Data Evaluation Report on the acute dietary toxicity of Acetamiprid insecticide to the Mallard Duck (Anas platyrhynchos)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 44651861

Data Requirement: : PMRA DATA CODE: 9.6.2.5-1
EPA DP Barcode:
OECD Data Point: IIA 8.1.2
EPA Guideline: US EPA Subdivision E Guideline 71-2

Test material: NI-25 **Purity (%):** 99.57%
Common name: Acetamiprid
Chemical name: N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine
IUPAC: (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine
CAS name: (*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N'*-cyano-*N*-methylethanimidamide
CAS No.: 160430-64-8
Synonyms: Pristine Brand RTU, Chipco Brand Tristar 70 WSP, Adjust Brand 70 WP and Assail Brand 70 WP

Primary Reviewer: Alison McLaughlin **Date:** January 25nd 2001
For PMRA

Secondary Reviewer(s): Hemendra Mulye, PhD **Date:** June 5, 2001
{EPA/OECD/PMRA}

Company Code: [For PMRA]
Active Code: [For PMRA]
Use Site Category:[For PMRA]
EPA PC Code:

CITATION: Johnson, A.J. 1994. NI-25 Subacute Dietary Toxicity (LC50) to the Mallard Duck, Huntingdon Research Center Ltd. (aka. Huntingdon Life Sciences Limited), Huntingdon, Cambridgeshire, England. Report No. NPS 60/942075, Sponsor: Nippon Soda Co., Tokyo, Japan. July 21 1994. Unpublished.

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EXECUTIVE SUMMARY:

The acute dietary toxicity of Acetamiprid (NI-25) to 8 day old Mallard Duck (Anas platyrhynchos) was assessed over 5 days in accordance with the US EPA Avian dietary LC_{50} test (EPA-540/9-85-008). NI-25 was administered to four controls and three treatment groups, each with ten birds. The treatment groups received 200, 1000 and 5000 mg a.i/kg dw (ppm) in diet respectively. The 5 day acute dietary LC_{50} was 5000 mg a.i/kg dw of diet (95% C.I.: 3700 - 6300 mg a.i./kg bw). The 5 day NOEC of NI-25 based on mortality was 200 mg a.i/kg dw of diet (95% C.I.: 0 - 1500 mg a.i./kg bw). According to the US EPA classification, NI-25 would be classified as slightly toxic to Mallard Duck (Anas platyrhynchos) on an acute dietary basis.

Under the conditions of this study, clinical signs of toxicity were noted in all birds treated at the 1000 and 5000 ppm dose levels. The birds became unsteady and imbalanced. Sublethal effects at these dose levels included significantly reduced food consumption and related depression of normal bodyweight increase. Post-mortem results for four birds dosed at the 5000 ppm level and one bird dosed at the 1000 ppm level indicated reduced muscle and subcutaneous fat.

This toxicity study is classified as supplemental and repeated because only three test concentrations were used and mortality (4 out of 10 birds) occurred at the highest test concentration (5000 ppm). The study was conducted during two different time periods. One of the test concentrations was evaluated two months after the other two test concentrations.

Results Synopsis

Test Organism: Mallard Duck (Anas platyrhynchos), 8 days old, mean weight 100 g.

LC_{50}: 5000 mg a..i./kg diet	95% C.I.: 3700 - 6300 mg a.i./kg bw
NOEC: 200 mg a.i./kg diet	95% C.I.: 0 - 1500 mg a.i./kg bw
Endpoint(s) Effected: mortality	

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The method followed was that given in the US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Series 71-1 Avian dietary LC_{50} test, dated Oct. 1982 and draft revised guideline dated Mar 1988.

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COMPLIANCE: It was stated that this study had been conducted according to GLP Standards under OECD Principles of GLP, OECD Environment Monograph No.45, 1992 and the US EPA, FIFRA, 40 CFR Part 160, 29 November 1983/ammended 17 August 1989. It was stated that the study also complied with the GLP standards of the UK Department of Health, the EC Council Directive and the Japan Ministry of Agriculture. Signed and dated GLP, Quality Assurance, and Signature Page were provided. There was also a signed and dated Statement of No Data Confidentiality Claim.

