Data Evaluation Report on the reproductive effects of Acetamiprid insecticide on avian species Bobwhite Quail (Colinus virginianus)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 449884-07

Data Requirement: PMRA DATA CODE: 9.6.3.1
EPA DP Barcode:
OECD Data Point: IIA 8.1.4
EPA Guideline: 71-4(a)

Test material: **NI-25** **Purity (%):** 100%
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:** February 1st 2001
For PMRA

Secondary Reviewer(s): Hemendra Mulye, PhD **Date:** June 8, 2001
{EPA/OECD/PMRA}

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CITATION: Taliaferro, M.C., Brewer, L.W. and V. Miller. 1997. Reproduction Study with Acetamiprid in the Northern Bobwhite (Colinus virginianus), EBA Inc., 2900 Quakenbush Rd., Snow Camp, NC 27349. Laboratory Project Identification No. 029604, Sponsor: Rhone-Poulenc Ag Company, NC, USA. October 29 1997. Unpublished.

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EXECUTIVE SUMMARY:

The one generation reproductive toxicity of acetamiprid (NI-25) to 3 treatment groups of 20 pairs each, male and female, and one control group of 20 pairs of Bobwhite Quail (Colinus virginianus) was assessed over 204 days (approximately 6 months) in accordance with an experimental protocol based on the US EPA Pesticide Assessment Guidelines (EPA 1982) and ASTM Standard Practice for Conducting Avian Reproduction Test, Draft No. 9 (1983). NI-25 was administered to the birds in the diet at 250, 500 and 1000 mg ai/kg dw diet for 166 days. Ten weekly sets of offspring, for a total of 1576 hatchlings, were housed to 14 days of age at which time surviving offspring were euthanized.

No mortality or intoxication was observed in adult Northern Bobwhites exposed to NI-25 at a dietary concentration of 250 mg ai/kg, 500 mg ai/kg or 1000 mg ai/kg for 23 weeks and five days (166 days). A significant difference was detected in adult male weights at weight interval 3 (start of egg laying) between the 1000 mg ai/kg treatment level and the control group.

There were significant differences detected at the 1000 mg ai/kg treatment level when statistically compared against the control group in several of the reproductive parameters tested including eggs laid and hatchling numbers. Hatchling weight was the most sensitive parameter. There was statistically significant depression of hatchling bodyweight at all treatment levels. The reproductive no observable effect concentration (NOEC) during the study was, therefore, 0 mg ai/kg dw diet (ppm), and the lowest observable effect concentration (LOEC) was 250 mg ai/kg dw diet (ppm).

This toxicity study is classified as supplemental and should be repeated. Birds from over 20 pens died during the study because of a "heat spike" in the laboratory. Also, the NOAEC was not determined because chick body weight was significantly reduced at all test concentrations. Other reproductive parameters such as fertile eggs, 14 day old survivors, viable embryos and number of hatchlings were also reduced compared to controls, although not significantly, at the lowest test concentration.

Results Synopsis

Test Organism Size/Age: Bobwhite Quail (Colinus virginianus), 18 weeks and one day old at start of test, mean weight 234 grams at start of test

NOEC: 0 mg a.i./kg dw diet
LOEC: 250 mg a.i./kg dw diet

Endpoint(s) Effected: The most sensitive parameter was hatchling bodyweight.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The method followed was an EBA Inc. laboratory protocol based on the US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Series 71-4, dated Oct. 1982 and ASTM Standard Practice for Conducting Avian Reproduction Test, Draft No. 9 1983. The protocol was provided in Appendix V and deviations to the protocol were listed in Appendix W. Deviations included the birds weight which ranged 50g heavier than the stated maximum, an accidental 1 hour heat spike which killed some of the test birds, and that humidity was not monitored for a portion of the study.

COMPLIANCE: It was stated that this study had been conducted according to GLP Standards under the US EPA, FIFRA, 40 CFR Part 160, with the exception that feed analysis for pesticides, PCBs and toxic metals would not be analyzed under GLP compliance as stated in the protocol but would be analyzed using standard US EPA procedures. Signed and dated GLP and Quality Assurance were provided. There was also a signed and dated Statement of No Data Confidentiality Claim.

