Data Evaluation Report on the reproductive effects of Acetamiprid insecticide on avian species Mallard Duck (Anas platyrhynchos)

PMRA Submission Number 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 449884-08

Data Requirement: PMRA DATA CODE: 9.6.3.2
EPA DP Barcode:
OECD Data Point: IIA 8.1.4
EPA Guideline: 71-4(b)

Test material: **Acetamiprid** **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.: 160430-64-8
Synonyms: NI-25, Pristine Brand RTU, Chipco Brand Tristar 70 WSP, Adjust Brand 70 WP and Assail Brand 70 WP

Primary Reviewer: Alison McLaughlin
For PMRA
Date: February 6th 2001

Secondary Reviewer(s): F. Nicholas Mastrota
EPA
Date: November 7, 2001

F. Nicholas Mastrota 11/7/01

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CITATION: Taliaferro, M.C., and V. Miller. 1999. Reproduction Study with Acetamiprid in the Mallard Duck (Anas platyrhynchos), EBA Inc., 2900 Quakenbush Rd., Snow Camp, NC 27349. Laboratory Project Identification No. 029708, Sponsor: Rhone-Poulenc Ag Company, NC, USA. February 1 1999. Unpublished.

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EXECUTIVE SUMMARY:

The one generation reproductive toxicity of acetamiprid (NI-25) to 4 treatment groups of 16 pairs each, male and female, and one control group of 16 pairs of Mallard Duck (Anas platyrhynchos) was assessed over 302 days (approximately 7 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). acetamiprid was administered to the birds in the diet at 62.5, 125, 250 and 500 mg ai/kg dw diet for 153 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 Mallard Ducks exposed to acetamiprid at a dietary concentration of 62.5, 125, 250 and 500 mg ai/kg dw diet for the duration of the experiment. s Significant differences were detected in change of adult female weights in the 250 and 500 ppm treatment levels when compared against the control group. The no observable adverse effect concentration (NOAEC) for chronic adult toxicity was, therefore, 125 mg ai/kg dw diet (ppm), and the low observable adverse effect concentration (LOAEC) was 250 ppm.

There were significant differences detected at the 500 mg ai/kg treatment level when compared against the control group in two of the reproductive parameters tested, namely number of live 3-week embryos and hatchling survivorship as measured by 14 day survivors / eggs hatched. These reproductive parameters were less sensitive than the effect on the bodyweights of the hens. The reproductive NOAEC during the study was, therefore, 250 mg ai/kg dw diet (ppm), and the LOAEC was 500 mg ai/kg dw diet (ppm).

This toxicity study is classified as as supplemental because of low hachability of eggs in all test groups, including the control, and other deficiencies related to inadequate reporting of results. This study does not satisfy the guideline requirement for a Mallard Duck (Anas platyrhynchos) reproductive toxicity study.

Results Synopsis

Test Organism Size/Age: Mallard Duck (Anas platyrhynchos), 27 weeks and one day old at start of test, mean weight 1056.45 grams at start of test.

NOEC: 125 mg a.i./kg dw diet
LOEC: 250 mg a.i./kg dw diet
Endpoint(s) Effected: The most sensitive endpoint overall was change in adult female

bodyweight. The most sensitive reproductive parameters were number of live 3-week embryos and hatchling survival as measured by the ratio of 14 day survivors/ eggs hatched, which were affected at the 500 ppm level.

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 W and deviations to the protocol were listed in Appendix X. Deviations included an oversight in the analysis of stability samples and a changed test substance expiration date.

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 Acetamiprid

Description: Pale yellow powder

Lot No./Batch No. : NFG-02

Purity: 99.9%

Stability of Compound Under Test Conditions: Results of the analytical chemistry report (Appendix D) indicate that acetamiprid was stable at nominal concentrations of 62.5 ppm, 125 ppm and 1000 ppm in the avian diet formulation assessed over a period of 28

days during storage under animal room conditions. The 125 ppm and 1000 ppm samples were also tested for stability at 90 days. No significant loss of acetamiprid occurred over the storage periods.

Storage Conditions of Test Chemicals: Prior to testing, acetamiprid was stored at ambient temperature in a chemical storage cabinet (Appendix A).

Table 1. Physicochemical properties of acetamiprid

Parameter	Values	Comments
Water solubility at 20°C	2.95 X 10^3 mg/L at pH 7	soluble
Vapour pressure	< 1 X 10^{-6} Pa	non-volatile
UV absorption	ϵ = 1.94 X 10^4 at 247 nm	-
pKa	0.7	will not dissociate
Kow	6.27	Bioconcentration is unlikely

2. Test organism:

Species (common and scientific names): Mallard Duck (Anas platyrhynchos)

Age at study initiation: 27 weeks and one day of age at experimental start

Weight at study initiation: mean: 1056.45 grams range: 849-1395 grams

Source: Whistling Wings, Hanover, IL 61041

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A range finding test was conducted from February 26 1997 (start of treated feed) through March 27 1997 with four treatment groups of six pairs each, male and female , and one control group of six pairs. The treatment levels were 125 ppm, 250 pmm, 500

ppm, and 1000 ppm. Birds were given treated feed for 29 days. Eggs were collected for four weeks, incubated for 14 days, candled for fertility, and discarded. 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:	34 days exposure prior to collection of first egg 25 days 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:	76 cm deep x 83 cm wide x 44 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:	16 pairs in the control group with 1 cage for each pair NA 16 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:	62.5, 125, 250, 500 62.45 ±10.47 (99.9% of nominal) 120.97 ±23.62 (96.8% of nominal) 227.61 ±38.04 (91.1% of nominal) 473.72 ±58.70 (94.7% 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:	Based on proposed labels, the maximum use rate per growing season is 0.6 lb a.i./A. Field residues are not expected to exceed 144 mg/kg.	

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 mixed directly into 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 21.6 °C (SD 1.8 °C) Mean 74.0% (SD 13.4%) 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 5 day period to 17 hours of light, 7 hours dark. Light exposure was an ranged between 6.3 and 17.5 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 13.1°C until being placed into the incubator. The storage period was not described. Humidity averaged 30.1%. There were 78 total egg laying days.	Humidity levels during egg storage were too low. *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	
When candling was done for viability?	On day 22 of incubation	

Parameter	Details	Remarks *Criteria*
		EPA requires: *bobwhite: approx. day 11* *mallard: approx. day 14* *OECD requires: 6-11 day*
When the eggs were transferred to the hatcher?	On day 22 of incubation	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*
Hatching conditions Temperature: Humidity: Photoperiod:	 36.9 °C ± 0.83 °C 59 - 75 % RH not reported	Humidity levels during hatching were at times too low. *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 5 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~75 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 22 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 no mortalities during the treated feed portion of the study. A few instances of cage abrasions were noted. No overt signs of treatment-related toxicity were reported.

