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_{50} Test. Species Tested: Mallard duck (*Anas platyrhynchos*).

4. **CITATION:** Hakin, B. and M. Rodgers. 1991. W140 Amitraz: Technical BTS 27919: Subacute Dietary Toxicity (LC_{50}) to Mallard Duck. Lab. Proj. ID No. TOX/91/179-198. Performed by Huntingdon Research Center, Huntingdon, Cambridgeshire, UK. Submitted by NOR-AM Chemical Co., Wilmington, DE. EPA MRID No. 421246-07.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 1/30/92

6. **APPROVED BY:**

Michael Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 1/30/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: Henry T. Craven
Date: 3/9/92

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_{50} was >5,200 ppm (based on nominal concentrations), which classifies BTS 27919 as practically non-toxic to mallard ducklings. The NOEC was determined to be 650 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:** Mallard ducklings (*Anas platyrhynchos*) were obtained from a supplier in Kent, 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 4 days. The birds weighed 43 g at the beginning of the acclimation period and were 7 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 brooder pens constructed of galvanized steel and wire mesh and measuring 84 x 57 x 27 cm. During the test, the mean daily temperature in the building was 25-28°C. The average relative humidity was 63 ±9.2%. A continuous photoperiod was used throughout the study.

The test diets were prepared by adding the test substance into the diet (standard chick diet in meal form) to create 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 163, 325, 650, 1,300, 2,600, and 5,200 ppm.

D. **Design:** Ten ducklings 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. 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 test 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 1,300 and 2,600 ppm test groups and a loss in body weight at the highest test concentration (5,200 ppm) during the treatment period. During the recovery period, the changes in body weight were similar to the controls (Table 1, attached). Food consumption was slightly reduced at 1,300 and 2,600 ppm and greatly reduced at the highest test concentration (5,200 ppm) during the exposure period (Table 2, attached). This trend continued during the observation period.

No 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 mallard ducklings 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.

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. Dunnett's test was used to compare reductions in feed consumption in comparison to the control. Although the authors did not report a no-observed-effect concentration (NOEC), the reviewer calculated that the NOEC was 650 ppm based on reductions in body weight gain and feed consumption during the exposure period (see attached printout).

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 mallard ducklings. The NOEC was determined to be 650 ppm, based upon effects

on body weight and feed consumption at concentrations ≥1,300 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 the 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_{50} of BTS 27919 to the Mallard duck. This value must be in excess of 5200 ppm, the maximum dose level used.

BODYWEIGHTS

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

TABLE 1

Group mean bodyweights and bodyweight changes (g/bird)

Group	Treatment		Days of study						
			Bodyweight				Bodyweight changes*		
			-4	0	5	8	-4 to 0	0 to 5	5 to 8
1	Control	0	43	83	179	258	40	96	79
2	Control	0	43	82	171	254	39	89	83
3	BTS 27919	163	43	85	179	240	42	94	61
4	BTS 27919	325	43	85	184	259	42	99	75
5	BTS 27919	650	43	85	182	255	42	97	73
6	BTS 27919	1300	43	82	154	230	39	72	76
7	BTS 27919	2600	43	80	120	196	37	40	76
8	BTS 27919	5200	43	85	67	134	42	-18	67

* All changes positive unless otherwise indicated

At the highest dose level, Group 8 (5200 ppm BTS 27919), there was a loss in bodyweight during the treatment period, Days 0 to 5. Reductions in bodyweight gain were observed in Groups 6 and 7 (1300 and 2600 ppm BTS 27919) during the treatment period when compared with control values. Remaining groups were unaffected.

Post-treatment bodyweight gains were comparable in all groups.

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							
		-4 to -1	1	2	3	4	5	1 to 5	6 to 8
1	Control 0	15	23	30	34	40	47	35	57
2	Control 0	15	24	30	32	38	43	33	57
3	BTS 27919 163	16	23	30	31	36	44	33	52
4	BTS 27919 325	17	24	29	32	37	46	34	53
5	BTS 27919 650	16	22	27	31	36	41	31	52
6	BTS 27919 1300	14	17	22	23	28	36	25	51
7	BTS 27919 2600	16	16	20	17	19	31	21	53
8	BTS 27919 5200	16	8	9	9	10	10	9	36

Food consumption in Groups 6 and 7 (1300 and 2600 ppm BTS 27919) was slightly reduced during the treatment period when compared with control values. A large reduction in food consumption was observed in Group 8 (5200 ppm BTS 27919) over the same period. During Days 6 to 8 all treated groups were lower than controls with Group 8 markedly lower.

MACROSCOPIC *POST-MORTEM* EXAMINATION

No abnormalities were detected in any bird.

mallard feed consumption

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control (concentration ppm)	10	34.1000	7.8804	23.1
2 163	5	32.8000	7.7910	23.8
3 325	5	33.6000	8.3845	25.0
4 650	5	31.4000	7.4364	23.7
5 1,300	5	25.2000	7.1903	28.5
6* 2,600	5	20.6000	6.0249	29.2
7* 5,200	5	9.2000	.8367	9.1

NOEC = 1,300 ppm
however, 26% wh. b.l.c. at this conc, the
NOEC = 650 ppm,

*) 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

Minumum detectable difference for
t-tests with Bonferroni adjustment = -7.998991
This difference corresponds to -23.46 percent of control

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* *
* 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 = 2776.475000 with 6 degrees of freedom.

Error mean square = 50.269697 with 33 degrees of freedom.

Bartlett's test p-value for equality of variances = .045