A. MATERIALS:

1. Test Material NI-25

Description: Pale brown powder

Lot No./Batch No. : NEJ/09

Purity: 100%

Stability of Compound Under Test Conditions: Results of the analytical chemistry report (Appendix D) indicate that NI-25 was stable at nominal concentrations

of 250 ppm and 1000 ppm in the avian diet formulation assessed over a period of 90 days during storage under animal room conditions.

Storage Conditions of Test Chemicals: Prior to testing, NI-25 was stored at ambient temperature in a chemical storage cabinet.

Physicochemical properties of NI-25.

Parameter	Values	Comments
Water solubility at 20°C		* reported elsewhere as 0.4% at 25°C
Vapour pressure		* reported elsewhere as <1.0 x 10-6 Pa ar 25°C
UV absorption		
pKa		
Kow		

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

2. Test organism:

Species (common and scientific names): Bobwhite Quail (Colinus virginianus)

Age at study initiation: 18 weeks and one day of age at experimental start

Weight at study initiation: mean: 234 grams range: 192.9-296.6 grams

Source: Quail Valley, 9219 Concord Highway, Indian Trail, North Carolina

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A range finding test was conducted from June 26 1996 (start of treated feed) through July 17 1996 with three treatment groups of 3 pairs each, male and female, and one control group of three pairs. Results of the range finding test were not provided. Nominal test

concentrations for the definitive portion of the study were determined at the conclusion of the range finding portion of the study.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions (same as test or not): Feeding: Health (any mortality observed):	14 days same as testing conditions feed and water were provided ad libitum no illness or mortality observed	*EPA recommends 2-3 week health observation period prior to selection of birds for treatment. Birds must be generally healthy without excess mortality. Sickness, injuries or mortality should be noted. Feeding should be ad libitum* *OECD requires acclimation of at least 2 weeks*
Test duration Pre-laying exposure: Egg-laying exposure: Withdrawal period, if used:	13 weeks 4 days exposure prior to collection of first egg 10 weeks 1 day exposure during egg laying no withdrawl period	*Pre-laying exposure duration* *EPA /OECD require at least 10 weeks prior to the onset of egg-laying.* *Exposure duration with egg-laying* *EPA requires at least 10 weeks.* *Withdrawal period* *EPA requires if reduced reproduction is evident, a withdrawal period of up to 3 weeks should be added to the test phase.*
Pen (for parental and offspring) Size: Construction materials: Number:	51 cm deep x 25 cm wide x 20-25 cm high (sloped floor) epoxy coated, galvanized, welded-wire cages birds were kept in pairs in battery breeding cages	

Parameter	Details	Remarks *Criteria*
		EPA requirements: *Pens* *Adequate room and arranged to prevent cross contamination* *Materials* *Nontoxic material and nonbinding material, such as galvanized steel.* *Number* *At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs. Chicks are to be housed according to parental grouping.*
Number of birds per pen (male:female)	one male, female pair per breeding cage	*EPA requires one male and 1 female per pen. For bobwhite, 1 male and 2 females is acceptable. For mallard, 2 males and 5 females is acceptable.*
Number of pens per group/treatment Negative control: Solvent control: Treated:	20 pairs in the control group with 1 cage for each pair NA 20 pairs in each treatment group with 1 cage for each pair	*EPA/OECD require at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.*
Test concentrations (mg ai/kg diet) Nominal: Measured:	250, 500, 1000 245.30 ±40.96 (98% of nominal) 483.84 ±86.12 (97% of nominal) 1026.01 ±205.21 (103% of nominal)	*EPA requires at least two concentrations other than the control; three or more are recommended. The highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. OECD requires measured concentration in diet should be at least 80% of nominal*
EEC/maximum labeled field residue anticipated and source of information:	not described	

Parameter	Details	Remarks *Criteria*
		EPA requires the highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. The source [i.e., maximum label rate (in lb ai/A & ppm), label registration no., label date, and site should be cited]
Solvent/vehicle, if used Type: Amount:	none. Test substance was sprinkled directly onto the feed.	*EPA /OECD require corn oil or other appropriate vehicle and not more than 2% of diet by weight*
Was detailed description and nutrient analysis of the basal diet provided (Yes/No)	Yes	*EPA requires a commercial breeder feed (or its equivalent) that is appropriate for the test species.*
Preparation of test diet	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. Dates of preparation were listed in Appendix C.	*A premix containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.*
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes. Results were satisfactory.	
Were concentrations in diet verified by chemical analysis (Yes/No)?	Yes.	
Feeding and husbandry	Feed and water were provided ad libitum.	