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. The number of eggs laid was significantly depressed at the 500ppm level, however, the effect was non-significant at the 250 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. 62.5 ppm	Test conc. 125 ppm	Test conc. 250 ppm	Test conc. 500 ppm
Number of Reproductive pairs	16	15	16	16	15
Total Eggs Laid	795	839	788	814	664
Eggs laid/hen	49.69	55.93	49.25	50.88	44.27
Eggs laid/hen/day	0.64	0.72	0.63	0.65	0.57
Eggs cracked	44	63	53	99	41
Eggs cracked/ Eggs Laid	0.06	0.08	0.07	0.12	0.06
Eggs set	683	705	663	641	557
Eggs set/hen	42.69	47.00	41.44	40.06	37.13
Shell thickness (mm ± SD)	0.361±0.027	0.362±0.018	0.356±0.020	0.361±0.024	0.358±0.020
Fertile Eggs	611	551	580	576	453
14-day old survivors/ hatchlings	0.94	0.96	0.99	0.96	0.78
Viable embryos	479	372	439	407	292
No. of hatchling/hen	14.75	7.6	15.81	10.06	10.8
No. of normal hatchlings	236	114	253	161	162
Hatchling weight	33.9±3.5 g	32.3 ±3.7 g	33.6±3.7 g	32.4 ±4.2 g	31.9 ±3.6 g
No. of 14-day old survivors	221	109	251	155	127
14-day old survivors weight	214.8±65.2 g	271.0±49.1 g	186.5±52.9 g	216.1±64.2 g	258.8±49.8 g
Mean food consumption (g / cage / week)	1985.8	1859.1	1947.0	2004.9	1924.4
Weight of females (parent) g At test initiation: At onset of egg laying: At test termination:	976.6 1061.2 1218.2	977.1 1001.3 1203.2	1016.9 1030.8 1194.9	999.6 1054.4 1164.4	963.0 991.5 1106.1
Weight of males (parent) g At test initiation: At onset of egg laying:	1138.8 1114.8	1164.1 1132.3	1128.6 1129.6	1126.0 1078.1	1073.8 1054.9

C. POST-MORTEM EXAMINATIONS:

All surviving adults were reportedly subject to a post-mortem examination following adult termination. During the range-finding portion of the study, notations were made regarding the presence of enlarged livers. These notations occurred in all treatment groups but in increasing numbers that correlated with the increase in treatment level ppms. A veterinarian's report was included in this study, but it was very brief as follows:

"The occurrence of liver lesions appeared to be related to test article concentration. In affected test subjects, the right lobe would appear enlarged. The mottling that was noted on the necropsy forms of several birds actually appeared less in those lobes that were enlarged. In retrospect, this mottling, which was not extraordinary in the least for production animals, was probably more related to birds being in active egg production rather than a toxic effect attributable to the test article. In general, most birds appeared to have normal livers irrespective of treatment group. At the higher levels of test article, a larger number of the test subjects presented with enlarged right liver lobes."

The mottled liver suggests a "fatty liver" change. "Fatty liver" occurs with several type of pathological processes, including toxins, and this would be a reasonable assumption. Note that no morphological diagnosis was given and that usually, pathologists do provide one. The results provided in the study were incomplete.

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 hatchlings, hatch weights, 14 day survivor weights, and eggshell thickness.

A significant difference was detected in adult female weights at the start of egg laying and at termination between the 500 ppm treatment level and the control group. There were significant differences detected at the 500 ppm treatment level when statistically compared against the control group in one of the 10 reproductive parameters tested: number of 14 day survivors/number of hatchlings. Significant differences were also reported between the 62.5 ppm treatment group and the corresponding control and also between the 500 ppm treatment group and the control for 14 day survivor weights. These differences in the 14 day survivor weights were not believed to be treatment related. The reproductive no observable effect concentration (NOEC) during the study was proposed to be 250 ppm.

The proponent used the Kruskal Wallace ANOVA by ranks followed by Dunn's Multiple Comparison testing for the analysis of Number of 14 Day Survivors/ Number of Hatchlings. Multiple range testing with Dunnett's test was used for adult male body weights and adult female

body weights. Multiple comparison testing with Tukey's test was used for total feed consumption, number eggs laid, number of eggs set, eggs cracked/eggs laid, number of eggs hatched/number of viable embryos, 14 day survivor weights, and eggshell thickness. Multiple comparison testing with Dunn's test was used for number of fertile eggs/number of eggs set, number of viable embryos/ number of fertile eggs, number of 14 day survivors/ number of eggs hatched, and hatchling weights.

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 comparisions and is not as powerful as the SNK (Zar 1974). Dunn's test (aka Bonferroni test) applies a correction for planned comparisons, which is based on the number of treatment levels (Keppel 1982). The result of a Dunn or 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 SAS, single factor ANOVA tests were preformed on the proponent's raw data from Appendix T. Counts of surviving chicks and hatchlings from cages in which no eggs were laid were included in the analysis as zeros. Ratios of counts were transformed with the arc-sin transformation. 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 for variables of interest and results of these tests were reproduced in Appendix 1 of this report. Paired t-tests were also performed for comparison and verification; 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.

Significant differences were confirmed between the 62.5 ppm treatment group and the corresponding control and also between the 500 ppm treatment group and the control for 14 day survivor weights. Closer examination of the data shows that the 62.5 ppm and 500 ppm treatment groups had higher mean bodyweights than the control group. The 125 ppm and the 250 ppm groups had bodyweights approximating those of the control group. It seems likely that this variation was the result of fewer chicks being produced at these treatment levels, and thus the chicks were raised under less crowded conditions. The proponent's assertion that these effects are not treatment related is accepted.

Effects on adult bodyweights were analyzed by calculating differences of final weight minus initial weights of individual ducks, and performing ANOVA and Dunnett's test on these differences.

