Toxanal program. The results obtained using the moving average method were more conservative than those obtained by probit analysis. The LC_{50} and 95% confidence interval were 1,276 ppm and 898 to 1,975 ppm, respectively.

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 1,276 ppm, which classifies BTS 27271.HCl as slightly toxic to bobwhite quail. The NOEC could not be determined due to reduced body weight gain in all treatment groups.

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 submission of satisfactory data regarding chemical analysis of the treatment diets.

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)
5200	10	10	100	9.765625E-02
2600	10	8	80	5.46875
1300	10	2	20	5.46875
650	10	1	10	1.074219
325	10	3	30	17.1875
163	10	0	0	9.765625E-02
81	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 163 AND 5200 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 1838.477

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	.1144044	1276.553	898.269	1974.618

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.6964933	2.436623	3.236288E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.253406
95 PERCENT CONFIDENCE LIMITS = .3727995 AND 4.134013

LC50 = 1361.503
95 PERCENT CONFIDENCE LIMITS = 407.5924 AND 8986.496

LC10 = 371.9009
95 PERCENT CONFIDENCE LIMITS = .8076587 AND 876.4453

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