9-22-98

MEMORANDUM

SUBJECT: **Feeding and Tissue Residue Study for Chlorfenapyr (AC 303,630)**

CHEMICAL CODE: **129093**

DP BARCODE: **D241963**

TO: **Ann Sibold, PM Team Reviewer**
Susan Lewis, Product Manager 03
Registration Division

FROM: **Arnet Jones, Chief**
ERB1
Environmental Fate and Effects Division (7507C)

09/22/98

GUIDELINE	MRID	TOXICITY	ACCEPTABILITY/ CLASSIFICATION
70-1(D) CL 303,630 Bobwhite Quail	444526-10	LOEC = 1.5 ppm NOEC = <1.5 ppm	Supplemental

Residues in the GI tract of birds exposed to AC 303,630 at concentrations of 1.5, 15, 150, and 1500 ppm were found. Residues in the liver were found in the 1500 ppm group that was capsule-dosed. Residues were also found in the skin and fat tissues of the 150 and 1500 ppm groups. In addition, blood tissue residues were found in the 1500 ppm group. No correlation was found between the amount of active ingredient consumed and the amount of residues found in the tissue samples. However, on average, the higher the concentration of active ingredient consumed the higher the residue in the tissues.

Birds in the 150 and 1500 ppm had significantly reduced feed consumption which most likely led to the significantly lower body weights from the control group. In addition, these two concentration groups exhibited the most abnormal behaviors that would indicate toxicity.

Due to the parent compound being quickly metabolized by MFO enzymes to the biologically active compound, it might have been more appropriate to test for residues of the metabolites/degradates in the tissues.

If you have any questions concerning this review please, contact Regina Hirsch (608-255-8606) or Arnet Jones (305-7416).

DATA EVALUATION RECORD
§ 70-1(D) -- Feeding and Tissue Residue Study

1. **CHEMICAL:** Chlorfenapyr (Pirate/Alert) PC Code No.:

2. **TEST MATERIAL:** AC 303,630 technical; Lot number AC 7504-59A. Purity: 94.9%

3. **CITATION**

Authors:	J. A. Gagne, L. W. Brewer, J. P. Sullivan, S. L. Tank, and L. C. Taliaferro
Title:	Feeding and Tissue Residue Study Using AC 303,630 Technical in Northern Bobwhite (*Colinus virginianus*)
Study Completion Date:	December 16, 1997
Laboratory:	Ecotoxicology and Biosystems Associates, Inc., Snow Camp, NC.
Sponsor:	American Cyanamid Company, Princeton, NJ
Laboratory Report ID:	954-94-132
MRID No.:	444526-10

4. **REVIEWED BY:** Regina M. Hirsch, Wildlife Ecologist, ERB1, EFED

Signature: Regina Hirsch **Date:** 4/9/98

5. **APPROVED BY:** Arnet Jones, Chief, ERB1, EFED Second Review

Signature: Arnet B. Jones **Date:** 09/22/98

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Colinus viginianus*
Age of Test Organisms at Test Initiation: 8 weeks at beginning of acclimation
Definitive Study Duration: 14 days

7. **CONCLUSIONS:**

LOEC = 1.5 ppm
NOEC = < 1.5 ppm

Residues in the GI tract of birds exposed to AC 303,630 at concentrations of 1.5, 15, 150, and 1500 ppm were found. Residues in the liver were found in the 1500 ppm group that was capsule-dosed. Residues were also found in the skin and fat tissues of the 150 and 1500 ppm groups. In addition, blood tissue residues were found in the 1500 ppm group. No correlation was found between the amount of active ingredient consumed and the amount of residues found in the tissue samples. However, on average, the higher the concentration of active ingredient consumed the higher the residue in the tissues.

Based on the known toxicities of this chemical's degradates, it might have been more pertinent to test for residues of the degradates in the tissues.

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rational: Not a required study and control group birds had detectable residues of active ingredient in GI tract tissues and blood tissues.