This analysis found that the mean weight change of male ducks in all of the treatment groups were not significantly different from the control group, which agrees with the results of the study authors. However, this analysis found that the change in bodyweights of female ducks were significantly different from the control group in both the 250 and 500 ppm group. Thus, NOAEC and LOEC were identified as 125 and 250 ppm, respectively, which were lower than the results obtained by the study authors.

The Dunnett's test determined a significant difference in the number of live three-week embryos in hte 500 ppm treatment group compared to the control group. The Dunnett's test with 14 day survivors/ eggs hatched data did not show a significant effect at the 500 ppm treatment level in comparison to the controls, although the effect was very close to being significant. The pairwise t-test did indicate a significant difference in this ratio for the comparison of the 500 ppm treatment group with the control group. I agree with the study author that the strong trend in this ratio probably represents a biologically significant effect of the acetamiprid on hatchling survival.

F. STUDY DEFICIENCIES:

1. The first weekly set of eggs produced was omitted from the reproductive data. There was no explanation for this omission, although it was reported that the data was archived and available for review upon request.

2. The gross pathology report was incomplete and uninformative.

3. No mortality was recorded, however data from two cages, one at the 62.5 ppm treatment level and one at the 500 ppm treatment level, were missing. There was no explanation as to why these data were omitted, but it appears that they were omitted because the pairs did not produce any eggs. These cages should have been included as zeros in the analysis of counts of surviving eggs and hatchlings.
4. Inappropriate statistical test methods, Dunn's test and Tukey's test, were used as the definitive statistical tests for most data. The result of a Dunn or 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).

5. Environmental conditions of egg storage deviated from the standards of the test guideline for this study. The EPA's Standard Evaluation Procedure states that eggs should be stored at 16 °C and 65% relative humidity, whereas eggs in this study were stored at 11.7 - 13.9 °C and 26 - 39% relative humidity. The temperature was generally within the recommended range stated in the

ASTM guidelines (12 to 16 °C). The low humidity level is a more significant deviation. The ASTM guidelines do not recommend humidity levels for egg storage; however, the laboratory's own protocol states that eggs should be stored with a relative humidity of 40 - 80%.

6. The humidity in the hatcher was at times lower than that required by the test guidelines. The relative humidity in the hatcher varied from 59 to 75%, whereas the EPA and OECD test guidelines state the relative humidity should be 70% and 70 - 85%, respectively.

7. The hatching success of eggs in the control group was very low. Only 34.6% of the eggs set hatched, and only 49.3% of the eggs with viable embryos hatched. Hatching success was also low in the treatment groups, especially the lowest dose group (62.5 ppm), in which only 16.2% of the eggs set hatched. As this dose is well below the NOEL obtained in this study (250 ppm), it appears likely that the low hatchability in this dose group was not treatment related, but was related to a equipment or husbandry problem.

G. REVIEWER'S COMMENTS: The hatching success of eggs in this study was low. The control ratios of viable embryos/fertile eggs (0.78) and hatchlings/viable embryos (0.49) were both very low compared to published historical control data (Piccirillo and Quesenberry, 1980). This low hachability might have been caused by the low relative humidity at which the eggs were stored (see deficiency #5), with possible additional contribution by slightly low humidity levels in the hatcher (see deficiency #6). The low hatching success in this study may have compromised the power of the statistical analysis for detecting significant treatment-related effects.

H. CONCLUSIONS: This toxicity study is classified as supplemental because of low hatching success and other deficiencies outlined above under section F. This study does not satisfy the guideline requirement for a Mallard Duck (Anas platyrhynchos) reproductive toxicity study.

NOEC: 125 mg a.i./kg dw diet
LOEC: 250 mg a.i./kg dw diet
Endpoint(s) Effected: The most sensitive endpoint overall was change in adult female bodyweight. The most sensitive reproductive parameters were number of live 3-week embryos and hatchling survival as measured by the ratio of 14 day survivors/ eggs hatched, which were affected at the 500 ppm level.

III. REFERENCES:

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MRID

EFFECTS OF ACETAMIPRID ON MALLARD REPRODUCTION (449884-08)

