438870-07

DATA EVALUATION RECORD
SIMULATED FIELD (PEN) TEST

1. **CHEMICAL:** PIRATE™ PC Code No.: 129093

2. **TEST MATERIAL:** AC 303,630 3SC Purity: 30.83% ai

3. **CITATION**

Author: Md. S. Ahmed and Dr. J A. Gagne
Title: A Simulated Field (Pen) Test with PIRATE Insecticide-Miticide 3SC Using Northern Bobwhite (*Colinus virginianus*)
Study Completion Date: December 21, 1995
Laboratory: Genesis Laboratories, Inc. Wellington, CO
Sponsor: American Cyanamid Company, Princeton NJ
Laboratory Report ID: 94011
MRID No.: 438870-07
DP Barcode: D222690

4. **REVIEWED BY:** John Eisemann, Wildlife Biologist, EEB, EFED

Signature: John D. Eisemann **Date:** 7/30/96

5. **APPROVED BY:** Ann Stavola, Head of Section (5), EEB, EFED

Signature: Ann Stavola **Date:** 9/13/96

6. **OBJECTIVES:**
To determine if exposures to residues of AC 303,630 resulting from typical applications of PIRATE 3SC in cotton cause mortality or intoxication in Northern Bobwhite.

7. **CONCLUSIONS:**

NOTE: This review incorporates data from both the interim report (MRID #434928-14) and the current final report (MRID # 438870-07).

The experimental design limits the information that can be used from this study. The most valuable information is the residue data on plant tissues and soils. Information concerning avian mortality, behavior, weight change, food consumption, and residue concentrations in avian tissues should not be used. Some of the endpoints assessed by this study are better evaluated using the American Cyanamid study 'Dermal Toxicity Study with AC 303,630 3SC in Northern Bobwhite (*Collinus virginianus*) (MRID #434928-14).

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: This is not a required study.

C. Repairability: No additional information is required.

9. METHODS

Site Description:
The 44 hectare study site was located in an area typical of cotton cultivation in central Louisiana. The site was a level field and consisted of silty loam soil (10% sand, 76% silt and 14% clay). Crops grown near the study site included cotton, soybeans and sorghum.

History of Pesticide Usage on the Study Site:
Information on pesticide usage, application rates, timings and tillage were listed for the 4 four years prior to the test. Conventionally tilled cotton was grown exclusively except that sorghum was grown just prior to the test.

Agronomic practices:
Stonville LA887 Cotton was planted on April 20, 1994. Warner 907 Sorghum was planted in the adjacent fields on April 23, 1994. Planting and initial fertilizer application was performed by the cooperating farmer where upon the management of the field was turned over to Genesis Laboratories.

Test Substance Mixing, Application and Concentration Verification:
The test substance (AC 303,630 3SC) was mixed directly in a tractor-mounted spray tank prior to application. Three applications were made at 0.35 lbs ai/acre at 7 day intervals beginning on June, 15 1994 (10 weeks after planting). A minimum of one pre- and post-application 500 ml sample was collected from the spray tank and stored in glass containers except for application number 1 when plastic containers were used for storage. Duplicate samples were collected and stored in plastic containers to compare concentrations between glass and plastic. Individual applications were made to the treated field three times over a two week period.

Spray cards were placed within the treated and untreated cotton, within the border region between cotton and sorghum and in the sorghum, 25 feet away from the cotton. Cards were placed at three heights, ground level, mid-canopy, and top-canopy.

Sampling Methods for Soil, Cotton Foliage and Sorghum Foliage: Duplicate samples of soil and cotton and sorghum foliage were taken immediately following applications. Foliage was clipped or manually removed from the plant. Soil was collected using an AMF Auger. Samples were stored in two zip-lock bags and frozen until analysis.

Test System:
Northern Bobwhite, twenty-one weeks old, were obtained from Barrett's Quail Farm, Houston, TX. The birds were de-beaked and held for three days prior to the 14 day acclimation period. After veterinary examination, the birds were introduced to the test pens the same day as the last application and held in the exposure pens for 21 days. Supplemental feed (Ranch-Way Turkey and Game Bird Grower) and water was provided *ad libitum* and located in both ends of the pens. Body weights and food consumption were recorded at weekly intervals. Gross necropsy was performed on birds which died during the test, as were 4 survivors taken from each pen at the conclusion of the exposure period.

Both the acclimation and exposure pens were 10 x 50 x 6 feet in dimension, and were equipped with accepted predator protection. They were situated in the field to represent high, low and control treatment levels. The low treatment group was placed in the adjacent sorghum field , 25 feet from the cotton, and represented residues resulting from spray drift. The control and high treatment pens were constructed with one half of the pen covering cotton and the other covering sorghum. The high treatment pen received three applications (0.35 lbs ai/acre) of the test solution at weekly intervals for the three week prior to beginning the exposure period. The birds were introduced into the exposure pens the same day as the last application period.