C. Repairability: No

9. GUIDELINE DEVIATIONS

1. Control group birds had detectable residues of active ingredient in GI tract tissues and blood tissues.

10. SUBMISSION PURPOSE: To determine the residues of AC 303,630 present in blood, GI tract, liver and fat tissues of Northern bobwhite following exposure to treated diet.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species: An upland game bird species, preferably the bobwhite (*Colinus virginianus*).	*Colinus virginianus*
Age at beginning of test:	8 weeks at acclimation
Supplier	Sand Prairie Quail Farm, Maquoketa, Iowa
Chicks appeared healthy and did not have excessive mortality before the test?	During the quarantine and acclimation periods, 18 birds died in their cages. These mortalities were primarily due to starvation, however several had physical injuries (broken wings and dislocated joints) as a result of frantic thrashing around in their cages. This was possibly due to the young birds being separated from their covey at too early of an age. By the end of acclimation period, the remaining birds were behaving normally.

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Guideline Criteria	Reported Information
Acclimation period: As long as possible.	approx. 2 weeks

B. Test System

Guideline Criteria	Reported Information
Pen size: about 35 x 100 x 24 cm	Birds housed separately in 50.8 cm X 30.5 cm with a sloping floor which gave the height of cage 20-25 cm.
Room temperature: 22-27°C (71-81°F)	71-78°F
Relative humidity: 30-80%	47-70%
Adequate ventilation?	Not reported
Photoperiod Minimum of 14 h of light.	16 hours light; 8 hours dark
Diet: A commercial diet for game birds.	Purina Game Bird Flight Conditioner

C. Test Design

Guideline Criteria	Reported Information
Definitive Test **Nominal concentrations:** Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 ppm.	1.5, 15, 150, 1500 ppm
Controls: Control group tested with diet containing the maximum amount of vehicle used in treated diets?	Yes

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Guideline Criteria	Reported Information
Number of birds per group: 10 (strongly recommended)	32 birds (16 males, 16 females) in each group, except the 1500 ppm group which had 23 birds. An additional 1500 ppm study was conducted using 8 birds (4 males, 4 females).
Vehicle: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	Acetone and corn oil
Vehicle amount (% of diet by weight): Not more than 2%	Not reported
Test duration:	14 days

Study Design:

Four concentrations of the test material mixed in feed were provided to four groups of 32 birds each for 14 days. One control group was maintained on commercial diet which contained an equal amount of corn oil and acetone as used in the test material and feed mixtures. Survival and behavioral observations were made on all birds and recorded twice per day on weekdays and once per day on weekends and holidays. Feed consumption and body weight were measured at scheduled intervals and eight birds of each of the five groups were randomly chosen for sacrifice on days 1, 3, 7, and 14 after treated diets were introduced. Any birds that died during the interim period leading up to the sacrifice date were included in that day's sample of tissue samples. If 4 or more males or females died in a particular group during the interim period, no birds were sacrificed for that respective period and group. Blood samples were collected immediately prior to euthanization. In addition, Gastrointestinal (GI) tracts (from crop to distal end of the large intestine), livers, and skin covering the breast and back (with fat) from each bird were taken during post-mortem examinations. Samples were labeled, frozen and shipped to McKenzie Laboratories for residue analysis.

At the 1500 ppm dietary concentration test bird feed consumption appeared to be very low. Therefore, in order to assure body tissues could be collected, an additional phase was added to the study. Eight birds (4 males, 4 females) from a different batch of 16-week old birds were caged individually in a separate room. These birds were dosed with size 0 gelatin capsules containing approximately 0.86 g of diet containing a nominal concentration of 1500 ppm AC 303,630. All birds were fasted for 15 hours prior to initial dosing and only diet in capsules was fed to them during the dosing period. Each bobwhite was scheduled to receive five capsules at approximately 9:00 am and all surviving birds were scheduled to receive five additional capsules at approximately 1:00 pm and 4:00 pm. This schedule was continued daily until all birds died or until 3 full days of dosing were complete. All capsule-dosed birds that died were collected upon

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observation of mortality and as soon as possible after death. Post-mortem exams were conducted and tissues were collected for analysis.