With Nonlayers Included as Zeros

09:24 Tuesday, November 20, 2001

Obs	LEVEL	EL	EC	ES	VE	LE	NH	HS	THICK	HATWT	SURVWT	FOOD	PREM	POSTM	PREF	POSTF
1	CONTROL	56	0	52	42	25	13	13	0.385	31.4	203.7	36740.3	1245	1278	935	1108
2	CONTROL	25	1	21	20	17	8	7	0.348	32.6	193.8	53146.2	1222	1598	1076	1262
3	CONTROL	65	0	60	57	44	11	11	0.351	31.9	192.5	47792.6	1085	1144	992	1323
4	CONTROL	10	1	7	7	1	0	0	0.345	.	.	38368.5	1171	1410	859	990
5	CONTROL	66	13	48	41	29	15	13	0.339	31.8	231.8	42582.2	904	977	905	1198
6	CONTROL	61	15	41	34	20	7	6	0.359	30.6	193.4	46972.1	986	1097	1023	1169
7	CONTROL	67	1	61	55	53	38	35	0.371	33.2	210.3	42500.2	1148	1266	980	1276
8	CONTROL	50	1	45	42	37	29	26	0.349	34.9	225.9	44048.7	1122	1366	975	1082
9	CONTROL	63	1	56	51	42	30	29	0.357	33.9	201.5	42574.6	1141	1189	908	1142
10	CONTROL	49	1	44	42	37	5	4	0.379	34.5	185.9	38965.4	1126	1342	970	1212
11	CONTROL	20	3	14	12	9	1	1	0.328	36.6	104.5	33461.1	1112	1166	945	1214
12	CONTROL	61	1	55	49	40	18	18	0.379	35.2	196.9	45729.6	1038	1137	963	1212
13	CONTROL	64	2	57	47	31	4	4	0.373	39.5	245.5	46545.2	1244	1313	953	1291
14	CONTROL	66	4	57	54	45	35	35	0.346	34.9	239.9	53300.5	1395	1274	1093	1385
15	CONTROL	37	0	34	30	26	10	10	0.392	36.1	211.4	37185.8	1065	1279	1021	1276
16	CONTROL	35	0	31	28	23	12	9	0.358	35.7	245.3	49098.6	1216	1540	1027	1351
17	TRT1	47	1	41	37	23	4	4	0.346	34.8	239.3	46505.3	1172	1215	1026	1238
18	TRT1	67	0	62	61	49	14	13	0.354	33.4	259.2	43744.0	1146	1257	1062	1207
19	TRT1	68	0	63	2	2	1	0	0.376	27.9	.	42117.5	1153	1144	958	1300
20	TRT1	49	25	19	11	10	5	5	0.367	34.5	277.7	39101.6	1258	1386	1065	1274
21	TRT1	59	3	51	45	30	4	4	0.375	34.2	243.8	39644.3	1155	1103	874	1107
22	TRT1	40	9	26	24	23	18	18	0.362	30.2	259.7	48908.1	1213	1143	945	966
23	TRT1	67	1	61	56	32	7	7	0.353	33.1	294.9	54513.7	1194	1139	976	1220
24	TRT1	60	2	53	50	27	5	5	0.350	32.8	265.6	32530.2	1192	1313	968	1191
25	TRT1	65	11	49	42	18	4	3	0.336	32.8	256.5	36318.0	1051	1140	1011	1226
26	TRT1	60	3	52	44	28	1	1	0.386	34.0	279.0	34029.1	971	1075	906	1170
27	TRT1	0	0	0	0	0	0	0	0.377	.	.	35080.8	1194	1198	981	1181
28	TRT1	68	0	64	36	33	1	1	0.351	30.4	232.5	44430.2	1117	1320	1032	1324
29	TRT1	62	1	56	49	32	14	13	0.358	31.8	282.7	37626.7	1312	1337	910	1234
30	TRT1	56	0	52	52	43	32	31	0.370	31.7	282.9	37261.2	1134	1222	976	1224
31	TRT1	23	3	16	15	5	1	1	0.372	33.7	243.0	38662.8	1175	1311	898	1148
32	TRT1	48	4	40	27	17	3	3	0.359	38.9	272.7	43919.5	1189	1297	1045	1241
33	TRT2	59	0	54	52	40	33	33	0.351	33.9	196.8	52349.3	1123	1125	1103	1144
34	TRT2	44	0	40	38	24	2	2	0.377	34.9	151.8	40773.4	1132	1304	1034	1302
35	TRT2	53	1	47	32	26	15	15	0.357	32.7	165.2	36861.4	1066	1108	948	1216
36	TRT2	60	0	55	29	18	2	2	0.363	33.1	236.3	38496.7	1015	1283	1022	1422
37	TRT2	56	4	47	44	40	37	37	0.352	34.1	192.9	49358.4	1111	1197	1018	1283
38	TRT2	57	1	51	49	41	23	23	0.367	34.8	190.9	42746.3	1192	1191	980	1295
39	TRT2	50	11	34	32	27	17	17	0.347	34.9	183.3	52522.9	1072	1165	987	1279
40	TRT2	58	5	48	43	21	11	11	0.338	31.2	160.3	36023.2	1123	1171	1106	1064
41	TRT2	64	2	57	50	38	25	24	0.354	30.4	197.9	47884.3	1377	1282	874	950
42	TRT2	48	14	29	26	16	5	5	0.363	32.3	144.2	46721.1	1177	1385	960	1182
43	TRT2	45	3	37	27	10	2	2	0.353	34.7	265.5	38966.5	1086	1358	946	1189
44	TRT2	58	7	46	43	37	27	26	0.372	34.8	188.1	39998.3	1124	1325	1093	1148
45	TRT2	60	1	54	52	46	25	25	0.360	33.8	180.3	43190.0	979	1040	947	1110
46	TRT2	22	1	19	19	19	16	16	0.363	34.3	170.8	34433.0	1211	1322	1160	1227
47	TRT2	17	1	14	13	10	6	6	0.318	32.6	182.8	41024.9	1278	1475	1121	1113
48	TRT2	37	2	31	31	26	7	7	0.347	35.7	203.6	44001.0	992	1215	972	1195
49	TRT3	57	11	41	37	11	0	0	0.369	.	.	42505.2	1102	1242	991	1259
50	TRT3	51	11	35	33	30	8	8	0.058	36.7	261.3	47593.5	1231	1172	970	1277
51	TRT3	46	1	40	38	33	22	21	0.343	30.5	182.1	39149.6	1115	1105	1048	1141
52	TRT3	41	0	37	30	27	4	4	0.374	32.1	242.1	46617.3	1109	1204	916	1124
53	TRT3	55	0	50	48	33	6	6	0.373	30.3	244.4	45420.1	1221	1226	1070	1203
54	TRT3	49	3	41	33	26	15	15	0.346	37.7	192.1	48351.9	1153	1114	1070	1085
55	TRT3	52	11	37	31	13	6	6	0.331	32.7	178.4	39944.9	1076	1066	954	1081
56	TRT3	68	7	56	51	45	35	35	0.350	30.8	217.7	39324.9	1077	1138	860	1013
57	TRT3	45	4	36	31	21	3	3	0.350	32.3	124.7	55543.6	1033	1054	1012	1111
58	TRT3	53	0	49	48	39	11	9	0.395	31.2	224.2	40605.0	1094	1318	1113	1350
59	TRT3	51	12	34	28	10	1	1	0.357	28.4	234.5	50207.5	1180	1285	912	1132
60	TRT3	62	11	46	41	29	13	12	0.363	32.4	194.8	34184.8	1021	1150	893	1065
61	TRT3	45	6	35	30	23	16	15	0.365	31.0	242.6	45718.7	1155	1343	1106	1298
62	TRT3	51	5	41	37	30	15	15	0.362	33.7	250.2	50532.6	1199	1268	985	1093

