Data Evaluation Report on the Reproductive Effects of AC 243997 on Avian Species Anas platyrhynchos ~~Colinus virginianus~~

PMRA Submission Number EPA MRID Number 45119714b

Data Requirement:

PMRA DATA CODE:
EPA DP Barcode: D275562
OECD Data Point:
EPA MRID: 45119714b
EPA Guideline: 71-4b

Test material: AC 243997 Technical **Purity:** 100%
Common name: Imazapyr
Chemical name: (IUPAC): 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid
CAS name: Not reported
CAS No.: 081334-34-1
Synonyms: AC 243997, CL 243997

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Secondary Reviewer(s): **Date:**
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Reference/Submission No.:

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EPA PC Code: 128821

Date Evaluation Completed:

CITATION: Ahmed, M.S. *et al.*, 1999. Avian Reproduction Studies on AC 243997 Technical. Unpublished studies performed by American Cyanamid Company, Princeton, NJ and Ecological Planning and Toxicology, Inc., Corvallis, OR. Laboratory Project ID: ~~ECO 97-147 and~~ ECO 97-146. Study submitted by American Cyanamid Company, Princeton, NJ. Study initiated March 18, 1997 and completed February 22, 1999.

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EXECUTIVE SUMMARY:

The one-generation reproductive toxicity of AC 243997 Technical to groups (16 pens/treatment level) of 1 male and 1 female of 30-week-old Mallard duck was assessed over 161 days. AC 243997 Technical was administered to the birds in the diet at measured concentrations of 0, 327, and 1670 ppm. Nominal concentrations were 0, 360, and 1800 ppm. The reviewer detected a significant reduction (10%) in the number of viable embryos/eggs set at the higher treatment level. There were no behavioral abnormalities or other signs of treatment-related toxicity.

This study is scientifically INVALID due to bacterial contamination and high embryonic mortality in the control group. Given this and other deviations from US EPA experimental procedure (e.g., reduced pre-egg laying exposure period), this study does not fulfill the requirements for an avian reproductive toxicity study with mallard duck (§71-4b). Another study should be conducted which conforms to the guidelines to determine the reproductive toxicity of AC 243997 to mallard duck.

Results Synopsis

Test Organism Size/Age : 30-weeks old at test initiation (males, 1023-1436 g; females, 908-1275 g)

Results are not reported; Invalid study
NOEC:
LOEC:
Endpoint(s) Affected:

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures of the United States Environmental Protection Agency (USEPA), FIFRA Guideline §71-4a, and OECD Guidelines for Testing Chemicals, 206, "Avian Reproduction Test". Deviations from §71-4a are:

1) The proportion of hatchlings per eggs set (43.6%) and hatchling per 3-week viable embryos (51.2%) in the control group was below the typically acceptable criterion for Mallard duck. A pathological examination of unhatched embryos revealed two bacterial contaminants that were putatively described as the cause of embryo death in the hatcher.

2) The Mallard were 30-weeks-old at test initiation. As a result, the pre-egg laying exposure period was only 8 weeks, instead of the 10 weeks required by guidelines. The first egg was laid only 2 days after the initiation of the extended photoperiod.

3) Following collection and prior to incubation, eggs were stored in a refrigerator; however, specific temperature and humidity levels were not reported.

4) Identical methods concerning the egg collection, handling, incubation, and hatching were employed for both the Bobwhite quail and Mallard duck experiments. Whereas, candling for embryotic viability was performed on Day 11 instead of Day 14 for the duck eggs. Furthermore, the eggs were transferred to the hatcher on the end of Day 20, instead of Day 23.

5) Relative humidity levels during incubation and hatching were not provided.

6) The average hatching temperature, 37.3 ± 0.9°C, was slightly less than the recommended value of 39°C.

7) The hatchling photoperiod was not specified.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material AC 243997 Technical

Description: White powder
Lot No./Batch No.: AC 10925-48
Purity: 100%

Stability of Compound Under Test Conditions: Samples of treated feed were stored in open feed containers and in bulk closed containers for 7 and 14 days at ambient conditions. Recoveries were 86-89% after 14 days of open storage and 95-101% after 14 days of closed-container storage. Additional frozen samples were stored for 81 days prior to analysis, with recoveries of 97-101%. OECD requirements were not reported.