Parameter	Details	Remarks *Criteria*
Test conditions (pre-laying) Temperature: Relative humidity: Photoperiod:	Mean 70.7 °F (SD 1.8 °F) Mean 52.1% (SD 15.8%) 7 hrs light, 17 hrs dark during acclimation and for the first 8 weeks of treated feed. At the beginning of week 9 lighting was gradually increased over a 6 day period to 17 hours of light, 7 hours dark. Light exposure was an average of 12.6 Foot Candles	*Temperature:* *EPA: about 21°C (70°F)* *OECD: 22 ± 5°C* *Relative humidity:* *EPA: about 55%* *OECD: 50-75%* *Lighting:* *EPA/OECD: first 8 weeks: 7 h per day* *Thereafter:* *EPA: 16-17 h per day.* *At least 6 footcandles at bird level* *OECD: 16-18 h per day*
Egg Collection and Incubation		
Egg collection and storage Collection interval: Storage temperature: Storage humidity: Storage period:	Eggs were collected daily Eggs were stored in a refrigerator at 44.9°F until being placed into the incubator. The storage period and humidity was not described. There were 71 total egg laying days.	*EPA requires eggs to be collected daily; egg storage temperature approximately 16°C (61°F); humidity approximately 65%.* *Collection interval: daily*
Were eggs candled for cracks prior to setting for incubation?	Yes.	*EPA requires eggs to be candled on day 0*
Were eggs set weekly?	Unclear when the eggs were set, although eggs were collected and stored daily.	
When candling was done for fertility?	On day 14 of incubation	*EPA requires:* *bobwhite: approx. day 11* *mallard: approx. day 14* *OECD requires: 6-11 day*

Parameter	Details	Remarks *Criteria*
When the eggs were transferred to the hatcher?	On day 21 of incubation	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*
Hatching conditions Temperature: Humidity: Photoperiod:	 99 °F ± 0.3 °F 52 - 66 % RH not reported	 *Temperature:* *EPA requires: 39°C (102°F)* *OECD requires: 37°C* *Humidity* *EPA requires: 70%* *OECD requires: 70-85%*
Day the hatched eggs were removed and counted	Eggs remained in the hatcher for 3 days and were allowed to hatch over an approximate 28 hr interval.	*EPA requires Bobwhite: day 24* *Mallard: day 27*
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes.	
Egg shell thickness No. of eggs used: Intervals: Mode of measurement:	 N~60 for each treatment group Sample eggs were collected weekly when available. Eggshells were measured with a micrometer at five points around the equator.	 *EPA requires newly hatched eggs be collected at least once every two weeks. Thickness of the shell plus membrane should be measured to the nearest 0.01 mm; 3 - 4 measurements per shell.*

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured		
Parental:	mortality, body weight, mean feed consumption	
Egg collection and subsequent development:	no. of eggs laid, no. of eggs cracked, shell thickness, no. of eggs set, no. of fertile eggs, no. of viable embryos, no. hatched, no. of 14-day survivors, average weight of 14-d old survivors,	*OECD requires that the mortality in the controls is not exceed 10% at the end of the test. The average number of 14 day-old survivors per pen in controls at least 14 and 12 for mallard and bobwhite, respectively. OECD requires average egg shell thickness for control group 0.34 and 0.19 for mallard and bobwhite, respectively* *EPA requires:* *body weight should be recorded at test initiation and a biweekly intervals up to week eight or up to the onset of egg laying and at termination.* • *Eggs laid/pen* • *Eggs cracked/pen* • *Eggs set/pen* • *Viable embryos/pen* • *Live 3-week embryos/pen* • *Normal hatchlings/pen* • *14-day-old survivors/pen* • *Weights of 14-day-old survivors (mean per pen)* • *Egg shell thickness* • *Food consumption (mean per pen)* • *Initial and final body weight (mean per pen)*