Samples were typically analyzed beginning with those from the highest feed concentration group and longest collection period (14-day) first, followed by the next collection period, etc., until no (or minimal) residue was detectable. The limit of quantification was 0.05 ppm for all tissues except blood, where it was <0.005 ppm. No residue (<0.05 ppm) was found in the control group samples. Since no birds in the 1500 ppm feeding group survived to day 14, there were no tissue samples for this group for day 14.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Body weights measured at beginning and end of study?	Body weight of each test animal was measured just prior to group assignment, immediately prior to first exposure to diet mixtures, and on test days 1, 3, 7, and 14.
Estimated consumption per pen reported for pretreatment, treatment, and observation periods?	Each day the feed in each feed tray was weighed and the weight subtracted from the feed weight recorded for the previous day. Fresh diet was provided daily.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Feed mixture Sample Analysis

Nominal Level	Mean Measure Conc.	Standard Deviation	% of Nominal
1.5 ppm	2.32	0.30	155
15 ppm	15.0	1.08	107
150 ppm	151	9.74	101
1500 ppm	1360	59.40	90.7

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Statistical Methods Used:

Body weights measured at time of group assignment and again just prior to test initiation were analyzed using Bartlett's Test for homogeneity of variance and ANOVA was run to evaluate differences in mean weight among groups.

Body weights collected on days 1, 3, 7, and 14 were tested for homogeneity of variance using the Bartlett's Test. If data sets had homogeneous variances, treatment groups were tested against the mean using Dunnett's Test. If the data sets did not have homogeneous variances, or were not normally distributed, the Kruskal-Wallis Test of pairs of treatments was used.

Linear Regressions were used to correlate the amounts of active ingredient consumed per bird to the amount of residue detected in those tissues in which the test material was consistently detected. The dependent variable was the residue level and the independent variable was the amount of a.i. consumed. The model for the regressions was:

$$Residue = Constant + \beta\, consumption$$

Statistical Results

GI Tract Tissue Residues:

1.5 ppm Dose Group: All periods, except day 7, had less than 0.05 ppm AC 303,630 residue. Of the 8 samples collected on day 7, six contained residue levels above 0.05 ppm to a maximum of 0.076 ppm AC 303,630.

15 ppm Dose Group: Five of 32 samples contained residues above 0.05 ppm. These levels ranged up to 0.59 ppm. Day 7 again had the highest residue levels of the 4 collection periods.

150 ppm Dose Group: All samples contained residues in excess of 0.05 ppm. The range was 0.29 ppm in day 3 samples to 8.30 ppm in day 7 samples. In this dose group day 7 samples contained the highest residues among the 4 sampling periods.

1500 ppm Dose Group: G.I. tract samples were available for only days 1, 3, and 7. Tissues from these sampling periods contained similar residue values ranging from 1.36 to 22.2 ppm.

Capsule-dosed Birds, 1500ppm: This group contained substantially higher G.I. tract residue levels than any other samples. Six of 8 samples had residue levels >50.0 ppm. These *birds Colinus virginianus* survived a maximum of 2 days after capsule administration.

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GI Tract Residues Data Summary

Collection Interval	Dosage Level	Mean GI Tract Residue (ppm)	GI Tract Residue Range (ppm)
Day 1	Control	0.0086 (n=8)	<0.005-0.00797
Day 3	Control	0.0099 (n=8)	<0.005-0.0168
Day 7	Control	0.0056 (n=8)	<0.005-0.0098
Day 14	Control	0.0078 (n=8)	<0.005-0.260
Day 1	1.5 ppm	<0.005 (n=8)	<0.005
Day 3	1.5 ppm	<0.005 (n=8)	<0.005
Day 7	1.5 ppm	0.049 (n=8)	<0.005-0.0764
Day 14	1.5 ppm	<0.005 (n=8)	<0.005
Day 1	15 ppm	0.218 (n=8)	0.0934-0.506
Day 3	15 ppm	0.095 (n=8)	<0.005-0.362
Day 7	15 ppm	0.452 (n=8)	0.224-0.591
Day 14	15 ppm	0.039 (n=8)	<0.005-0.0708
Day 1	150 ppm	2.07 (n=8)	1.31-2.91
Day 3	150 ppm	1.93 (n=8)	0.291-4.97
Day 7	150 ppm	5.28 (n=8)	2.69-8.30
Day 14	150 ppm	1.01 (n=8)	0.330-2.93
Day 1	1500 ppm	7.22 (n=8)	1.62-19.7
Day 3	1500 ppm	7.52 (n=8)	0.623-21.1
Day 7	1500 ppm	7.55 (n=7)	1.48-22.2
Day 1	1500 ppm (capsule)	93.2 (n=6)	0.239-193
Day 2	1500 ppm (capsule)	207.5 (n=2)	164.0-251