Storage conditions of test chemicals: Dry conditions at room temperature in the dark.

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2. Test organism:

Table 1. Test organism.

Parameter	Details	Remarks *Criteria*
Species (common and scientific names):	Mallard duck (*Anas platyrhynchos*)	*EPA requires: a wild waterfowl species, preferably the mallard, Anas platyrhynchos, or an upland game species, preferably the northern bobwhite, Colinus virginianus.*
Age at Study Initiation:	30 weeks, approaching their first breeding season.	*EPA requires: birds should be approaching their first breeding season.*
Body Weight: (mean and range)	Males: range: 990 - 1648 g, mean: 1257.0 g. Females: range: 869 - 1371 g; mean: 1196.0 g	Recorded at the beginning of the acclimation period, bi-weekly during the first 8 weeks of the treatment period, and at study termination. *EPA requires that body weights should be recorded at test initiation and at biweekly intervals up to week eight or up to the onset of egg laying and at termination.*
Source:	Whistling Wings Hanover, IL.	*EPA requires that all birds should be from the same source.*

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Not performed.

b) Definitive Study

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Table 2. Experimental Parameters.

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	20 days Environmental conditions during acclimation were identical to test conditions. Water and feed were provided *ad libitum*. None	One bird was removed from the study due to an injured penis. Two birds were eliminated because they were too heavy (>1300 g). Ducks were fed Purina Game Bird Breeder Layena, and tap water from the city of Corvallis. *EPA recommends a 2-3 week health observation period prior to selection of birds for treatment. Birds must be generally healthy without excess mortality. Feeding should be ad libitum, and sickness, injuries or mortality be noted.*
Test duration pre-laying exposure: egg-laying exposure: withdrawal period, if used:	8 weeks 15 weeks None	*EPA requires* ***Pre-laying exposure duration*** *At least 10 weeks prior to the onset of egg-laying.* ***Exposure duration with egg-laying*** *At least 10 weeks.* ***Withdrawal period*** *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:	Parents (one pair) were housed in 90- x 70- x 46-cm pens. Offspring (by set and group) were housed in 72- x 90- x 23-cm brooders. Parental and offspring pens were constructed of galvanized steel. 16 pens	Number of pens needed for offspring housing was not specified. *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.*

Parameter	Details	Remarks *Criteria*
Number of birds per pen (male:female)	2 birds/pen (1 male:1 female)	*EPA requires one male and 1 female per pen. For quail, 1 male and 2 females is acceptable. For ducks, 2 males and 5 females is acceptable.*
Number of pens per group/treatment negative control: solvent control: treated:	16 pens N/A 16 pens/treatment	Pen number is theoretical. Actual number was less due to mortality. *EPA requires at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.*
Test concentrations (ppm) nominal: measured:	0, 360, and 1800 ppm 0, 327, and 1670 ppm	*EPA requires at least two concentrations other than the control are required; three or more are recommended.*
Maximum labeled field residue anticipated and source of information:	360 ppm based upon application rate of 1.5 lb a.i./acre using Kenaga nomogram conversion.	*EPA requires that 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:	Acetone and corn oil 5.4% (v:w) and 2% (w:w), respectively	Although the acetone concentration exceeded 2% of the diet, the acetone was completely evaporated during the 15- to 20-minute mixing period. *EPA requires corn oil or other appropriate vehicle not more than 2% of diet by weight*

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Parameter	Details	Remarks *Criteria*
Was detailed description and nutrient analysis of the basal diet provided? (Yes/No)	Yes	Basal diets contained ≥20% protein, ≥2.5% fat, and ≤7% fiber. *EPA requires a commercial breeder feed (or its equivalent) that is appropriate for the test species.*
Preparation of test diet	The test material was dissolved in acetone and mixed for 5-6 minutes, and this solution was mixed in corn oil for an additional 5-6 minutes prior to incorporation into the Layena feed. Duplicate batches were prepared and sampled independently and maintained separately. Mixed feed was placed in 1 gallon resealable bags and stored frozen.	The acetone was completely evaporated during the 15- to 20-minute mixing process. *A premixed 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	
Were concentrations in diet verified by chemical analysis?	Yes	
Did chemical analysis confirm that diet was stable and homogeneous?	Yes	
Feeding and husbandry	Feeding and husbandry conditions appeared to be adequate, given guideline recommendations.	