A. MATERIALS:

1. Test Material NI-25

Description: Pale yellow powder

Lot No./Batch No. : NNI-03

Purity: >99 %

Stability of Compound Under Test Conditions: Results of the analytical chemistry report (Appendix 1) indicate that NI-25 was stable at nominal concentrations of 200 ppm, 1000 ppm and 5000 ppm in the avian diet formulation assessed over a period of 6 days during storage under animal room conditions.

Storage Conditions of Test Chemicals: Prior to testing, NI-25 was stored at 4°C in the dark; the test substance analysis certificate reported that NI-25 is stable for 1 yr in the dark at 50°C, and stable for 4 yrs in the dark at -20°C.

Physicochemical properties of [test material].

Parameter	Values	Comments
Water solubility at 20°C	not reported	* reported elsewhere as 0.4% at 25°C

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Parameter	Values	Comments
Vapour pressure	not reported	* reported elsewhere as <1.0 x 10-6 Pa ar 25°C
UV absorption	not reported	
pKa	not reported	
Kow	not reported	

* These results come from the Salinity Challenge Study in this same data submission

2. **Test organism**:

Species: Mallard Duck (Anas platyrhynchos)

Age at study initiation: 8 days old at the introduction of the test diet

Weight at study initiation: (mean and range): mean 100 g, range was not reported

Source: Country Game Farms, Ashford, Kent, England

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A preliminary range finding test had been performed on birds at 0, 1000 and 5000 ppm dose levels, but no results were provided. There was no indication of how the results from the preliminary study were used to determine the conditions for the definitive study.

b) Definitive Study: The definitive study was conducted in two parts. The first part included a test of two negative controls and nominal doses of 1000 and 5000 ppm. Two months later, a second portion of the study was conducted which included two controls and one nominal dose of 200 ppm. The nominal dose of 200 ppm was prepared with a methanol vehicle, however, we have no indication if two accompanying controls were prepared with methanol or if they were also "negative" untreated controls. Results for both parts of the study were combined.

Table 1: Experimental Parameters

Parameter	Details	Remarks

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		Criteria
Acclimation Period: Conditions (same as test or not): Feeding: Health (any mortality observed):	 4 days acclimation yes standard chick diet *ad libitum* none reported	acceptable *OECD requires at least 7 days of acclimation*
Pen size and construction materials	wire floor pens 1.80 x 1.22 m, with low level feeder and drinking fount	acceptable *EPA requires: about 35 x 100 x 24 cm; OECD requires: 300 cm^2 for bobwhite and 600 cm^2 for mallard*
Test duration	4 days pre-treatment, 5 days treatment, 3 days post-treatment	acceptable *EPA/OECD requires: 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations Nominal: Measured:	 200 *mg ai/kg diet* 1000 *mg ai/kg diet* 5000 *mg ai/kg diet* 192 *mg ai/kg diet* 990 *mg ai/kg diet* 5010 *mg ai/kg diet*	 *Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 mg ai/kg diet.* Measured conc. should be 80% of the nominal
Solvent/vehicle, if used Type: Amount:	The test substance, NI-25, was added as the solid test material when inclusion levels ≥1000 ppm, or as a solution in methanol when inclusion levels were < 1000 ppm.	The EPA guidelines are not clear on policy for the addition of dry powder to a test diet. *EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.*
Diet preparation and feeding	Treated diets were prepared by mixing the test substance with the untreated basal diet. Diets were prepared one day prior to use and during the treatment period, all diets were stored in labeled polythene bags at room temperature.	 *EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets*
Was detailed description and nutrient analysis of the basal diet provided (Yes/No)	Yes. The composition of the diet was described.	acceptable