Obs	LEVEL	EL	EC	ES	VE	LE	NH	HS	THICK	HATWT	SURVWT	FOOD	PREM	POSTM	PREF	POSTF
63	TRT3	33	1	29	27	13	3	3	0.352	36.2	212.0	37931.3	1086	1249	1036	1130
64	TRT3	55	16	34	33	24	3	2	0.383	37.2	291.5	42087.4	1164	1242	1057	1268
65	TRT4	56	0	51	39	34	17	14	0.371	31.0	230.2	51988.7	1099	1149	914	1055
66	TRT4	47	2	39	30	2	0	0	0.382	.	.	40844.2	1004	1075	865	1053
67	TRT4	31	0	28	25	18	1	1	0.358	42.0	231.0	35714.5	1057	1218	988	1306
68	TRT4	38	7	26	19	12	3	3	0.363	36.6	250.5	49567.9	1145	1123	974	1114
69	TRT4	37	7	25	19	11	6	6	0.347	33.7	300.5	37984.5	1113	1291	1063	1225
70	TRT4	21	4	13	10	7	2	1	0.360	36.3	265.0	42145.0	1017	1147	1092	1205
71	TRT4	67	1	61	54	28	7	3	0.377	28.3	275.5	43488.2	984	1089	932	953
72	TRT4	20	2	16	14	12	3	3	0.336	34.8	250.8	49195.5	1215	1360	849	1063
73	TRT4	53	7	41	39	31	26	23	0.347	30.8	269.9	49721.7	1028	1205	924	1008
74	TRT4	50	0	46	40	32	30	25	0.343	30.5	258.2	43107.0	1011	1238	908	1062
75	TRT4	42	9	29	27	21	17	12	0.361	31.6	246.0	39102.3	886	1002	984	989
76	TRT4	41	0	36	34	31	18	13	0.342	33.2	262.5	38081.4	1026	1216	900	1250
77	TRT4	56	0	52	46	14	3	3	0.363	37.5	224.7	40273.2	1081	1120	1045	1132
78	TRT4	0	0	0	0	0	0	0	.	.	.	36550.2	1121	1261	970	1074
79	TRT4	54	1	49	48	38	28	20	0.365	33.1	265.6	41709.5	1149	1079	962	1079
80	TRT4	51	1	45	9	1	1	0	0.350	23.9	.	37928.7	1245	1464	1038	1129

	LEVEL				
	CONTROL	TRT1	TRT2	TRT3	TRT4
	Mean	Mean	Mean	Mean	Mean
EL	49.69	52.44	49.25	50.88	41.50
EC	2.75	3.94	3.31	6.19	2.56
ES	42.69	44.06	41.44	40.06	34.81
VE	38.19	34.44	36.25	36.00	28.31
LE	29.94	23.25	27.44	25.44	18.25
NH	14.75	7.13	15.81	10.06	10.13
HS	13.81	6.81	15.69	9.69	7.94
ES/EL (%)	84.68	82.28	83.78	79.19	82.09
(EL-EC)/EL (%)	94.30	91.30	93.13	88.37	92.63
VE/ES (%)	90.00	79.45	88.66	89.60	81.38
LE/VE (%)	74.70	69.97	75.07	69.33	62.91
NH/EL (%)	27.12	14.08	32.54	19.23	23.02
NH/ES (%)	31.11	18.01	38.72	23.71	28.03
NH/LE (%)	42.70	30.84	52.44	35.03	48.36
HS/ES (%)	28.82	17.41	38.48	22.80	22.16
HS/NH (%)	92.89	90.51	99.52	95.33	75.80
THICK	0.36	0.36	0.36	0.34	0.36
HATWT	34.19	32.95	33.64	32.88	33.09
SURVWT	205.49	263.54	188.17	219.51	256.18
FOOD	43688.23	40899.56	42834.42	44107.39	42337.66
POSTM	1273.50	1225.00	1246.63	1198.50	1189.81
POSTF	1218.19	1203.19	1194.94	1164.38	1106.06

---------- LEVEL=CONTROL ----------

The MEANS Procedure

Variable	Label	N	Mean	Std Dev	Coeff of Variation
EL		16	49.688	18.596	37.427
EC		16	2.750	4.539	165.044
ES		16	42.688	16.887	39.559
VE		16	38.188	15.219	39.854
LE		16	29.938	13.988	46.724
NH		16	14.750	12.019	81.488
HS		16	13.813	11.537	83.524
THICK		16	0.360	0.018	5.025
HATWT		15	34.187	2.349	6.872
SURVWT		15	205.487	34.500	16.790
FOOD		16	43688.225	5778.346	13.226
PREM		16	1138.750	115.510	10.144
POSTM		16	1273.500	159.799	12.548
PREF		16	976.563	61.926	6.341
POSTF		16	1218.188	103.872	8.527
ES_EL	ES/EL (%)	16	84.681	9.079	10.722
NH_EL	NH/EL (%)	16	27.124	18.853	69.508
ENC_EL	(EL-EC)/EL (%)	16	94.298	7.640	8.102
VE_ES	VE/ES (%)	16	89.996	5.492	6.103
NH_ES	NH/ES (%)	16	31.115	20.862	67.047
HS_ES	HS/ES (%)	16	28.822	19.618	68.067
LE_VE	LE/VE (%)	16	74.700	19.226	25.738
NH_LE	NH/LE (%)	16	42.702	25.037	58.633
HS_NH	HS/NH (%)	15	92.887	8.387	9.029

---------- LEVEL=TRT1 ----------

Variable	Label	N	Mean	Std Dev	Coeff of Variation
EL		16	52.438	18.547	35.370
EC		16	3.938	6.475	164.451
ES		16	44.063	19.140	43.438
VE		16	34.438	19.204	55.765
LE		16	23.250	14.026	60.328
NH		16	7.125	8.516	119.518
HS		16	6.813	8.296	121.781
THICK		16	0.362	0.013	3.721
HATWT		15	32.947	2.495	7.574
SURVWT		14	263.536	19.012	7.214
FOOD		16	40899.563	5876.077	14.367
PREM		16	1164.125	77.540	6.661
POSTM		16	1225.000	94.856	7.743
PREF		16	977.063	60.022	6.143
POSTF		16	1203.188	83.316	6.925
ES_EL	ES/EL (%)	15	82.284	14.894	18.100
NH_EL	NH/EL (%)	15	14.084	16.400	116.451
ENC_EL	(EL-EC)/EL (%)	15	91.302	13.554	14.845
VE_ES	VE/ES (%)	15	79.448	25.090	31.580
NH_ES	NH/ES (%)	15	18.011	20.856	115.797
HS_ES	HS/ES (%)	15	17.414	20.677	118.738
LE_VE	LE/VE (%)	15	69.966	19.692	28.145
NH_LE	NH/LE (%)	15	30.839	23.385	75.829
HS_NH	HS/NH (%)	15	90.506	25.891	28.607

