Data Evaluation Report on the Acute Oral Toxicity of Acetamiprid to the Passerine Zebra Finch (*Taeniopygia guttata*)

EPA MRID Number 484077-01

Data Requirement:	EPA DP Barcode	D388026
	EPA MRID	484077-01
	EPA Guideline	850.2100

Test material:	**NI-25**	**Purity:** 99.9%
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.:	135410-20-7	
Synonyms:		

Primary Reviewer: Scott Glaberman **Date:** 7 April 2011
Scott Glaberman, Biologist, PhD, Environmental Risk Branch 4 (ERB4), Environmental Fate and Effects Division (EFED), U. S. Environmental Protection Agency

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CITATION: Hubbard, P. 2011. Acetamiprid: An Acute Oral Toxicity Study with the Zebra Finch (*Taeniophygia guttata* aka *Poephila guttata*[1]). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Project ID: 437-119. Study sponsored by Nippon Soda Co., Ltd., Chiyoda-ku, Tokyo, Japan.

[1] http://elibrary.unm.edu/sora/Auk/v106n04/p0750-p0750.pdf

EXECUTIVE SUMMARY:

The acute oral toxicity of acetamiprid (NI-25) to 4-to-8-month-old zebra finches (*Taeniopygia guttata* aka *poephila guttata*) was assessed over 14 days in accordance with EPA Ecological Effects Test Guidelines OPPTS Number 850.2100. Acetamiprid was administered to birds by gavage (oral intubation) at nominal doses of 1.8, 2.5, 3.6, 5, 7, and 10 mg a.i./kg bw. The 14-day acute oral LD_{50} is 5.7 mg a.i./kg bw. The 14-day NOAEL for mortality is 2.5 mg a.i./kg bw. The 14-day NOAEL for mean body weight and mean feed consumption are both 10 mg a.i./kg bw. The 14-day NOAEL based on other clinical signs, including ruffled appearance and abnormal behavior, is < 1.8 mg a.i./kg bw. According to US EPA classification, acetamiprid would be classified as **VERY HIGHLY TOXIC** to zebra finches on an acute oral exposure basis.

No mortalities occurred in the control group or the lowest acetamiprid (1.8 mg a.i./kg bw) dosage group. Two mortalities (out of 10 individuals) occurred in the 2.5 mg a.i./kg bw dosage group, but both were attributed to non-treatment related causes and were not included in LD_{50} or NOAEL calculations. NOAELs were determined by visual inspection of the data and were not determined statistically. There was 10% (1 of 10) mortality in the 3.6 mg a.i./kg bw dosage group, 20% (2 of 10) mortality in the 5 mg a.i./kg bw dosage group, 80% (8 of 10) mortality in the 7 mg a.i./kg bw dosage group, and 100% (10 of 10) mortality in the 10 mg a.i./kg bw dosage group.

No apparent effects were observed in mean body weight or mean feed consumption of acetamiprid-treated birds relative to controls throughout the test period. At least one clinical sign of toxicity or observation of abnormal behavior was recorded in all treatment groups, but not in the control group. Symptoms ranged from transient ruffled appearance in the lowest dose group (1.8 mg a.i/kg bw) to lethargy, wing droop, prostrate posture, loss of coordination, loss of righting reflex, depressed behavior, and minor muscle fasciculation in higher dose groups.

This study is classified as **ACCEPTABLE**; it is scientifically sound and is consistent with the test guidelines for an acute avian oral toxicity study with a passerine species (i.e., *T. guttata*).

Results Synopsis

Test Organism Size/Age: Weight range 10.2-16.2 g (combined sexes; Day 0); Age range approximately 5-9 months old.

LD_{50}: 5.68 mg a.i./kg bw 95% C.I.: 4.9 to 6.7 mg a.i./kg bw
Probit slope: 8.6 95% C.I.: 4.4 to 12.8
NOAEL: < 1.8 mg a.i./kg bw (based on transient ruffled feather appearance)
Endpoint(s) Affected: The endpoint for LD_{50} was mortality. The endpoint for NOAEL was any one sign of nonlethal clinical toxicity.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in FIFRA Subdivision E, Section 71-1 and U.S. EPA Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.2100.