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Parameter	Details	Remarks *Criteria*
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes. Homogeneity and stability were acceptable.	acceptable
Feed withholding period	none	
Number of birds per replicate/groups For negative control: For vehicle control: For treated:	 10 for each of four replicates NA 10 for each treatment level	 *EPA requires: 10 birds each (strongly recommended)*
Number of replicates/group (if used) For negative control: For vehicle control: For treated:	 4 NA 1 at each treatment level	
Test conditions Temperature: Relative humidity (%): Photoperiod:	 *21-24 °C* in the animal room An infra-red heat lamp was suspended over each box to provide additional heat 44 % 14 hour	 *Brooder temperature:* *EPA: about 35 °C (95 °F)* *Room temperature:* *EPA: 22-27 °C (71-81 °F);* *OECD: range of 22-38 °C based on bird age and species (see OECD 205)* *Relative humidity:* *EPA: 30-80%* *OECD: 50-85% based on bird species (see OECD 205)* *Photoperiod:* *EPA: Minimum of 14 h of light* *OECD: 12-16 h of light*

b) Analytical Chemistry Report analysis for the Measured Dose Concentrations:

Table 2. Calculation of the Measured Dose

Nominal conc. (mg ai/kg dw) (ppm):	Mean analyzed conc. (mg ai/kg dw) (ppm):	Relative mean error as measured concentration deviation from nominal	Variation of NI-25 in diet formulation samples
200	192	-4.0%	2.32%

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Nominal conc. (mg ai/kg dw) (ppm):	Mean analyzed conc. (mg ai/kg dw) (ppm):	Relative mean error as measured concentration deviation from nominal	Variation of NI-25 in diet formulation samples
1000	990	+2.0 %	7.31%
5000	5010	+4.2 %	1.67%

Results of the Analytical chemistry report confirmed that the nominal concentrations of the dietary formulation were very accurate. The report confirmed that the dietary formulations were stable over a period of six days. The report also showed that the dietary formulations were homogeneous at the time of preparation, however, there were no results for homogeneity at study completion.

2. Observations:

Table 3: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured (mortality/body weight/ mean feed consumption/ others)	mortality body weight feed consumption	*OECD : the mortality in the controls should not be exceed 10% at the end of the test.*
Indicate the stability and homogeneity of test chemical in the diet	Stable over 6 days Homogeneous at study initiation	
Indicate if the test material was regurgitated	No regurgitation was reported	
Treatments on which necropsies were performed	Ten control birds and ten birds from the highest treatment level, as well as ten birds from the lowest treatment level were subject to macroscopic post mortem examination.	
Observation intervals (days)	Bodyweight: -4, 0, 5, 8 Food consumption: -4 to -1 pre-test, daily during testing (1,2,3, 4, 5), and 6 to 8 post-testing	
Were raw data included?	Raw analytical data, but not	

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	raw data for bodyweight, food consumption and necropsy.	

II. RESULTS AND DISCUSSION:

A. MORTALITY: Two birds at the 5000 ppm treatment level were found trembling and unable to stand early in the test on Day 3; both of these birds were sacrificed on humane grounds. These birds will be considered treatment related mortalities since their described condition suggests that recovery would have been highly unlikely, not to mention consideration of the increased susceptibility to predation of any bird in this condition. Two other mortalities were noted in this group the following day. One mortality was noted at the 1000 ppm treatment level.

Table 4: Effect of NI-25 on mortality of Mallard Duck (Anas platyrhynchos).

Treatment (mg a.i. kg diet)	No. of birds per treatment	Mortality (Days of Study)							
		-3 to -1	1	2	3	4	5	6 to 8	Total
control (for 1000, 5000 ppm)	10								
control (for 1000, 5000 ppm)	10								
control (for 200 ppm)	10								
control (for 200 ppm)	10								
Test concentration 200	10								0
Test concentration 1000	10				1				1
Test concentration 5000	10				2	2			4
LC_{50}	estimated at 5000 ppm								
NOEC	200 ppm								

B. SUB-LETHAL TOXICITY ENDPOINTS:
There were clinical signs of toxicity such as loss of balance was reported in all birds treated at the 1000 and 5000 ppm levels. Examination of the food consumption data would suggests a sub-lethal toxic effect. Food avoidance and related bodyweight appear to have been a significant factor at the 1000 ppm and 5000 ppm levels although this was not demonstrated statistically. Note that the reported values were all rounded off to the nearest gram.