---------- LEVEL=TRT2 ----------

The MEANS Procedure

Variable	Label	N	Mean	Std Dev	Coeff of Variation
EL		16	49.250	13.655	27.726
EC		16	3.313	4.110	124.089
ES		16	41.438	13.054	31.502
VE		16	36.250	11.964	33.004
LE		16	27.438	11.558	42.126
NH		16	15.813	11.485	72.630
HS		16	15.688	11.371	72.483
THICK		16	0.355	0.014	3.951
HATWT		16	33.638	1.471	4.374
SURVWT		16	188.169	30.233	16.067
FOOD		16	42834.419	5603.930	13.083
PREM		16	1128.625	103.428	9.164
POSTM		16	1246.625	114.612	9.194
PREF		16	1016.938	79.736	7.841
POSTF		16	1194.938	111.173	9.304
ES_EL	ES/EL (%)	16	83.778	8.653	10.328
NH_EL	NH/EL (%)	16	32.536	21.576	66.313
ENC_EL	(EL-EC)/EL (%)	16	93.129	8.159	8.761
VE_ES	VE/ES (%)	16	88.655	12.981	14.642
NH_ES	NH/ES (%)	16	38.722	24.946	64.424
HS_ES	HS/ES (%)	16	38.476	24.815	64.494
LE_VE	LE/VE (%)	16	75.067	16.502	21.983
NH_LE	NH/LE (%)	16	52.442	25.995	49.569
HS_NH	HS/NH (%)	16	99.519	1.317	1.323

---------- LEVEL=TRT3 ----------

Variable	Label	N	Mean	Std Dev	Coeff of Variation
EL		16	50.875	8.197	16.111
EC		16	6.188	5.218	84.334
ES		16	40.063	7.085	17.685
VE		16	36.000	7.474	20.762
LE		16	25.438	10.073	39.598
NH		16	10.063	9.183	91.261
HS		16	9.688	9.090	93.833
THICK		16	0.342	0.077	22.631
HATWT		15	32.880	2.834	8.620
SURVWT		15	219.507	40.779	18.577
FOOD		16	44107.394	5599.784	12.696
PREM		16	1126.000	62.978	5.593
POSTM		16	1198.500	87.798	7.326
PREF		16	999.563	77.445	7.748
POSTF		16	1164.375	98.174	8.431
ES_EL	ES/EL (%)	16	79.189	9.373	11.837
NH_EL	NH/EL (%)	16	19.229	15.730	81.803
ENC_EL	(EL-EC)/EL (%)	16	88.373	9.477	10.724
VE_ES	VE/ES (%)	16	89.602	5.787	6.458
NH_ES	NH/ES (%)	16	23.714	18.819	79.359
HS_ES	HS/ES (%)	16	22.804	18.510	81.171
LE_VE	LE/VE (%)	16	69.328	19.823	28.593
NH_LE	NH/LE (%)	16	35.026	24.113	68.844
HS_NH	HS/NH (%)	15	95.333	9.388	9.848

LEVEL=TRT4

The MEANS Procedure

Variable	Label	N	Mean	Std Dev	Coeff of Variation
EL		16	41.500	16.931	40.798
EC		16	2.563	3.162	123.380
ES		16	34.813	16.384	47.063
VE		16	28.313	15.624	55.183
LE		16	18.250	12.715	69.670
NH		16	10.125	10.770	106.366
HS		16	7.938	8.675	109.296
THICK		15	0.358	0.013	3.727
HATWT		14	33.093	4.392	13.270
SURVWT		13	256.185	20.819	8.127
FOOD		16	42337.656	5164.919	12.199
PREM		16	1073.813	91.091	8.483
POSTM		16	1189.813	117.535	9.878
PREF		16	963.000	70.571	7.328
POSTF		16	1106.063	97.849	8.847
ES_EL	ES/EL (%)	15	82.090	10.660	12.986
NH_EL	NH/EL (%)	15	23.018	20.750	90.149
ENC_EL	(EL-EC)/EL (%)	15	92.628	8.477	9.152
VE_ES	VE/ES (%)	15	81.383	18.772	23.066
NH_ES	NH/ES (%)	15	28.029	24.324	86.779
HS_ES	HS/ES (%)	15	22.163	19.564	88.275
LE_VE	LE/VE (%)	15	62.908	26.915	42.784
NH_LE	NH/LE (%)	15	48.362	32.460	67.120
HS_NH	HS/NH (%)	14	75.803	28.748	37.925

The GLM Procedure

Type I Estimable Functions

Effect		-Coefficients- LEVEL
Intercept		0
LEVEL	CONTROL	L2
LEVEL	TRT1	L3
LEVEL	TRT2	L4
LEVEL	TRT3	L5
LEVEL	TRT4	-L2-L3-L4-L5

The GLM Procedure

Dependent Variable: LE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1264.67500	316.16875	2.00	0.1027
Error	75	11836.81250	157.82417		
Corrected Total	79	13101.48750			

R-Square	Coeff Var	Root MSE	LE Mean
0.096529	50.52915	12.56281	24.86250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	4	1264.675000	316.168750	2.00	0.1027

The GLM Procedure
Least Squares Means

LEVEL	LE LSMEAN	LSMEAN Number
CONTROL	29.9375000	1
TRT1	23.2500000	2
TRT2	27.4375000	3
TRT3	25.4375000	4
TRT4	18.2500000	5

Least Squares Means for effect LEVEL
Pr > |t| for HO: LSMean(i)=LSMean(j)

Dependent Variable: LE

i/j	1	2	3	4	5
1		0.1364	0.5752	0.3142	0.0103
2	0.1364		0.3488	0.6238	0.2639
3	0.5752	0.3488		0.6538	0.0420
4	0.3142	0.6238	0.6538		0.1098
5	0.0103	0.2639	0.0420	0.1098	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The GLM Procedure

Dunnett's One-tailed t Tests for LE

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	75
Error Mean Square	157.8242
Critical Value of Dunnett's t	2.19721
Minimum Significant Difference	9.7592

Comparisons significant at the 0.05 level are indicated by ***.

