9/1/94

MRID No. 421449-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorpyrifos
Shaughnessey Number: 059101

2. **TEST MATERIAL:** Chlorpyrifos (Pyrinex 4E) 48.9% purity (Batch No. B23-11) liquid emulsion

3. **STUDY TYPE:** 71-5(a) Avian Simulated Field Study (Large Pen)
Species Tested: Bobwhite Quail (Colinus virginianus)

4. **CITATION:** Booth, Gary M. 1989. A simulated field study (using large pens) on the effect of Pyrinex 4E (Chlorpyrifos) on bobwhite quail. Study performed by Environmental Labs Inc., Springville, Utah. Laboratory Project ID. ELI/MAA-88. Submitted by Makhtashim-Agan (America) Inc. EPA MRID No. 421449-03.

5. **REVIEWED BY:**

William S. Rabert, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)
Signature: William S. Rabert
Date: September 1, 1994

6. **APPROVED BY:**

Dan Rieder, Section Head
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)
Signature: Dan Rieder
Date: 9-1-94

7. **CONCLUSIONS:** Thirty-day exposures to two applications of 3 lbs ai/A (two weeks apart) or one application of 6 lbs ai/A of chlorpyrifos on turf and ton seed treated at about 12 and 30 ppm, respectively, produced no statistically significant effects on bobwhite quail brain Ache levels, body weight, brain/body weight, liver/body weight crop/body weight, gonad/body weight, eggs/pen and eggs/bird day. Of the 6.2 % mortality occurring in the controls, 75 % died in the last five days of the study suggesting some source of stress. These control deaths interfere with interpretation of lethal effects in treatments. Only 29 % of the 10 % mortality in the 6-lbs ai/A treatment died during the same 5-day period. Statistically significant number of birds in the 6-lbs ai/A treatment showed behavioral deficits. The 3 + 3-lb ai/A treatment birds did not show significantly more abnormal behavior compared to the control. This study indicates a LOEL is 6 lbs ai/A and NOEL is 3 lbs ai/A for Pyrinex 4E use on turf for bobwhite quail.

An abbreviated review of this study indicates that the study is scientifically sound and would fulfill a guideline requirement for a simulated terrestrial field, if required.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** N/A

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bobwhite quail (Colinus virginianus) used in the study were pen-reared stock purchased from Oakridge Game Farm, Gravette, Arkansas. The birds were acclimated in the large pens constructed on turf on site of a turf farm in Goshen, Utah for 15 days prior to initiation of the test. The birds were adults at the initiation of the test.

B. **Dose/Diet Preparation/Food Consumption:** Turf in the pens were sprayed with two applications of 3 lbs ai/A or one application of 6 lbs ai/A and the seed diets were prepared by mixing chlorpyrifos directly into the feed. Chlorpyrifos was sprayed by a hand sprayer at the rate of 1 gallon of water/1000 ft^2 with treatments of 0, 3 or 6 lbs ai/A to each pen on June 11, 1988. The pens treated at 3 lbs ai/A were sprayed again on June 25. The test ended on July 11, 1988, when all the birds were sacrificed.

The control diet and two test concentrations (12 and 30 ppm) were prepared at the beginning of the treatment. After preparation, the diets were spread on six wire-meshed-covered trays on the roof of a building and weathered after the initial drying. Seeds used in the 3 lbs ai/A treatment were retreated at the end of 14 days corresponding to the second spray treatment.

The basal diet for adult birds was Gro-scratch (9% protein contained wheat, milo, sorghum, and cracked corn) a commercial feed obtained from Intermountain Farmers Association, Provo, Utah. Food and water were provided daily. After the spray treatment, 640 g of seed was scattered onto the turf in each pen using a Whirley-Bird applicator. The birds were then released into their respective pens.

Samples of turf and treated seed were sampled for chlorpyrifos residue concentrations according to a

2

randomized sampling schedule. For the 6 lbs ai/A treatment, samples were collected at 0 (immediately after treatment), 1, 2, 3, 4, 5, 6, 10, 20, and 30 days post-treatment. For the 3 lb ai/A treatments, samples were collected at 0 (immediately after treatment), 1, 2, 3, 4, 5, 6, 10 and 14 days after the first treatment and collected at 0 (immediately after treatment), 1, 2, 3, 4, 5, 6, 10, 14 and 16 days after second treatment.

Homogeneity and stability samples were taken from the treatment diets (16.5 ppm and 33 ppm) and spray treated turf in each pen at specific intervals. Analyses were performed using gas-liquid chromatography.

C. **Design:** The bobwhite quail were randomly assigned to 28 pens (50 x 10 X 6 feet high). The pens for the three groups were placed in a randomized block design.

Chlorpyrifos Nominal Concentration	Number of Pens	Birds Per Pen Males	Females
Control (0 ppm)	9	8	8
3 lb ai/A twice + 16.5 ppm	9	8	8
6 lb ai/A once + 33 ppm	9	8	8

Extra replacement birds were held in a 28th pen.