Table 5: Group mean bodyweights and bodyweight changes (g)

Treatment (mg a.i. kg diet)	No. of birds per treatment	Days of Study						
		Bodyweight				Bodyweight increase		
		-4	0	5	8	-3 to 0	0 to 5	5 to 8
control (for 1000, 5000 ppm)	10	51	101	218	311	50	117	93
control (for 1000, 5000 ppm)	10	51	99	215	309	48	116	94
control (for 200 ppm)	10	51	104	213	286	53	109	73
control (for 200 ppm)	10	51	97	203	278	46	106	75
Test concentration 200	10	51	102	201	291	51	99	90
Test concentration 1000	10	51	98	138	228	47	40	90
Test concentration 5000	10	51	101	90	174	50	-11	84

Table 6: Group mean food consumption (g/bird/day)

Treatment (mg a.i. kg diet)	No. of birds per treatment	Days of Study							
		-4 to -1	1	2	3	4	5	1 to 5	6 to 8
control (for 1000, 5000 ppm)	10	24	36	40	47	53	55	46	67
control (for 1000, 5000 ppm)	10	24	36	43	48	52	58	47	74
control (for 200 ppm)	10	26	39	35	48	59	60	48	62
control (for 200 ppm)	10	23	34	40	49	55	56	47	52
Test concentration 200	10	30	39	45	46	54	59	48	60
Test concentration 1000	10	23	18	28	31	35	37	29	62
Test concentration 5000	10	24	10	14	17	19	21	15	57

C. REPORTED STATISTICS: No statistical analysis was performed. The proponent stated that it was not possible to calculate an LC_{50} value with the data available, but that the value must

be in excess of 5000 ppm.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: There was no evidence that the mortalities and severe debilitation of birds at the 5000 ppm level was not treatment related. Conversely, the clinical signs of toxicity in birds treated at 1000 and 5000 ppm would suggest that the mortalities were treatment related. Statistical analysis of the limited data set for mortality was performed (Appendix I). Using trend analysis, the LC_{50} value based on nominal dose concentrations was found to be 6176 mg ai/kg bw (C.I. 4800 - 7500 mg/kg), but this statistic was derived from only three treatment levels, including one treatment level that had been "retrofitted" into the study. Based on direct observation of the results, the actual LC_{50} value might well have been as low as 5000 mg ai/kg bw (C.I. 3700 - 6300 mg/kg). In view of the poor data sample submitted, the more conservative LC_{50} value of 5000 mg ai/kg bw (ppm) was selected. The proposed NOEC of 200 mg ai/kg bw (ppm) was accepted.

E. STUDY DEFICIENCIES:

1) The US EPA protocol for the Avian Dietary LC_{50} Test (EPA-540/9-85-008) states that studies should be designed to establish an actual LC_{50} and 95% C.I.. In lieu of this, a study may demonstrate that the LC_{50} is greater than 5000 ppm. When any mortality is observed at 5000 ppm, however, sequentially lower doses must be tested (with ten birds per level) in order to get a dose-response series which includes at least one "no-effect" (no mortality) level.

This study clearly used an inadequate number of treatment levels which confounded calculation of a statistically sound dose-response curve. The study had no range finding test and was poorly designed. Consequently, the data includes one treatment level (200 ppm) that was "retrofitted" subsequent to test completion of the other two treatment levels. The proponent stated that the data was insufficient for the calculation of an LC_{50} value. While it is true that the data set is not ideal, an LC_{50} value and 95% confidence intervals could be estimated, and was estimated for the purpose of this review. A conservative estimate was made in view of the limited data set.

2) The proponent should be asked to clarify what type of controls (negative or vehicle) were used for the 200 ppm test treatment level.