The deviations from the OPPTS Guideline No. 850.2100, avian acute oral toxicity test included:

1) Fasting time of 2-3 hours was shorter than recommended time of at least 15 hours.
2) Relative humidity levels of 22% ± 10% in test environment was below 45-70% recommended in guidelines.
3) Gross pathology was not performed on at least two birds dying per dosage level as specified in guidelines.
4) A single regurgitating individual from the 10 mg a.i./kg bw dosage group was removed from the study on day 0 and replaced with a new individual. Guidelines do not specify this as appropriate practice.
5) Age of animals used in study was estimated to be between 4 and 8 months, which is greater than the allowable age variation of ± 1 week described in guidelines.

COMPLIANCE: Signed and dated GLP and Quality Assurance statement were provided. This study was conducted in accordance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Part 160).

A. MATERIALS:

1. Test Material Acetamiprid (NI-25)

Description: Solid, light yellow powder

Lot No./Batch No. : NFG-02

Purity: 99.9% active ingredient

Stability of compound under test conditions: Stable

Storage conditions of test chemicals: Stored at room temperature

Physicochemical properties of Acetamiprid: Not reported

2. Test Organism:

Species (common and scientific names): Zebra Finch (*Taeniopygia guttata*; also known as *Poephila guttata*)

Age at study initiation: Approximately 5-9 months

Weight at study initiation (mean and range): Mean: 12.1 g; Range: 10.2-16.2 g

Source: Maryland Exotic Birds, Pasadena, Maryland

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Study found total mortality at 10 mg a.i./kg bw. No further study details were provided.

b. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	19 days Same as test conditions Not described, but assumed to be same as test conditions 3 mortalities out of 150 birds in same lot used for testing	**Acceptable** *The recommended acclimation period is a minimum of 15 days. OECD recommends a minimum of 7 days.*
Pen size and construction materials	Pen size: 58 x 26 cm with 31 cm ceiling. Construction: external walls, ceiling, floors made of coated wire; side walls made of fiberglass. Each pen contained 3 perches made of wood, cement, cuttlebone	**Acceptable.** Appropriate pen size for passerine birds not described in guidelines, but experimental pen size meets specifications for northern bobwhite quail. In addition, no mortality in control animals suggests pen size and conditions were sufficient. *Pen size and construction should conform to good husbandry practices and should not create crowding stress.* *OECD recommends that pens be suitable for the captive rearing of that species.*
Test duration	14 days	**Acceptable** *Recommended test duration is one day for dosing and at least 14 days observation.*
Dose preparation	The appropriate dose of test substance was weighed and dissolved in reverse osmosis water	**Acceptable**
Mode of dose administration	Gavage via oral intubation	**Acceptable** *Gavage or gelatin capsule is recommended*

Parameter	Details	Remarks *Criteria*
Dose levels nominal: measured:	Nominal: 1.8, 2.5, 3.6, 5, 7, 10 mg a.i./kg bw No measured values	**Acceptable** *Dose levels should be a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2000 mg ai/kg*
Solvent/vehicle, if used type: amount/bw:	Type: reverse osmosis water Amount: 10 ml/kg bw (1%)	**Acceptable** *The test material should be administered without a vehicle if possible. Maximum vehicle should not exceed 0.1 to 1.0% of body weight.*
Number of birds per groups/treatment for negative control: for solvent/vehicle control: for treated:	Not performed 10 (5 male, 5 female) 10 (5 male, 5 female)	**Acceptable** Note: 5 pens of 2 birds (1 male, 1 female) in each treatment group and vehicle control group *Recommended number of birds in a treatment group is 10 and 10 birds for each control and vehicle group.*
No. of feed withholding days before dosing	Feed withheld 2 to 3 hour fast before dosing	**Shorter than specified in guidelines, but sufficient for passerines** *Food should be withheld for at least 15 hours prior to dosing.*
Test conditions Temperature: Relative humidity: Photoperiod:	23.2 °C ± 1.2 °C 22% ± 10% 8.5 hours light/dim light; 15.5 hours dark	**Relative humidity below guideline specifications.** No mortality in control animals suggests conditions were sufficient. *The recommended photoperiod is 10 hours of light and 14 hours of dark.*
Reference chemical, if used name: concentrations tested:	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured (mortality/individual body weight at test initiation and termination/ mean feed consumption/ others)	-Mortality -Signs of toxicity -Abnormal behavior -Mean body weight -Estimated mean feed consumption (g/bird/day)	**Acceptable** *Body weight should be measured at test initiation, on day 14 and at the end of the test if the test is extended beyond 14 days. Mortality should not be more than 10% in controls.* *Feed consumption should be measured as average daily food consumption.*
Indicate if the test material was regurgitated	One female in the 10 mg a.i./kg dosage level regurgitated and was removed from the study and replaced with another female	**Acceptable** *Regurgitation is an indication that the dose was rejected. If this problem persists, the test should be repeated.*
Groups on which necropsies were performed	Two individuals from 2.5 mg a.i./kg dosage group that were indicated to have died from causes unrelated to test substance were examined. Exterior of birds were examined as well as thoracic and abdominal cavities, including cardiovascular and respiratory systems, liver, spleen, gastrointestinal tract, urogenital systems.	**Did not perform necropsies on two dying individuals per dosage group as specified in guidelines** (see discussion of study deficiencies [Section II(E)]) *Gross necropsies should be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*
Observation intervals	-During acclimation period: observed daily for abnormal behavior or physical injury -Observed for at least one hour after dosing for signs of regurgitation -Observed multiple times on day 0 of test -During test period: observed 2 times per day -Body weight measured at test initiation and on days 3, 7, 14 -Feed consumption	**Acceptable**