Parameter	Details	Remarks *Criteria*
Indicate if the test material was regurgitated	no regurgitation reported	
Observation intervals (for various parameters)	Body weight data was collected at 4 intervals. Feed consumption data was collected by cage at 23 weekly feeding intervals.	*Body weights and food consumption must be measured at least biweekly.*
were raw data included?	Yes. Appendix T.	

II. RESULTS AND DISCUSSION:

A. MORTALITY: There were several non-treatment related mortalities during the treated feed portion of the study. A female in the controls died due to pair aggression during the 2nd week of treated feed. A female at the 250 ppm level died due to pair aggression during the 8th week of treated feed A female at the 1000 ppm level died due to cage injury during the 17th week of treated feed. During the 9th week of treated feed, a heat spike occurred in the adult quail room which resulted in mortality in 21 cages. Mortality was spread among all treatment levels (5 cages - control, 6 cages - 250 ppm group, 6 cages 500 ppm group, 4 cages 1000 ppm group). Only the top row of cages had mortalities and the remaining birds continued to feed normally and to produce eggs. A sufficient number of birds remained within all treatment levels to continue the study. There were no reported signs of treatment related intoxication.

B. REPRODUCTIVE AND OTHER ENDPOINTS: Direct observation of the data suggests a pattern of NOEC = 250 ppm and LOEC = 500 ppm based on parameters measuring egg production and fertility, but when subject to statistical testing the significance of this observation did not hold up. The number of eggs laid was significantly depressed at the 1000ppm level, however, the effect was non-significant at the 500 ppm level (Appendix 1). By contrast, hatchling weight was found to be an exceptionally sensitive endpoint and all treatment levels were found to be significantly affected. Although hatchling bodyweights were significantly depressed in all of the treatment groups, the weights of 14 day old offspring did not appear to be affected. Shell thickness also tested normal in all groups.

Table 3. Reproductive and other parameters - Results for each test group

Parameter	Control 0 ppm	Test conc. 250 ppm	Test conc. 500 ppm	Test conc. 1000 ppm	NOEC ppm	LOEC ppm
Number of Reproductive pairs	14	13	13	15		
Total Eggs Laid	696	643	539	421		
Eggs laid/hen	49.71	49.46	41.46	28.07	500	1000
Eggs laid/hen/day	0.70	0.70	0.58	0.40		
Eggs cracked	15	0	6	3		
Eggs cracked/ Eggs Laid	0.02	0.00	0.01	0.01		
Eggs set	618	584	471	357	1000	NA
Eggs set/hen	44.14	44.92	36.23	23.80		
Shell thickness (mm ± SD)	0.209±0.019	0.211±0.017	0.210±0.014	0.205±0.017	1000	NA
Fertile Eggs	534	485	402	282		
14-day old survivors/ hen	28.29	26.23	20.00	13.60		
Viable embryos	526	481	398	277		
No. of hatchling/hen	36	34	29	17		
No. of normal hatchlings	502	442	378	254	500	1000
Hatchling weight	7.2 ±0.60 g	6.6 ±0.65 g	6.7 ±0.80 g	6.3 ±0.68 g	0	250
No. of 14-day old survivors	396	341	260	204		
14-day old survivors weight	33.9±6.82 g	33.1±6.86 g	34.0±8.05 g	34.2±6.05 g	1000	NA
Mean food consumption (g / cage / week)	261.5	266.2	258.2	249.1	1000	NA
Weight of females (parent) g At test initiation: At onset of egg laying: At test termination:	 230.8 250.1 259.3	 234.4 248.4 248.2	 236.0 242.6 253.1	 236.5 236.6 236.6		
Weight of males (parent) g At test initiation: At onset of egg laying: At test termination:	 231.8 248.4 243.3	 231.6 245.0 246.3	 233.4 246.0 244.2	 237.8 230.5 226.2		