Parameter	Details	Remarks *Criteria*
Test conditions (pre-laying) temperature: relative humidity: photoperiod:	Average temperature was 20˚C (range of 17-22˚C). Average humidity was 78% (range of 47-100%). 7 hr light/day up to Week 8 and increased to 17 hr light/day thereafter.	An average of 6.6 footcandles of illumination was maintained at bird level. *EPA Requires* *Temperature: About 21°C (70°F)* *Relative humidity: About 55%* *Lighting* *First 8 weeks: 7 h per day.* *Thereafter: 16-17 h per day.* *At least 6 footcandles at bird level.*
Egg Collection and Incubation		
Egg collection and storage collection interval: storage temperature: storage humidity:	Eggs were collected daily. Not reported Not reported	*EPA requires eggs to be collected daily; egg storage temperature approximately 16°C (61°F); humidity approximately 65%.*
Were eggs candled for cracks prior to setting for incubation?	Yes	*EPA requires eggs to be candled on day 0*
Were eggs set weekly?	Yes	
Incubation conditions temperature: humidity:	37.5 ± 0.2˚C (dry bulb) Not specified	Humidity was not specified; however, the wet bulb temperature was reported as 29.1 ± 1.0˚C.
When candling was done for fertility?	Day 11 for fertility and Day 18 for viability.	*EPA requires:* *Quail: approx. day 11* *Ducks: approx. day 14*
When the eggs were transferred to the hatcher?	End of Day 20	*EPA requires:* *Bobwhite: day 21* *Mallard: day 23*

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Parameter	Details	Remarks *Criteria*
Hatching conditions temperature: humidity: photoperiod:	37.3 ± 0.9°C Not specified Not specified	Humidity was not specified; however, the wet bulb temperature was reported as 32.4 ± 0.8°C. *EPA requires:* *temperature of 39°C (102°F)* *humidity of 70%*
Day the hatched eggs were removed and counted	Day 28	*EPA requires Bobwhite: day 24* *Mallard: day 27*
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes, shells were washed and air-dried for at least 48 hours.	
Egg shell thickness no. of eggs used: intervals: mode of measurement:	164 of the 192 eggs collected were measured for thickness Collections occurred on 9/4/97, 9/18/97, 10/2/97, 10/9/97, and 11/6/97. Five points around the equatorial circumference were measured to the nearest 0.001 mm.	Eggs collected on 11/6/97 were never measured for egg shell thickness. *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.*
Reference chemical, if used	None used	

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2. Observations:

Table 3: 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 viable embryos, no. of live 3 week embryos, no. hatched, no. of 14-day survivors, average weight of 14-d old survivors, mortality, gross pathology, others)	- mortality - body weight - food consumption - signs of toxicity - necropsy - eggs laid - eggs cracked - eggs set - eggshell thickness - viable embryos - live 3-week embryos - hatchling success - normal hatchlings - hatchling body weight - 14-day-old survivors - 14-day-old survivor body weight - signs of toxicity	*EPA requires:* • *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* • *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)*
Indicate if the test material was regurgitated	No indications of dietary regurgitation.	
Observation intervals (for various parameters)	Mortality and signs of toxicity were recorded once daily for parents and hatchlings. Parental body weights were recorded bi-weekly through the start of photostimulation (6/17/97, 7/1/97, 7/16/97, 7/29/97, and 8/12/97), and at adult termination (11/26/97). Parental food consumption was determined each Monday, Wednesday, and Friday. Gross pathological examinations were performed on all decedent ducks (and their penmates) and on half of the test animals from control and treatment groups that survived to study termination.	*Body weights and food consumption must be measured at least biweekly.*
Were raw data included?	Yes	

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II. RESULTS AND DISCUSSION:

A. MORTALITY:

One mortality occurred during the treatment-phase of the experiment. During the 22nd week, a female from the control group died from strangulation after catching her head in the cage. Necropsies of her and her penmate revealed no remarkable internal findings. The cage was removed from all subsequent analyses. No other mortalities during the experiment.