Daily observations were made for utilization of the study pen cover types, evidence of intoxication and environmental conditions. Three times a day during the 21 day exposure period the perimeter of the pens were walked and the number of birds in each cover type (cotton, sorghum or border) were counted as an indicator of habitat utilization.

A hygrothermograph was installed at the test site to record temperature, humidity and rainfall.

Statistical Analysis:
Body weight, food consumption, mortality and residue levels were compared between treatment by ANOVA. Dunnett's means comparison test was used if significant effects were observed in the ANOVA.

GLP:
Guidelines as specified by the U.S. EPA's Good Laboratory Practice Standards 40 CFR Part 160 were followed.

Quality Assurance:
EPA GLP Standards and SOP's of the test facility were followed. The Quality Assurance Unit of the test facility will conduct inspection and provide timely reports to the Study Director and the Study Director's management.

10. DEVIATIONS

No reported deviations impacted the integrity of this study.

11. RESULTS:

Twenty-six pesticides were used on the study site from 1991 to 1994, totaling of 66 application periods.

Calculated application rates showed the actual application rates were 8.03, 7.95, and 7.85 gallons per acre for the first, second and third applications, respectively. All applications were within 2% of the target 8.0 gallons per acre. Mixture concentrations averaged 77.5%, 88.1%, and 85.7% of the target 5.25 mg/L concentration for the first, second and third application, respectively. Calculated treatment rates were 0.35 lbs/acre.

Spray cards placed both in the treated cotton and at the border between the cotton and sorghum showed 100% impingement by spray droplets. Cards place 25 feet into the sorghum showed no spray droplets. Spray cards were observed to discolor when placed at the test site. No speculation was made as the effect of this discoloration.

Average body weights by treatment group were not statistically different at the start of the exposure period or between groups at 15 and 36 days. Average body weights declined in all treatment groups an average of 2.8% during the acclimation period. Average body weights increased in all treatment groups an average of 3.81% during the exposure period and 6.98% over the entire 36 day period.

The mean feed consumption for control, low dose,and high dose treatments was 15.5, 16.0, and 15.8 grams/bird/day, respectively. No statistical difference was found.

Observational data collected on usage of cover types also showed no statistical difference. Thirty-six percent of birds

in the control pens were observed in the cotton, 66% were observed in the sorghum as opposed to the high dose pens where 56% of the birds were observed in the cotton and 43% were in the sorghum.

No evidence of intoxication was recorded in the control and low dose pens. One bird in the low dose pens was unkempt which may have been treatment related.

No birds died in the control pen during the exposure. However, one bird died in the low dose pens on day 36 (the same bird which exhibited intoxication) and 2 birds died in the high dose one and three days after the beginning of the exposure period. No statistical differences were observed in mortality.

Gross pathological exam of the birds surviving to day 36 of the test showed no notable abnormalities. One bird which died in the high dose group had unusually red breast muscle. The other bird in the high dose group suffered a swollen wing near the attachment point of the patagial tag. The bird which died in the low dose group was emaciated (31% decrease in body weight) and had intestines filled with fluid and gas. A white chalky excreta was reported around the anus of this bird.

Rainfall was recorded for the test period. During the test substance application phase, days 1 to 15 (6-15-94 to 6-29-94)), 2.30 cm fell. During the exposure phase, days 15 to 36 (6-30-94 to 7-20-94) of the study, 8.03 cm fell. Historically, the 10 year average for entire months of June and July are 13.38 cm and 12.19 cm, respectively.

RESIDUES

Only three birds had detectable residues of AC 303,630 in their tissues. These birds were all in the high dose group. No residues were detected in the livers. One bird had 0.012 ppm in the skin and sub-cutaneous body fat. Two birds had detectable residues in the gastrointestinal tract (0.0802 and 0.0726 ppm). Recovery of AC 303,630 from fortified liver and GI tract samples was 103.4% and 100.2%, respectively.

Table 1. Mean AC 303,630 residue concentrations detected on cotton and sorghum foliage and soil. Day 1 represents the first application period. Day 15 represents the third application. Day 36 represents the end of the exposure period.

		Control	Low Dose	High Dose
Cotton Foliage (ppm)	Day 1	n/a	ND	83.7
	Day 15	<0.5	ND	65.0
	Day 36	<0.5	ND	1.75
Sorghum Foliage (ppm)	Day 1	n/a	n/a	<0.5
	Day 15	<0.5	1.63	2.25
	Day 36	<0.5	<0.5	4.65
Soil (ppb)	Day 1	n/a	n/a	154
	Day 15	<10	<10	380
	Day 36	<10	<10	251

n/a - Data not available due to technician error.
ND - No Data (Only sorghum was planted in the low dose pens.)