C. POST-MORTEM EXAMINATIONS:

All surviving adults were reportedly subject to a post-mortem examination following adult termination. A majority of birds in all groups were noted to have fatty tissue, the males to have enlarged testes and the females to have eggs present, typical of sexually active birds. Miscellaneous signs of cage injuries and pair aggression such as foot abrasions, scraped legs and pecked heads were noted across all groups. Many birds in each group were reported to have gaseous intestines. A few birds in each group were noted to have small growths on the head area. One bird in the 1000 ppm was noted to have dark green intestines. Another bird in the 1000 ppm group was noted to have black intestines. None of the above post-mortem findings were considered to be treatment related. No other details of the post-mortem were provided.

D. REPORTED STATISTICS: The reproductive parameters which the proponent analyzed included number of eggs laid, number of eggs set, number of eggs cracked/number of eggs laid, number of fertile eggs/number of eggs set, number of viable embryos/number of fertile eggs, number of eggs hatched/ number of viable embryos, number of 14 day survivors/ number of eggs hatched, hatch weights, 14 day survivor weights, and eggshell thickness.

The proponent had reported that an initial ANOVA for hatchling weights indicated a significant difference in mean hatchling weights between the 250 ppm group and the control group. They identified one cage in the 250 ppm group which exhibited an apparent fertility problem (cage 17) and removed it from the data. They then reported that when the ANOVA test was rerun, there was no significant difference between the 250 ppm treatment group and the control. Nor did their ANOVA reveal any statistical difference between the 500 ppm treatment group and the control.

A significant difference was detected in adult male weights at weight interval 3 (start of egg laying) between the 1000 ppm treatment level and the control group. There were significant differences detected at the 1000 ppm treatment level when statistically compared against the control group in three of the 10 reproductive parameters tested: eggs laid, eggs set and hatchling weights. The reproductive no observable effect concentration (NOEC) during the study was proposed to be 500 ppm.

The proponent used multiple comparison testing (although not multiple range testing) for the definitive determination of significant difference. In particular, Tukey's test was used for all data. Tukey's test is identical to the commonly used multiple range test known as Student-Newman-Keuls test (SNK) except that it uses a single critical value for all comparisons and is not as powerful as the SNK (Zar 1974). The result of a Tukey's test partly depends upon the significance of the difference between the means of different treatment level data. This between dose level comparison data is not relevant to the present concern.

E. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical Method: Using Excel, single factor ANOVA tests were preformed on the proponent's raw data from Appendix T. Since it is desirable in this case to compare a control mean to each of the other level means, one may employ a Dunnett's test (Zar 1974). ANOVA and Dunnett's tests were performed and, for the sake of comparison, a Tukey's test was also performed. Results are reproduced in the appendix of this report. T-test statistics for groupwise comparisons were also performed; these were set as two tailed with an assumption of equal variance. Dunnett's is normally used to determine whether or not a significant difference exists between control and treatment sample means for multiple ranges.

Most parameters, such as the number of eggs laid clearly indicated that the 1000 ppm treatment level had a significant effect. Hatchling weight was found to be an exceptionally sensitive endpoint. ANOVA, Student's t-tests, Dunnett's and Tukey's tests were run on data for hatchling weights (Appendix 1). The Dunnett's test with cage 17 data (the lowest hatchling weights) omitted showed that there was a significant effect on hatchling weight at all treatment levels in comparison to the controls. The results at 250 ppm were significant ($p<0.05$) as were the results at 500 ppm ($p<0.05$) and at 1000 ppm ($p<0.01$). These results were supported by independent t-test calculations.

NOEC: 0 ppm
LOEC: 250 ppm
Most Sensitive endpoint(s): Hatchling Weight

F. STUDY DEFICIENCIES:

1. The egg storage period and humidity was not described, and it is unclear when the eggs were set.

2. The gross pathology report was incomplete and uninformative.

3. There was one omission at the 500ppm level which was unaccounted for. Final data was provided for thirteen pairs of birds at this level; if only six cages were dropped from the data set following the accidental heat spike and the experiment started with twenty cages, there is still one cage unaccounted for. This appears to have been cage 9, which was dropped from the data set because the hen did not lay eggs. A statement should have appeared in the report explaining this.