Table 4: Effect of AC 243997 Technical on mortality of *Anas platyrhynchos*.

Treatment (ppm)	Observation Period					
	Week 8		Week 16		Week 23	
	No. Dead		No. Dead		No. Dead	
	Male	Female	Male	Female	Male	Female
Control	0	0	0	0	0	1
327 (360)	0	0	0	0	0	0
1670 (1800)	0	0	0	0	0	0

Nominal concentrations are in parentheses.

B. REPRODUCTIVE AND OTHER ENDPOINTS:

There were no overt signs of toxicity during the study. In addition, no treatment-related gross pathological findings were present at necropsy of adult birds at study termination. Findings were observed in 4 of 48 animals examined: one control male had a discolored liver; one control female had a tumorous liver, a fatty heart, a discolored pancreas, and its abdomen was filled with liquid; one female from the 327-ppm group had a fatty heart; and one female from the 1670-ppm group had a mottled and discolored liver and a fatty heart.

No treatment-related differences were detected for adult body weight (gender specific), adult food consumption, or any reproductive parameter. Body weights (of hatchlings and 14-day-old survivors) and survivability of offspring from treatment groups also did not significantly differ from controls.

There was considerable variability in the number of eggs produced in each group, with 777 eggs laid in the control group, 924 in the 327-ppm group, and 969 in the 1670-ppm group. One pair of ducks in the control group laid only one egg during the test. For the remaining pairs, 19 to 92 eggs were laid per cage. The average number of eggs laid per cage was 51.8 in the control group, 57.8 in the 327-ppm group, and 60.6 in the 1670-ppm group. Due to the variability within treatments, there were no statistically-significant differences among treatments with respect to the number of eggs laid.

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Table 5. Reproductive and other parameters.

Parameter	Control	327 ppm	1670 ppm	NOAEC/ LOAEC
Eggs laid/pen	51.8	57.8	60.6	1670 >1670
Eggs laid/hen/day	0.49	0.55	0.58	---
Eggs cracked	0.53	0.31	0.38	1670 >1670
Eggs cracked/eggs laid (%)	1.07	0.69	0.55	1670 >1670
Eggs set/pen	47.6	53.4	55.5	1670 >1670
Shell thickness (mm ± SD)	0.394 ± 0.023	0.379 ± 0.025	0.401 ± 0.022	1670 >1670
Viable embryos/hen	44.7	49.6	48.3	1670 >1670
Live 3-week embryos/hen	44.1	49.4	48.1	1670 >1670
No. of hatchling/hen	22.6	24.4	21.6	1670 >1670
No. of normal hatchlings	There were no abnormally formed hatchlings observed in the study.			
Hatchling weight (g)	37.5	37.2	38.6	1670 >1670
14-day old survivors/hen	19.8	20.0	17.9	1670 >1670
14-day old survivors weight (g)	242.4	237.1	240.1	1670 >1670
Mean food consumption (g/bird/day)	157.5 ± 28.2	162.2 ± 25.1	167.4 ± 25.1	1670 >1670
Weight of adult males, g at start of treatment: at start of photostimulation: at termination:	 1210 ± 72 1207 ± 108 1255 ± 79	 1209 ± 96 1206 ± 89 1300 ± 123	 1184 ± 81 1183 ± 83 1216 ± 81	 1670 >1670
Weight of adult females, g at start of treatment: at start of photostimulation: at termination:	 1073 ± 101 1059 ± 106 1207 ± 106	 1074 ± 86 1075 ± 90 1163 ± 134	 1110 ± 102 1127 ± 123 1218 ± 126	 1670 >1670

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Parameter	Control	327 ppm	1670 ppm	NOAEC/ LOAEC
Gross pathology	No treatment-related findings.			1670 >1670

ND = Not determined

C. REPORTED STATISTICS:

Analyses were conducted using Statistica for Windows (1994). Variables included adult body weights (biweekly prior to egg laying and termination weight), feed consumption, total eggs laid per hen, percentage of eggs cracked, eggshell thickness, fertile eggs (per cage and as percent of eggs set), live 18-day-old embryos (per cage and as a percentage of fertile eggs), normal hatchlings (as percent of 18-day-old viable embryos), 14-day-old survivors per hen, 14-day-old survivors per cage and as percent of normal hatchlings, hatchling weight (day of hatch and 14 days post-hatch).