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Liver Tissue Residues:

No samples from the 4 feeding groups or the controls contained residues of AC 303,630 above the quantification limit (0.05 ppm). However, residues levels in the capsule-dosed birds ranged from <0.05 ppm to 0.672 ppm (Mean = 0.239 ppm).

Skin and Fat Tissue Residues:

Analyses were conducted on tissue from the control group, 150 ppm and 1500 ppm group. No residues were detected in the control group. Residues in the 150 ppm ranged from <0.05 to 0.28 ppm. Nine of 23 samples from the 1500 ppm contained quantifiable residue levels that ranged from 0.52 ppm to 0.41 ppm. Capsule dosing samples ranged from 0.35 ppm to 2.26 ppm.

Skin and Fat Study Analysis Results

Collection Interval	Dosage	Mean Residue (ppm)	Residue Range (ppm)
not reported	Control	<0.005 (n=8)	<0.005
Day 1	150 ppm	0.0802 (n=8)	<0.05-0.220
Day 3	150 ppm	0.068 (n=8)	<0.05-0.141
Day 7	150 ppm	0.121 (n=8)	0.0592-0.275
Day 14	150 ppm	0.114 (n=8)	<0.05-0.281
Day 1	1500 ppm	0.071 (n=8)	<0.05-0.198
Day 3	1500 ppm	0.058 (n=8)	<0.05-0.118
Day 7	1500 ppm	0.138 (n=7)	<0.05-0.414
Day 1	1500 ppm (capsule)	1.056 (n=6)	0.358-2.26
Day 2	1500 ppm (capsule)	0.898 (n=2)	0.506-1.29

Blood Tissue Residues:

One of 16 samples in the 1500 ppm group contained quantifiable residues of 0.566 ppm. No residues were found in the 150 ppm group. **Three of 7 control samples were reported to have residues of 0.031, 0.032 and 0.038 ppm, respectively**. All other samples were reported as < 0.005 ppm. No blood samples were drawn from the capsule-dosed birds as all of them were found dead in their cages and none were euthanized.

Correlation of Tissue Residues and Consumption of Test Material:

Residues were detected consistently only in the GI tract in both the 1500 ppm groups (feeding group and capsule-dosed). A correlation was performed for the total amount of active ingredient consumed per bird to the amount of residue detected in the GI tract for all birds in the 1500 ppm feeding group (squared multiple R = 0.011 (P = 0.63)); no correlation was found. The same regression was performed using the amount of active ingredient consumed during the last day before death for each bird (squared multiple R = 0.005 (P = 0.75)); again no correlation. A regression was performed on the capsule-dosed birds and amount of active ingredient consumed (squared multiple R = 0.247 (P = 0.21)); indicating a very weak correlation. In addition, a regression was performed on the liver residues for the capsule-dosed birds and the amount of active ingredient consumed (squared multiple R = 0.020 (P = 0.74)); indicating no correlation.

Bird Body Weights:

The pre-exposure body weights of birds in all test groups, taken at the beginning of the acclimation period and again just prior to the exposure period, had homogenous variances (P = 0.01). Post-exposure body weights were taken on days 1, 3, 7, and 14. On days 1 and 3 body weights were significantly lower than the controls for both the 150 and 1500 ppm groups ($P<0.05$). On day 7 there were too few 1500 ppm birds remaining, however the 150 ppm group was still significantly lower than the controls for both days 7 and 14 ($P<0.05$).