4. An inappropriate statistical test method, Tukey's test, was used as the definitive statistical test. The result of a Tukey's test partly depends upon the significance of the difference between the

means of different treatment level data. This between dose level comparison data is not relevant to the present concern. Since it is desirable in this case to compare a control mean to each of the other level means, one may employ a Dunnett's test (Zar 1974).

G. REVIEWER'S COMMENTS: This toxicity study is classified as supplemental and should be repeated. Birds from over 20 pens died during the study because of a "heat spike" in the laboratory. Also, the NOAEC was not determined because chick body weight was significantly reduced at all test concentrations. Other reproductive parameters such as fertile eggs, 14 day old survivors, viable embryos and number of hatchlings were also reduced compared to controls, although not significantly, at the lowest test concentration.

H. CONCLUSIONS: This toxicity study is classified as supplemental and should be repeated.

III. REFERENCES:

ASTM.1983. *Draft Standard Practice For Conducting Avian Reproductive Toxicity Tests.* American Society for Testing and Materials.

Finney, D.J.1971. *Statistical Methods in Biological Assay,* 2nd edition, Griffin Press, London.

U.S. Environ. Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Subsection 71-4.* U.S. EPA, Office of Pesticide Programs.

TOXSTAT, 1994. *Users Manual for Toxstat 3.4,* D.D.Gulley, ED., West, Inc., Cheyenne, WY.

Williams, D.A. 1971. A Test for Differences Between Treatment Means When Several Dose Levels Are Compared With a Zero Dose Control. Biometrics 27: 103-117.

Williams, D.A. 1972. The Comparison of Several Dose Levels With a Zero Dose Control. Biometrics 28:519-531.

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J., U.S.A.

Appendix 1.

Hatchling weights

0ppm	250ppm	500ppm	1000ppm	250ppm
5.2	6.6	5.8	5.9	6.6
7.4	4.3	7.3	6.5	6.6
7.4	6.6	6.9	6.3	6.3
7.5	6.3	7.2	5.8	6.9
7.2	6.9	6.3	6.6	7.1
6.9	7.1	6.8	6	6.1
7.4	6.1	6.5	6.4	6.3
7.7	6.3	6.3	6.6	6.8
6.9	6.8	6.6	5.8	6.2
7.1	6.2	6.5	6.9	7
7.5	7	6.99	6.4	6.9
7.5	6.9	6.8	6.6	7.4
7.1	7.4	6.8	5.7	
6.9			6.5	
			5.9	

0.044508

t-test, two tails, equal variance

0.027441	0.036226	8.62914E-05

ANOVA for Hatchling Weights
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0ppm	14	99.7	7.121429	0.3725824
250ppm	13	84.5	6.5	0.585
500ppm	13	86.79	6.676154	0.1647256
1000ppm	15	93.9	6.26	0.1411429

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.680873606	3	1.893625	6.1060412	0.001247	2.78623
Within Groups	15.81627912	51	0.310123			
Total	21.49715273	54				

Since F > Critical F we must reject the null hypothesis that all groups are equal ($p<0.05$)

Hatchling Weight Multiple Comparison Tukey's Test

RESULTS
TUKEY'S TEST

Q values from pairwise means.

	Names	0 ppm	250 ppm	500 ppm	1000 ppm
	0 ppm		4.08770	2.90095	5.87683
P	250 ppm	p<0.05		-1.16537	1.60836
	500 ppm	n.s.	n.s.		2.81463
v	1000 ppm	p<0.01	n.s.	n.s.	

The same Tukey's test with data from cage 17 removed

RESULTS
TUKEY'S TEST

Q values from pairwise means.

	Names	0 ppm	250 ppm	500 ppm	1000 ppm
	0 ppm		3.43983	3.51336	7.11747
P	250 ppm	n.s.		0.00000	3.33519
	500 ppm	n.s.	n.s.		3.40882
v	1000 ppm	p<0.01	n.s.	n.s.	