LEVEL Comparison		Difference Between Means	Simultaneous 95% Confidence Limits		
TRT2	- CONTROL	-2.500	-Infinity	7.259	
TRT3	- CONTROL	-4.500	-Infinity	5.259	
TRT1	- CONTROL	-6.688	-Infinity	3.072	
TRT4	- CONTROL	-11.688	-Infinity	-1.928	***

The GLM Procedure

Class Level Information

Class	Levels	Values
LEVEL	5	CONTROL TRT1 TRT2 TRT3 TRT4

Number of observations 80

The GLM Procedure

Type I Estimable Functions

Effect		-Coefficients- LEVEL
Intercept		0
LEVEL	CONTROL	L2
LEVEL	TRT1	L3
LEVEL	TRT2	L4
LEVEL	TRT3	L5
LEVEL	TRT4	-L2-L3-L4-L5

The GLM Procedure

Dependent Variable: HS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	932.700000	233.175000	2.38	0.0588
Error	75	7336.687500	97.822500		
Corrected Total	79	8269.387500			

R-Square	Coeff Var	Root MSE	HS Mean
0.112789	91.68506	9.890526	10.78750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	4	932.7000000	233.1750000	2.38	0.0588

The GLM Procedure
Least Squares Means

LEVEL	HS LSMEAN	LSMEAN Number
CONTROL	13.8125000	1
TRT1	6.8125000	2
TRT2	15.6875000	3
TRT3	9.6875000	4
TRT4	7.9375000	5

Least Squares Means for effect LEVEL
Pr > |t| for HO: LSMean(i)=LSMean(j)

Dependent Variable: HS

i/j	1	2	3	4	5
1		0.0489	0.5934	0.2419	0.0971
2	0.0489		0.0132	0.4136	0.7486
3	0.5934	0.0132		0.0903	0.0297
4	0.2419	0.4136	0.0903		0.6182
5	0.0971	0.7486	0.0297	0.6182	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The GLM Procedure

Dunnett's One-tailed t Tests for HS

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	75
Error Mean Square	97.8225
Critical Value of Dunnett's t	2.19721
Minimum Significant Difference	7.6833

Comparisons significant at the 0.05 level are indicated by ***.

LEVEL Comparison		Difference Between Means	Simultaneous 95% Confidence Limits	
TRT2	- CONTROL	1.875	-Infinity	9.558
TRT3	- CONTROL	-4.125	-Infinity	3.558
TRT4	- CONTROL	-5.875	-Infinity	1.808
TRT1	- CONTROL	-7.000	-Infinity	0.683

The GLM Procedure

Type I Estimable Functions

Effect		-Coefficients- LEVEL
Intercept		0
LEVEL	CONTROL	L2
LEVEL	TRT1	L3
LEVEL	TRT2	L4
LEVEL	TRT3	L5
LEVEL	TRT4	-L2-L3-L4-L5

EFFECTS OF ACETAMIPRID ON MALLARD REPRODUCTION (449884-08)
13. ANALYSIS OF 14-DAY HATCHLING SURVIVORS/NORMAL HATCHLINGS
****

09:24 Tuesday, November 20, 2001

The GLM Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4454.56921	1113.64230	3.85	0.0069
Error	70	20235.35387	289.07648		
Corrected Total	74	24689.92309			

R-Square	Coeff Var	Root MSE	RESPONSE Mean
0.180421	21.45288	17.00225	79.25392

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	4	4454.569210	1113.642303	3.85	0.0069

EFFECTS OF ACETAMIPRID ON MALLARD REPRODUCTION (449884-08)
13. ANALYSIS OF 14-DAY HATCHLING SURVIVORS/NORMAL HATCHLINGS
****

09:24 Tuesday, November 20, 2001

The GLM Procedure
Least Squares Means

LEVEL	RESPONSE LSMEAN	LSMEAN Number
CONTROL	78.8686440	1
TRT1	79.2224606	2
TRT2	88.5498111	3
TRT3	83.0736692	4
TRT4	64.9839388	5

Least Squares Means for effect LEVEL
Pr > |t| for HO: LSMean(i)=LSMean(j)

Dependent Variable: RESPONSE

i/j	1	2	3	4	5
1		0.9547	0.1176	0.5004	0.0313
2	0.9547		0.1314	0.5371	0.0274
3	0.1176	0.1314		0.3732	0.0003
4	0.5004	0.5371	0.3732		0.0055
5	0.0313	0.0274	0.0003	0.0055	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

EFFECTS OF ACETAMIPRID ON MALLARD REPRODUCTION (449884-08)
13. ANALYSIS OF 14-DAY HATCHLING SURVIVORS/NORMAL HATCHLINGS
****

09:24 Tuesday, November 20, 2001

The GLM Procedure

Dunnett's One-tailed t Tests for RESPONSE

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	289.0765
Critical Value of Dunnett's t	2.19998

Comparisons significant at the 0.05 level are indicated by ***.

LEVEL Comparison		Difference Between Means	Simultaneous 95% Confidence Limits	
TRT2	- CONTROL	9.681	-Infinity	23.124
TRT3	- CONTROL	4.205	-Infinity	17.863
TRT1	- CONTROL	0.354	-Infinity	14.012
TRT4	- CONTROL	-13.885	-Infinity	0.015

EFFECTS OF ACETAMIPRID ON MALLARD REPRODUCTION (449884-08)

Change in Adult Body Weights

16:21 Tuesday, November 20, 2001

Obs	MDIFF	FDIFF
1	33	173
2	376	186
3	59	331
4	239	131
5	73	293
6	111	146
7	118	296
8	244	107
9	48	234
10	216	242
11	54	269
12	99	249
13	69	338
14	-121	292
15	214	255
16	324	324
17	43	212
18	111	145
19	-9	342
20	128	209
21	-52	233
22	-70	21
23	-55	244
24	121	223
25	89	215
26	104	264
27	4	200
28	203	292
29	25	324
30	88	248
31	136	250
32	108	196
33	2	41
34	172	268
35	42	268
36	268	400
37	86	265
38	-1	315
39	93	292
40	48	-42
41	-95	76
42	208	222
43	272	243
44	201	55
45	61	163
46	111	67
47	197	-8
48	223	223
49	140	268
50	-59	307
51	-10	93
52	95	208
53	5	133
54	-39	15
55	-10	127
56	61	153
57	21	99
58	224	237
59	105	220
60	129	172
61	188	192
62	69	108

Obs	MDIFF	FDIFF
63	163	94
64	78	211
65	50	141
66	71	188
67	161	318
68	-22	140
69	178	162
70	130	113
71	105	21
72	145	214
73	177	84
74	227	154
75	116	5
76	190	350
77	39	87
78	140	104
79	-70	117
80	219	91