Feed Consumption

Significant differences in feed consumption among groups for days 1-14 as determined by Dunn's pair-wise comparison of means. Groups not sharing at least on similar letter are significantly different for that particular day.

Day	Control	1.5 ppm	15 ppm	150 ppm	1500 ppm
1	A	A	A	B	B
2	A	A	A	B	B
3	AB	A	A	BC	C
4	A	AB	AB	BC	C
5	A	A	A	B	B
6	AB	B	B	A	A
7	A	A	A	B	ND
8	A	A	A	A	ND
9	A	A	A	A	ND

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10	A	AB	AB	B	ND
11	A	A	AB	B	ND
12	A	AB	AB	B	ND
13	A	AB	A	B	ND
14	A	A	A	A	ND

ND = no data

Behavioral Observations:

The number and percent of birds in each group observed to exhibit behavioral abnormalities during at least 2 observation periods following introduction of the test material. The percent, in parentheses, is based on the initial number of birds in each group.

Group (ppm)	Unkempt	Piloerection	Wing Droop	Tremors	Ataxia[1]	Inactive	Prostrate
Control	2 (6.3)	0	0	0	0	0	0
1.5	4 (12.5)	4 (12.5)	0	0	0	0	0
15	4 (12.4)	2 (6.3)	1 (3.0)	2 (6.3)	1 (3.0)	2 (6.3)	0
150	7 (21.9)	2 (6.3)	5 (15.6)	1 (3.0)	15 (47)	6 (19.0)	4 (12.5)
1500	0	10 (43.5)	8 (34.8)	2 (8.7)	8 (34.8)	3 (13.0)	0

[1] The ataxia observed involved the birds displaying an unsteadiness while on their feet.

Mortality:

One bird died in the 15 ppm group on day 4 post-exposure, upon necropsy it was found that this male had an abnormality in its intestinal tract which caused the small intestine and cloaca to become impacted. This condition did not appear to be related to the test material, as no other birds exhibited this condition. Two birds in the 150 ppm group died on day 14 post-exposure. Both of these birds exhibited ataxia for several days before becoming inactive and finally lying prostrate before dying. The post-mortem of these birds showed only obvious abnormality to be lower than normal body fat deposits. There were seven post-exposure mortalities in the 1500 ppm group: one female on day 2, one male on day 3, one male and female on day 4, one male on day 5, one male on day 6, and one female on day 7. All these birds were emaciated with no body fat present during the post-mortem examination.

Capsule Dosing of Northern Bobwhite with 1500 ppm Feed Mix:

During the first and second dose all eight birds received approximately 4.35 g of 1500 ppm feed and test material mix. At the initiation of the third dose on day 1, three birds were dead. Two others were sick and were not given the scheduled 5 capsules, due to inability to get the 5 capsules into the crop. On day 2, three additional birds were dead. The remaining two birds were dosed with approximately 4.35 g of 1500 ppm. By that afternoon, these two birds were dead. Post-mortem exams conducted on all eight birds in this study indicated no abnormalities.

14. REVIEWER'S COMMENTS:

This study does not appear to be scientifically sound as control birds contained detectable amount of active ingredient residues in both GI tract tissues and blood. It is interesting to note, that only the control group and the highest concentration group (1500 ppm) had detectable residues in their blood tissues.

Birds in the 150 and 1500 ppm had significantly reduced feed consumption which most likely led to the significantly lower body weights from the control group. In addition, these two concentration groups exhibited the most abnormal behaviors that would indicate toxicity.

Overall, the higher the concentration of the active ingredient consumed the higher the residue in the GI tract tissues, skin and fat tissues and blood tissues. However, the amount of a.i. consumed was not directly correlated with the amount of residue found in the tissues, as some birds had very little residues where others contained a greater amount for the same concentration level.

Based on the known toxicities of this chemical's degradates, it might have been more pertinent to test for residues of the degradates in the tissues.