	observed by pen at 24-hr intervals for 3 days post-treatment; average feed consumption then calculated for 3-7, 7-10, and 10-14 days	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

All mortalities took place on day 0 or 1 of the test period as described in **Table 3**, except two mortalities in the 2.5 mg a.i./kg dosage group that that took place on days 7 and 9. These two deaths were reported as incidental and unrelated to the test compound. This conclusion was based on necropsy results and the later timing of death within the study test period. The male individual that died on day 7 had injuries suggestive of cage mate aggression. The female individual that died on test day 9 had necropsy results suggestive of an incidental infection in the abdominal cavity.

Table 3: Effect of NI-25 on Mortality of *Taeniopygia guttata*

Treatment (mg a.i./kg bw)	No. of Birds	Cumulative Mortality	
		Day 1	Total
Control	10	0	0
1.8	10	0	0
2.5	8[a]	0	0
3.6	10	1	1
5	10	2	2
7	10	8	8
10	10	10	10
NOAEL	2.5 mg a.i./kg bw		
LD_{50}	5.68 mg a.i./kg bw (95% CI: 4.9 to 6.7 mg a.i./kg bw)		

[a] The two mortalities recorded in the 2.5 mg a.i./kg bw dosage group were not included in the table or NOAEL calculation since they were attributed to non-treatment related causes.

B. SUBLETHAL TOXICITY ENDPOINTS:

Body weights of each individual were observed at test initiation and on days 3, 7, and 14. The mean weight was then calculated for each dosage group, sex, and day in which individual weights were collected (**Table 4**). Mean weight changes between observation days were also calculated (**Table 4**). According to the study author, no notable changes in weight, relative to controls, occurred between observation days or between test initiation and termination based on a qualitative (visual inspection) rather than a quantitative statistical examination of the

data. Since no notable weight changes were observed, relative to controls for any dosage group, the NOAEL for mean body weight for this study is the highest dose tested (i.e., 10 mg a.i./kg bw).

Table 4: Sublethal Effect of Acetamiprid on Mean Body Weight of *Taeniopygia guttata*

Mean Body Weight (and Change), g								
Nominal Treatment Group (mg a.i./kg bw)	**Males**				**Females**			
	Day 0	**Day 3**	**Day 7**	**Day 14**	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Control	11.8	12.5 (0.8)	12.8 (0.3)	12.4 (-0.4)	11.1	12.0 (0.9)	12.2 (0.2)	12.1 (-0.2)
1.8	12.3	13.3 (1.1)	13.4 (0)	13.3 (-0.1)	11.8	12.5 (0.7)	12.6 (0.1)	12.7 (0)
2.5	11.9	12.9 (1.0)	13.0 (0.2)	12.5 (-0.5)	12.7	13.7 (1.0)	13.2 (-0.4)	14.3 (0.2)
3.6	11.9	12.4 (0.5)	12.5 (0.1)	12.4 (-0.1)	12.6	12.9 (0.4)	13.1 (0.2)	13.0 (-0.2)
5	12.7	13.5 (0.7)	13.5 (0)	13.4 (-0.1)	12.7	13.0 (0.6)	13.1 (0.1)	13.1 (0)
7	12.0	12.1 (0.9)	12.2 (0.1)	11.8 (-0.4)	12.0	-	-	-
10	11.8	-	-	-	11.9	-	-	-
NOAEL	10 mg a.i./kg bw				10 mg a.i./kg bw			

Feed consumption was recorded by pen (each pen contained two individuals; one male, one female) for the intervals between days 0, 1, 2, 3, 10, and 14 (**Table 5**). Mean feed consumption was calculated for each time interval per dosage group. As with body weight, visual inspection of the data did not identify notable changes in mean feed consumption relative to controls between observation intervals for any dosage group, although there was no quantitative or statistical treatment of the data. The NOAEL for mean feed consumption is equivalent to the highest dosage tested (i.e., 10 mg a.i./kg bw).