EFFECTS OF ACETAMIPRID ON MALLARD REPRODUCTION (449884-08)
1. ANALYSIS OF MALE WEIGHT GAIN 16:21 Tuesday, November 20, 2001
****

	LEVEL				
	CONTROL	TRT1	TRT2	TRT3	TRT4
	Mean	Mean	Mean	Mean	Mean
MDIFF Change in male wts	134.75	60.88	118.00	72.50	116.00
FDIFF Change in female wts.	241.63	226.13	178.00	164.81	143.06

The GLM Procedure

Class Level Information

Class	Levels	Values
LEVEL	5	CONTROL TRT1 TRT2 TRT3 TRT4

Number of observations 80

The GLM Procedure

Type I Estimable Functions

Effect		-Coefficients- LEVEL
Intercept		0
LEVEL	CONTROL	L2
LEVEL	TRT1	L3
LEVEL	TRT2	L4
LEVEL	TRT3	L5
LEVEL	TRT4	-L2-L3-L4-L5

The GLM Procedure

Dependent Variable: MDIFF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	65178.8000	16294.7000	1.72	0.1546
Error	75	710800.7500	9477.3433		
Corrected Total	79	775979.5500			

R-Square	Coeff Var	Root MSE	MDIFF Mean
0.083996	96.93965	97.35165	100.4250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	4	65178.80000	16294.70000	1.72	0.1546

The GLM Procedure
Least Squares Means

LEVEL	MDIFF LSMEAN	LSMEAN Number
CONTROL	134.750000	1
TRT1	60.875000	2
TRT2	118.000000	3
TRT3	72.500000	4
TRT4	116.000000	5

Least Squares Means for effect LEVEL
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: MDIFF

i/j	1	2	3	4	5
1		0.0351	0.6279	0.0745	0.5875
2	0.0351		0.1012	0.7365	0.1135
3	0.6279	0.1012		0.1902	0.9538
4	0.0745	0.7365	0.1902		0.2102
5	0.5875	0.1135	0.9538	0.2102	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The GLM Procedure

Dunnett's One-tailed t Tests for MDIFF

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	75
Error Mean Square	9477.343
Critical Value of Dunnett's t	2.19721
Minimum Significant Difference	75.626

Comparisons significant at the 0.05 level are indicated by ***.

LEVEL Comparison		Difference Between Means	Simultaneous 95% Confidence Limits	
TRT2	- CONTROL	-16.75	-Infinity	58.88
TRT4	- CONTROL	-18.75	-Infinity	56.88
TRT3	- CONTROL	-62.25	-Infinity	13.38
TRT1	- CONTROL	-73.88	-Infinity	1.75

The GLM Procedure

Class Level Information

Class	Levels	Values
LEVEL	5	CONTROL TRT1 TRT2 TRT3 TRT4

Number of observations 80

The GLM Procedure

Type I Estimable Functions

Effect		-Coefficients- LEVEL
Intercept		0
LEVEL	CONTROL	L2
LEVEL	TRT1	L3
LEVEL	TRT2	L4
LEVEL	TRT3	L5
LEVEL	TRT4	-L2-L3-L4-L5

The GLM Procedure

Dependent Variable: FDIFF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	111185.0750	27796.2687	3.32	0.0148
Error	75	628510.8750	8380.1450		
Corrected Total	79	739695.9500			

R-Square	Coeff Var	Root MSE	FDIFF Mean
0.150312	47.99745	91.54313	190.7250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
LEVEL	4	111185.0750	27796.2687	3.32	0.0148

The GLM Procedure
Least Squares Means

LEVEL	FDIFF LSMEAN	LSMEAN Number
CONTROL	241.625000	1
TRT1	226.125000	2
TRT2	178.000000	3
TRT3	164.812500	4
TRT4	143.062500	5

Least Squares Means for effect LEVEL
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: FDIFF

i/j	1	2	3	4	5
1		0.6334	0.0530	0.0202	0.0032
2	0.6334		0.1412	0.0620	0.0123
3	0.0530	0.1412		0.6848	0.2838
4	0.0202	0.0620	0.6848		0.5036
5	0.0032	0.0123	0.2838	0.5036	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The GLM Procedure

Dunnett's One-tailed t Tests for FDIFF

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	75
Error Mean Square	8380.145
Critical Value of Dunnett's t	2.19721
Minimum Significant Difference	71.114

Comparisons significant at the 0.05 level are indicated by ***.

LEVEL Comparison		Difference Between Means	Simultaneous 95% Confidence Limits		
TRT1	- CONTROL	-15.50	-Infinity	55.61	
TRT2	- CONTROL	-63.63	-Infinity	7.49	
TRT3	- CONTROL	-76.81	-Infinity	-5.70	***
TRT4	- CONTROL	-98.56	-Infinity	-27.45	***

This page is from the study report.

Table 1

Mean Adult Body Weight (g)
from a Mallard Duck Reproduction Study with Acetamiprid
EBA Study 029708

Experimental Group (ppm)	Sex	5/13/97 WT 1 Start of Acclimation	Change	5/27/97 Wt 2 Start of Test Feed	Change	7/29/97 Wt 3 Start of Egg Laying	Change	10/23/97 Wt 4 Adult Termination	Total Change Wt 2 - Wt 4
Control	Male	1181.7	-42.9	1138.8	-24.0	1114.8	158.8	1273.5	134.8
	Female	1015.6	-39.0	976.6	84.6	1061.2	157.0	1218.2	241.6
62.5 ppm	Male	1183.5	-19.4	1164.1	-31.8	1132.3	92.7	1225.0	60.9
	Female	1010.5	-33.4	977.1	24.2	1001.3	201.9	1203.2	226.1
125 ppm	Male	1168.6	-40.0	1128.6	1.0	1129.6	117.0	1246.6	118.0
	Female	1041.7	-24.8	1016.9	13.9	1030.8	164.1	1194.9	178.0
250 ppm	Male	1176.9	-50.9	1126.0	-47.9	1078.1	120.4	1198.5	72.5
	Female	1039.9	-40.3	999.6	54.8	1054.4	110.0	1164.4	164.8
500 ppm	Male	1106.2	-32.4	1073.8	-18.9	1054.9	134.9	1189.8	116.0
	Female	1011.5	-48.5	963.0	28.5	991.5 *	114.6	1106.1 *	143.1

* Significantly different from the controls.