12. REVIEWER'S COMMENTS

Problems with the study design exclude the use of much of this data. The most useful portion of this study is the plant tissue and soil residue data. It can be used to support residue data in a prior small scale exposure study.

The protocol provided states in section 15.4.1 "Bobwhite will not have the test substance applied directly to them, they will be forced to utilize the treated cotton and thereby be exposed to the residues of AC 303,630". The nature of the design does not 'force' the birds to utilize the cotton. The birds were always presented with the opportunity to stay in the untreated sorghum. To meet the stated objective using a total of nine pens, the 'High Dose' pens should have been located entirely within the treated cotton and 'Control' pens should have been located entirely within the untreated cotton.

Due to the density of birds in the test pens, supplemental feeding is recommended. The provision of untreated feed may lead to preferential foraging on the supplemental feed and reduce the impacts of the chemical at normal application rates. EPA Pesticide Assessment Guidelines: Subdivision E, Section 71-5, p 63, details methods utilized to eliminate this bias. It

is suggested that feed provided be treated with the chemical at the anticipated residue levels from normal application procedures. This can be accomplished by pre-mixing the diet to expected levels. These levels can be determined from previous application data if available. If that information is not available feed could be spread in a thin layer in the field prior to application and collected after application and provided for the birds. No mortality was observed throughout the test. In fact the mean body weight of birds increased in all treatment groups. This can be explained by the availability of clean feed *ad libitum* throughout the study.

Removing a portion of the bird's beak two weeks prior to test initiation could severely impact the birds natural foraging ability. This makes it difficult to predict natural exposure based upon this data. It might in fact lead to increased foraging on the easily obtained supplemental feed.

An average of 83.7 ppm was reported on cotton foliage in the high dose treatment pens after one treatment. This value is 1.8X the concentration predicted by Fletcher (1994).

Residues were not detected on the sorghum in the high dose pen after the first application indicating little deposition from drift. Fletcher estimated residues from direct application to long grass at 38.5 ppm (at 0.35 lbs/acre). Assuming 1% drift from ground boom application, residues resulting from spray drift in the adjacent sorghum would be 0.68 ppm (38.5 ppm x 1.7 x 0.01) which is slightly above detection limits. Residues increased more than expected on days 15 and 36. Sorghum in the low dose pens, 25 feet from the treated field, received little detectable active ingredient.

REFERENCES

Fletcher, J.S., J.E. Nellessen and T.G. Pfleeger. 1994. Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, an instrument for estimating pesticide residues on plants. Environmental Toxicology and Chemistry, No. 9, pp 1383-1391.

Author: Rick Petrie
Date: 10/07/96 07:06 AM
Priority: Normal
TO: Bill Evans
TO: Nicholas Mastrota
CC: Michael Davy
CC: David Bays
CC: William Rabert
CC: Andrew Bryceland
CC: Laura Dye
Subject: Re[2]: Issue with vegetative vigor test

---------- Message Contents ----------

Nick, Bill,

To respond, on page 39 of Subdivision J, I quote "The phrase [the maximum label rate] means the maximum recommended amount of active ingredient in the recommended MINIMUM quantity of carrier such as water to be used per land area." While this definition is in the Tier I section, I believe that it carries over to the Tier II testing when they test up to the maximum label dosage. The recommended minimum quantity of carrier would be stated on the label.

Rick P.

Nick,

I appreciate the information. As I read the DER, I found nothing in the Guideline Criteria indicating how much water should be applied. I believe that even if our current guidelines (i.e the SEPs) don't address this issue, we should still list it in the DER and point it out as a deviation. It is only through your note that I am made aware of this problem.

How much water should be applied with the test material? Shouldn't we point out to how much water should have been used to Springborn?

---------- Reply Separator ----------

Subject: Issue with vegetative vigor test
Author: Nicholas Mastrota at dcopp1
Date: 10/3/96 9:57 AM

PRET,

Attached is a DER I did for a formulated product containing cyclanilide, a new plant growth regulator. As explained in my previous method, I am calling the study invalid because the test material was applied with way too much water (approximately 900 gallons/A). There is not time for me to wait for the next PRET meeting, so I would appriciate any comments/input from the PRET team via cc:Mail. If you have time, please review the Conclusions, Rationale, and

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Reviewer's Comments sections of the DER. Thanks.

This study was conducted by Springborn Laboratories. You should be on the lookout for similar problems with other vegetative vigor studies done by this lab.

Nick