Table 5: Sublethal Effect of Acetamiprid on Mean Feed Consumption of *Taeniopygia guttata*

Mean Feed Consumption, g/bird/day						
Nominal Treatment (mg a.i./kg bw)	**Day 0-1**	**Day 1-2**	**Day 2-3**	**Day 3-7**	**Day 7-10**	**Day 10-14**
Control	5	5	5	4	5	5
1.8	5	5	6	5	5	5
2.5	5	5	6	5	6	6
3.6	5	5	6	5	6	4
5	4	6	8	5	6	5
7	4	6	3	5	6	5
10	-	-	-	-	-	-
NOAEL	10 mg a.i./kg bw					

Observations of other clinical signs of toxicity and behavioral abnormalities were recorded for each individual animal twice per day for the entire study period. All observations were provided in the appendix of the study report. Endpoints included: ruffled appearance, lethargy, wing droop, prostrate posture, loss of coordination, loss of righting reflex, depression, and minor muscle fasciculation. None of these symptoms was observed in the control group. In the 1.8 mg a.i./kg bw dosage group, transient ruffled appearance was the only clinical sign recorded and was observed in a single individual. In the 2.5 mg a.i./kg bw dosage group, ruffled appearance was more widespread (4 of 10 individuals), and wing droop and loss of coordination were each observed in a single individual. In all higher dosage groups (3.6, 5, 7, and 10 mg a.i./kg bw groups), clinical toxicity signs were more widespread with multiple signs of toxicity observed in multiple individuals. NOAEL values for each specific endpoint are reported in **Table 6.**

Table 6: NOAEL Values for Acetamiprid on Clinical Toxicity and Behavioral Endpoints

Clinical Toxicity or Abnormal Behavior Endpoint	NOAEL Values
Depression	2.5 mg a.i./kg bw
Wing droop	1.8 mg a.i./kg bw
Loss of coordination	1.8 mg a.i./kg bw
Prostrate posture	2.5 mg a.i./kg bw
Loss of righting reflex	2.5 mg a.i./kg bw
Ruffled appearance	<1.8 mg a.i./kg bw
Lower limb weakness	3.6 mg a.i./kg bw
Lethargy	3.6 mg a.i./kg bw
Minor muscle fasciculation	2.5 mg a.i./kg bw
Gaping	5 mg a.i./kg bw

Note: Mortality-related symptoms in two dying individuals from 2.5 mg a.i./kg bw dosage group were not included in NOAEL calculation since these individuals were suggested to have died from non-treatment related causes.

C. REPORTED STATISTICS:

The study author used probit analysis to calculate the LD_{50} value for mortality, 95% confidence interval, and probit dose-response slope using the computer program of C.E Stephan (Stephan, 1978). The LD_{50} value for zebra finch exposed to acetamiprid as a single oral dose was reported to be 5.68 mg a.i./kg bw (95% CI: 4.9 to 6.7 mg a.i./kg bw). The slope of the dose-response curve was 8.6 and the Chi-square value was 1.582. The no-treatment-related mortality level was reported as 2.5 mg a.i./kg bw. The study author did not conduct any statistical tests on body weight or feed consumption endpoints, as well as clinical toxicity or behavioral endpoints.

D. VERIFICATION OF STATISTICAL RESULTS:

Results were verified using the TOXWIN program which calculates LD_{50} values using probit analysis. The program results output is shown below:

```
RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS      G          H      GOODNESS OF FIT PROBABILITY
5               .2380986   1      .8120546
SLOPE = 8.635674
95 PERCENT CONFIDENCE LIMITS = 4.421868 AND 12.84948
INTERCEPT=-6.512657
LC50 = 5.677502
95 PERCENT CONFIDENCE LIMITS = 4.851956 AND 6.672499
LC25 = 4.742978
95 PERCENT CONFIDENCE LIMITS = 3.701309 AND 5.45532
LC10 = 4.034128
95 PERCENT CONFIDENCE LIMITS = 2.781846 AND 4.745703
LC05 = 3.661677
95 PERCENT CONFIDENCE LIMITS = 2.325694 AND 4.401978
```

The above results match those calculated by the study author and confirm their findings for the mortality endpoint.