Hatchling Weight Multiple Comparison Dunnett's Test

RESULTS
DUNNETT'S TEST

Q values from pairwise means.

	Names	0 ppm	250 ppm	500 ppm	1000 ppm
	0 ppm		2.89044	2.05128	4.15555
P	250 ppm	p<0.05			
	500 ppm	n.s.			
v	1000 ppm	p<0.01			

The same Dunnett's test with data from cage 17 removed

RESULTS
DUNNETT'S TEST

Q values from pairwise means.

	Names	0 ppm	250 ppm	500 ppm	1000 ppm
	0 ppm		2.43233	2.48432	5.03281
P	250 ppm	p<0.05			
	500 ppm	p<0.05			
v	1000 ppm	p<0.01			

Hatchlings

0ppm	250ppm	500ppm	1000ppm
2	39	35	29
23	4	52	12
44	12	28	13
11	37	50	1
37	58	32	20
56	13	34	4
58	52	24	26
13	15	38	26
41	39	14	10
34	47	20	28
59	53	4	12
53	51	25	25
43	22	22	27
28			5
			16

0.795805 0.286473 0.001579

Eggs laid

0ppm	250ppm	500ppm	1000ppm
7	52	51	47
30	66	65	26
61	49	37	21
21	45	65	4
67	65	43	31
68	25	48	9
68	59	67	34
35	19	44	39
58	48	20	15
41	60	25	59
69	67	9	23
69	60	36	45
61	28	29	33
41			6
			29

t-test, two tails, equal variance
0.972045 0.278034 0.003444

14 day weights

0ppm	250ppm	500ppm	1000ppm
27.2	30.9	28.3	36.7
32	35.9	37.6	39.4
32.8	30.6	34.2	31.2
30.3	36.4	40.4	33.5
34.1	29.3	34.2	34.7
34.9	33.2	33.2	32.2
32	27.2	30.5	31.7
39.4	32.2	30	37.9
33.3	29.5	32.6	34
35.5	32.5	28.9	34.5
32.3	37.3	34.2	32.3
37.1	33.8	36.6	33.2
3.6			30.5
32.6			30.9
			33

t-test, two tails, equal variance

0.652825	0.418606	0.286482

eggshell thickness

0ppm	250ppm	500ppm	1000ppm
0.16	0.208	0.201	0.216
0.186	0.2	0.236	0.207
0.228	0.215	0.205	0.204
0.218	0.176	0.221	0.203
0.209	0.212	0.209	0.214
0.218	0.233	0.196	0.22
0.211	0.214	0.223	0.201
0.193	0.206	0.226	0.21
0.225	0.189	0.2	0.2
0.203	0.198	0.198	0.21
0.193	0.218	0.206	0.213
0.204	0.21	0.197	0.218
0.226	0.201	0.22	0.213
0.22			0.221
			0.198

t-test, two tails, equal variance

0.93145	0.540875	0.553491

adult food consumption

0ppm	250ppm	500ppm	1000ppm
5543.8	6967	6661	6065.2
5734.5	6195	5987.8	6262.8
6488.9	7069.2	6672	6848.7
5927.1	5473.3	6728.2	5551.7
6776.7	6466.7	7046.5	6125.3
7003.8	7325.9	6238	5539.9
6833.5	6821.2	6928.4	5521.2
6517.8	5385.5	6008	6226.5
6263.6	6052	5485.6	5574.3
5788	5937.3	5590.2	6217.1
6139.8	6300.6	5609.5	6165.3
6051.3	6713.6	6027.7	6436.7
6266.2	6334.9	5993.1	5557.9
6529.3		5775.3	5801.5
			5780

t-test, two tails, equal variance
0.579913 0.666305 0.066578

Eggs Laid Multiple Comparison Dunnettt's Test

RESULTS DUNNETT'S TEST		Q values from pairwise means.			
	Names	0 ppm	250 ppm	500 ppm	1000 ppm
	0 ppm		0.03669	1.21080	3.29179
P	250 ppm	n.s.			
	500 ppm	n.s.			
v	1000 ppm	p<0.01			