D. **Pen Facilities:** Adult birds were housed outdoors in large pens constructed of hardware cloth over turf areas. Pens measured 50' x 10' x 6' high. Each pen had a 3' x 6' piece of plywood placed on the windward (south-side) of each pen to reduce violent wind exposure. Roof-top shading was provided in one end of each pen using a nylon mesh.

E. **Adult Observations/Gross Pathology:** Observations were made daily for each pen during the application period for signs of toxicity including: behavior, mortality, and egg laying. Gross pathological examinations and measurements were conducted on all birds at the end of the study. At the end of the test, all birds were individually weighed and sexed, and the brain, liver, crop, and gonads were removed and weighed. A total of 271 brains were immediately processed for Ache analysis, while 113 brains (by block) were frozen and analyzed for Ache the next day.

F. **Statistics:** The General Linear Model was used (RUMMAGE Software) to analyze the following response variables:

Eggs/pen, eggs/bird days, mortality/ pen by sex, Ache, final body weight, brain weight/body weight, liver weight/body weight. crop weight/body weight, gonad weight/body weight. Least square means were obtained with the above model. Behavioral deficits were analyzed using the binomial distribution.

12. **REPORTED RESULTS**:

A. **Residue Analyses on Grass and Seed**:

Analyses of the Gro-scratch treated with Pyrinex 4E on day 0 indicated 30 ppm (91% of 33 ppm theoretical level) on seeds treated at 6 lb ai/A and 12 ppm (73 % of 16.5 ppm theoretical level) following the first treatment at 3 lb ai/A. Chlorpyrifos residues on seeds changed little over the 7-day sampling period indicating the photodegradation was minimal.

Analyses of chlorpyrifos residues in grass samples taken from 6 lb ai/A treatment pens ranged from a mean average of 903 $\pm$ 310 ppm on day 0 to an average of 9 $\pm$ 8 ppm on day 30 post-treatment. It is of interest that the half-life of the day 0 residues was less than 24 hours, but the half-life of the day 1 residues was greater than 3 days. Chlorpyrifos residues in grass samples taken from the 1st and 2nd applications from the 3 + 3 lb treated pens ranged from 306 $\pm$ 95 ppm on day 0, to a mean average of 18 $\pm$ 8 ppm on day 14 for the first application and from 361 $\pm$ 167 ppm on day 0, to a mean average of 38 $\pm$ 24 on day 16 for the second application. The dissipation pattern was generally the same for the 3 lb ai/A treated pens.

Treatment of turf with 3 versus 6 lbs ai/A would be expected to yield a 2-fold difference in residue levels on grass (6 lbs/3 lbs = 2X). However, comparison of the initial chlorpyrifos levels on grass for 3 and 6 lbs ai/A (306 ppm versus 903 ppm) shows a 3-fold difference rather than the predicted 2-fold difference.

B. **Adult Mortality and Behavioral Reactions**: Control mortality was 8 females and 1 male (6.25 % of the 144 control birds) with 66.7 % of the deaths occurring during the last five days in the 30-day study (i.e., July 7 and 11). Mortality in the 3 + 3 lb ai/A was 8 females and 3 males (7.6 %) with 82 % of the deaths occurring during the same 5-day period, July 7 and 11. Mortality in the 6 lb ai/A treatment was 9 females and 5 males (9.7 %) with only 29 % occurring during the

last 5 days. 71 % of the deaths occurred between June 17 and July 4 preceding the period of most deaths in the controls and 3 + 3 lbs ai/A treatment from July 7 to 11. Mortality was higher in females (11.57%) than males (4.17%).

The death of one bird in the 6-lb ai/A treatment group was video-taped and showed many symptoms of Ache depression. This bird also had the lowest AChE level of any bird that died (i.e., 4.384 umoles/min./g). Mean cholinesterase levels in dead birds were 6.13 umoles/min./g in controls, 5.78 (6 % AChE reduction) in the 3 + 3 lbs ai/A treatment, and 5.66 (8 % AChE reduction) in the 6 lbs ai/A treatment. Reduction in AChE levels showed a dose-response pattern. Ache levels in dead birds did not show any pattern of decrease during the test.

A total of 1 male (3 + 3-lbs ai/A) and 1 male and 5 females (6-lbs ai/A) exhibited behavioral deficits. Controls showed no abnormal behaviors. Behavioral deficits were statistically significant ($P > 0.05$) in the 6-lb treatment. Description of the abnormal behavior were not reported.

C. **Adult Body Weight and Food Consumption**: Mean body weights 30 days after treatment for controls, 3 + 3 and 6 lb ai/A treatments were 189.7, 188.3, and 183.5 grams, respectively. The average losses of body weight for the control, 3 + 3, and 6-lb ai/A treatments were 12.01, 13.14, and 18.15 grams, respectively. Losses in adult body weights show a dose-response relationship, but the differences compared to controls were not statistically significant.