An additional analysis was run including the two individuals that died in 2.5 mg a.i./kg bw dosage group as treatment-related mortalities; results are as follows:

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RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS      G          H      GOODNESS OF FIT PROBABILITY
5               .1819038   1      5.531639-2
SLOPE = 5.100219
95 PERCENT CONFIDENCE LIMITS = 2.924966 AND 7.275471
INTERCEPT=-3.685006
LC50 = 5.278605
95 PERCENT CONFIDENCE LIMITS = 4.335613 AND 6.566993
```

Since the onset of mortality and behavioral effects of birds in the 2.5 mg a.i./kg bw treatment group do not appear to be dose related, the two mortalities are not considered by the reviewers to be treatment related.

E. STUDY DEFICIENCIES:

There were several deviations from OPPTS Guideline No. 850.2100 as follows:

1) The animals in this study underwent a fasting period of 2-3 hours before the test substance was administered. This is shorter than the recommended time of at least 15 hours described in the guidelines. However, these guidelines were not designed for passerine birds, which includes *T. guttata*. It has also been shown that fasting induces stress in zebra finches (Lynn *et al.*, 2010). The two hour fasting time may be sufficient for digestive tract clearance in passerines.

2) Relative humidity levels of 22% ± 10% in test environment was below 45-70% recommended in guidelines. Since there were no mortalities or adverse clinical symptoms observed in control animals, this deviation from guidelines is not sufficient to invalidate the study or alter interpretation of results.

3) Gross pathology was not performed on any animals in the study that were presumed to die of treatment-

related causes. Guidelines specify that at least two birds dying per dosage level should be examined. Results of necropsies would not change LD_{50} values for this study, but could provide useful information about the causes of death or other toxicological effects. Pathological examinations were only performed on two individuals in the 2.5 mg a.i./kg bw dosage group that were presumed to have died of non-treatment related causes. Necropsies for these two individuals were performed as specified in the guidelines and the findings confirmed, to the study author's satisfaction, that the mortalities were due to causes other than exposure to the test compound. Since these deaths took place later in the study period (days 7 and 9), whereas all other mortalities in the study took place on days 0 and 1, this conclusion appears plausible. However, from the necropsy results reported by the study author, it is difficult to make any definitive conclusion as to the cause of death. Therefore, statistical analyses were also performed in section II(D) under the scenario that the two individuals in the 2.5 mg a.i./kg bw dosage group died of treatment-related causes. Addition of these two individuals to the analysis did not substantially alter the LD_{50} value (less than 1 mg a.i./kg bw difference).

4) A single regurgitating individual from the 10 mg a.i./kg bw dosage group was removed from the study on day 0 and replaced with a new individual. Guidelines do not specify this as appropriate practice. However, this does not appear to be significant since it is limited to one individual. Moreover, there are no specifications in the guidelines on what to do when a regurgitating animal is removed from a study.

5) The estimated age variation of animals (approximately 5-9 months of age) was much higher than the ± 1 week of variation specified in the guidelines. However, there is no passerine-specific guidance for allowable age variation.

Although there were several deviations from the guideline protocol, none of these appear sufficient to invalidate the study or alter interpretation of results.

F. REVIEWER'S COMMENTS:

No additional comments.

G. CONCLUSIONS:

This study is classified as **ACCEPTABLE**; it is scientifically sound and is consistent with the recommended guidelines for an acute avian oral toxicity study with a passerine species (i.e., *T. guttata*).

LD_{50}: 5.68 mg a.i./kg bw 95% C.I.: 4.9 to 6.7 mg a.i./kg bw
Probit slope: 8.6 95% C.I.: 4.4 to 12.8
NOAEL: < 1.8 mg a.i./kg bw (based on transient ruffled appearance)
Endpoint(s) Affected: The endpoint for LD_{50} was mortality. The endpoint for NOAEL was any one sign of nonlethal clinical toxicity.

III. REFERENCES:

Lynn, S.E., Stamplis, T.B., Barrington, W.T., Weida, N., Hudak, C.A. 2010. Food, stress, and reproduction: short-term fasting alters endocrine physiology and reproductive behavior in the zebra finch. *Hormones and Behavior* 58(2):214-22.

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. *Personal Communication.*