F. REVIEWER'S COMMENTS:

This toxicity study is classified as supplemental and repeated because only three test concentrations were used and mortality (4 out of 10 birds) occurred at the highest test concentration (5000 ppm). The study was conducted during two different time periods. One of the test concentrations was evaluated two months after the other two test concentrations. Also,

methanol was used as a carrier, which is generally not accepted by EPA.

G. CONCLUSIONS: This toxicity study is classified as supplemental. The LC_{50} value was found to be 5000 mg ai/kg bw (C.I. 3700 - 6300 mg a.i./kg bw). The NOEC value was 200 mg ai/kg bw (C.I.: 0 - 1500 mg a.i./kg bw). On the basis of these results, Acetamiprid (NI-25) would be classified as slightly toxic to Mallard Duck. There were sub-lethal clinical signs of toxicity, such as loss of balance, reported in all birds treated at the 1000 and 5000 ppm levels.

III. REFERENCES:

Anderson, A., Dawe, I.S., and L. Martin. Analytical Chemistry Report. NI-25: The Analysis in Avian Diet Formulations. NPS 59/932525.

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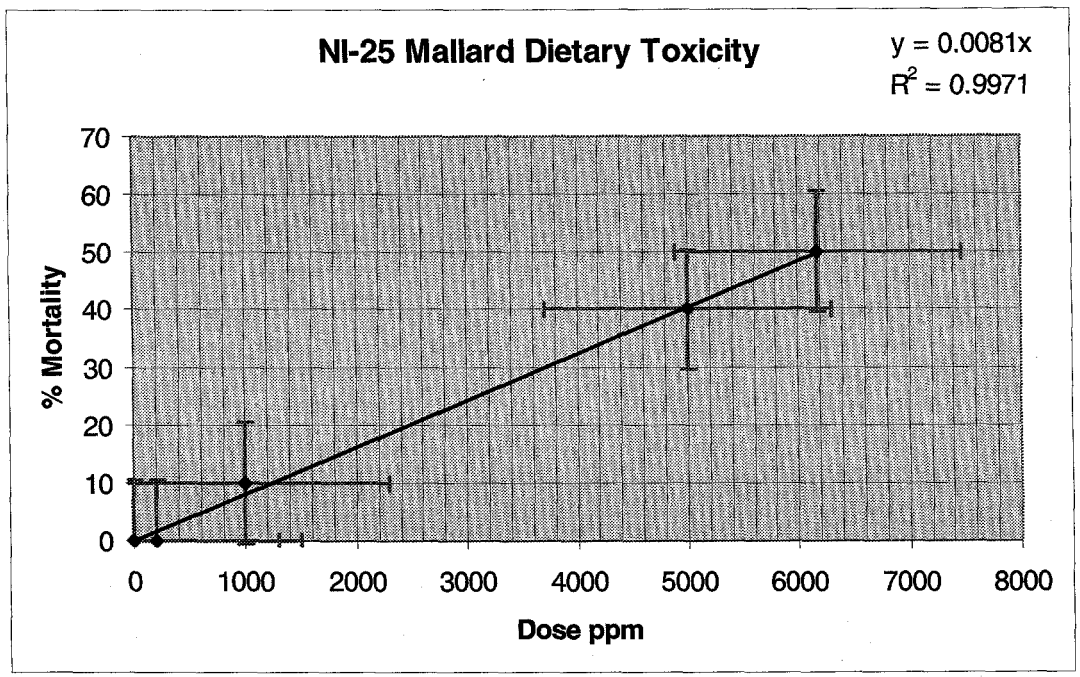

Appendix I:

Limited statistics were performed on the available data. There were only three treatment levels and a control level from which to extrapolate.

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NOEC and LC_{50} values were extrapolated through a trend line. A conservative estimate of the LC_{50} was made in view of the limited data set. Using Excel software. Standard error values were calculated and are shown in red. The equation for the slope of the line appears next to the title.

Accordingly

LC_{50}: 5000 mg a..i./kg bw 95% C.I.: 3700 to 6300 mg a.i./kg bw
NOEC: 200 mg a.i./kg 95% C.I.: 0 to 1500 mg a.i./kg bw

Approved 04/01/01 C.K.