Levene's and Kolomogrov-Smirnov tests were conducted on variables to determine homogeneity of variances and normality, respectively. Percentage data were arcsine transformed. If the data conformed to assumptions of normality of distribution, then a standard Analysis of Variance (ANOVA) was used for examination of each parameter to determine whether or not there were differences among treatments. When ANOVA detected significant differences among groups, a Least Significant Difference (LSD) test was run as a means separation procedure. If data violated ANOVA assumptions (normality, equal variances), the Kruskal-Wallis ANOVA by ranks and median test was used.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Analyses were conducted using "chicks.sas" (Ver. 3; March 2002), a SAS program provided by USEPA/OPP/EFED. Data for all endpoints were examined graphically using box plots to determine if they exhibited a dose-dependent response, which was ultimately used to select the multiple comparison test to detect the LOEC and NOEC. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests and data that did not satisfy these assumptions were subjected to the nonparametric MannWhitney-U (with a Bonferroni adjustment) and Jonckheere's tests. See Appendix I for output of reviewer's statistical verification and graphs for affected endpoints to support any reviewer-generated conclusions that may differ from those reported in the study.

The reviewer's analysis detected a significant reduction in the ratio of viable embryos/eggs set at the higher treatment level (1670 ppm) using Jonckheere's test. This ratio was reduced 10% from the control value.

Results are not reported; Invalid study
NOEC:
LOEC:
Endpoint(s) Affected:

E. STUDY DEFICIENCIES:

Two significant deficiencies were encountered in this study.

- The proportion of hatchlings per viable eggs in the control group (43.6%) was slightly below the typically acceptable criterion of 50 to 90% for Mallard duck. The comparison of hatchlings per 3-week

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viable eggs raised the hatch rate to 50.3%. The study authors reported that the hatchling rate for the Mallards has consistently been on the low end of the acceptability criterion in other reproduction studies conducted previously in this laboratory. In the 327- and 1670-ppm groups, the proportion of hatchlings per viable eggs were 48.4 and 46.6%, respectively, and the proportion of hatchlings per 3-week viable eggs were 48.8 and 46.8%, respectively.

- A pathological examination of unhatched embryos revealed two bacterial contaminants that were putatively described as the cause of embryo death in the hatcher.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions differed from those of the study authors. The study authors did not detect any treatment related effects of AC 243997 on any adult or reproductive parameters. Whereas, the reviewer detected a significant reduction in the ratio of viable embryos/eggs set at the higher treatment level. However, these results are not reliable because there were several deviations from US EPA guidelines, which may have impacted the study results.

The timing to candling for embryonic visibility and hatching were identical to the bobwhite quail test protocols. Whereas, candling for embryotic viability was performed on Day 11 instead of Day 14 for the duck eggs. Furthermore, the eggs were transferred to the hatcher on the end of Day 20, instead of Day 23. This leads the reviewer to assume reduction in viable embryos when candling was performed on Day 11 instead of Day 14 to allow maximum embryonic development of the ducks. It is recommended to follow the mallard duck test protocols on the appropriate days for candling, transferring from incubation to the hatcher, and hatching to prevent falling below acceptable criterions for the mallard ducks.

It was unclear if the chemical name provided for AC 243997 was based on IUPAC or CAS nomenclature.

G. CONCLUSIONS:

This study is scientifically INVALID due to bacterial contamination and high embryonic mortality in the control group. Given this and other deviations from US EPA experimental procedure (e.g., reduced pre-egg laying exposure period), this study does not fulfill the guideline requirements for an avian reproductive toxicity study with mallard duck (§71-4b). Another study should be conducted which conforms to the guidelines to determine the reproductive toxicity of AC 243997 to mallard duck.

Results are not reported; Invalid study
NOEC:
LOEC:
Endpoint(s) Affected:

III. REFERENCES:

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