Comparisons of some organ weights to body weight were analyzed for toxic effects. Statistically significant differences from controls were not found for brain/boy weight, liver/body weight, crop/body weight, and gonad/body weight. No pattern of toxicological effect on organ weight/body weights was seen for any of these four endpoints.

Food consumption was not measured, because the seeds were scattered onto the turf where the birds had to search for it and the seeds could not be recovered to determine the amount eaten.

D. **Reproduction**: The only measure of reproduction potential measured in this study were eggs/pen and

eggs/bird. Statistically significant differences from controls were not found. The mean number of eggs/pen for controls, 3 + 3 and 6 lb ai/A treatments were 15.79, 20.448, and 16.893, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

"Under the conditions of these experiments, Pyrinex 4E sprayed on turf at 3 + 3 lbs ai/A (two treatments 2 weeks apart) and 6 lbs ai/A and on seed at about 12 and 30 ppm chlorpyrifos respectively, did not significantly affect bobwhite quail mortality, brain AChE levels, body weight, brain/body weight, liver/body weight, crop/body weight, gonad/body weight, eggs/pen, and eggs/bird day. However, there was a significant number of birds (total of 6) in the 6-lb ai/A treatment that showed behavioral deficits. The 3-lb ai/A treatment birds did not show significantly more abnormal behaviors compared to the control."

"Based on these data, it can be concluded that application of Pyrinex 4E to turf at 3 and 6 lbs ai/A will not be expected to have deleterious effects on grazing bird populations which have feeding behavior similar to bobwhite quail."

14. **Reviewer's Discussion and Interpretation of the Study:**

A. **Test Procedure:** The test procedures appeared to be acceptable, but questions were raised in the following areas:

Spray application of chlorpyrifos on turf resulted in highly variable levels on grass between pens within each treatment. For example, chlorpyrifos applied to grass at 3 and 6 lbs ai/A initially ranged from 170 to 470 ppm (a 2.8-fold difference) and from 440 to 1400 ppm (a 3.2-fold difference), respectively. These residue variation within treatments were greater than the 2-fold difference between 3 and 6 lbs ai/A treatments.

The predicted initial residue level on short grass using the monograph value of 240 ppm/lb ai/A would be 720 ppm and 1440 ppm for 3 and 6 lbs ai/A treatments, respectively. These predicted values approximated the highest residue levels measured in both treatments. But, the mean measured residues on grass were only 42 and 63 % of nominally predicted levels, respectively.

Chlorpyrifos was measured on turf in the controls as high as 3 ppm. The presence of chlorpyrifos in the controls complicates the measurement of AChE inhibition comparison with birds from treated areas.

Mortality occurring in the controls, especially during the last five days of the study, suggest that the control birds were stressed. It should be noted that many birds died in the last five days of the study and that deaths occurred in contrrols and all treatments the day the study ended. There was no apparent cessation in deaths at the end of the study.

Control deaths make the interpretation of mortality in treatments difficult. Since most deaths in the low treatment also occurred during the last five days, the deaths may have been due to the same stress as in the controls. But, since only 29 percent of the dead birds in the high treatment died during that 5-day period, the earlier deaths might be attributed to chlorpyrifos. Evidence of AChE-type syptoms were video-taped of one bird that died in the 6-lbs ai/A treatment, which suggests that chlorpyrifos may have caused its death.

Omitting all deaths occurring during the last five days, a statistical analysis may show statistically significant differences between the 10 bird deaths in the 6 lbs ai/A treatment and the 3 control deaths. If so, the NOEL would remain 3 lbs ai/A and mortality would be added to abnormal behavior as a statistically significant effect compared to controls. As noted above, more female birds than males died in all groups. Consequently, adverse effects on populations might be greater because of the higher female losses.

The study did not include description of the abnormal behavior reported in the study. Knowledge of these abnormal behaviors may give some insight into their possible causes and likelihood for survival and their ability to successfully breed and reproduce in the wild. These observations should submitted.

B. **Statistical Analysis:** Statistical analyses of the study parameters were not checked by the reviewer in this abbreviated review.

C. **Discussion/Results:** The only statistically significant effect reported in the study was abnormal behavior in the 6 lbs/A treatment group. Hence, the NOEL and LOEL values for the study are 3 and 6 lb ai/A, respectively,

7

for Pyrindex 4E sprayed on turf. If the mortality occurring during the last five days of the study were deleted from all groups, the mortality that occurred in the 6 lb/A treatment group may also be statistically significant. Descriptions of abnormal behavior cited in the study should be submitted to assess possible effects on survival and reproduction in the wild.

The study is scientifically sound and might fulfill the guideline requirement for a simulated terrestrial field study with bobwhite quail, if a simulated field study were required for turf uses.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental

(2) **Rationale:** A simulated field study for bobwhite quail has not been required for any uses. And descriptions of the abnormal behavior should be submitted to assess possible effects in the wild.

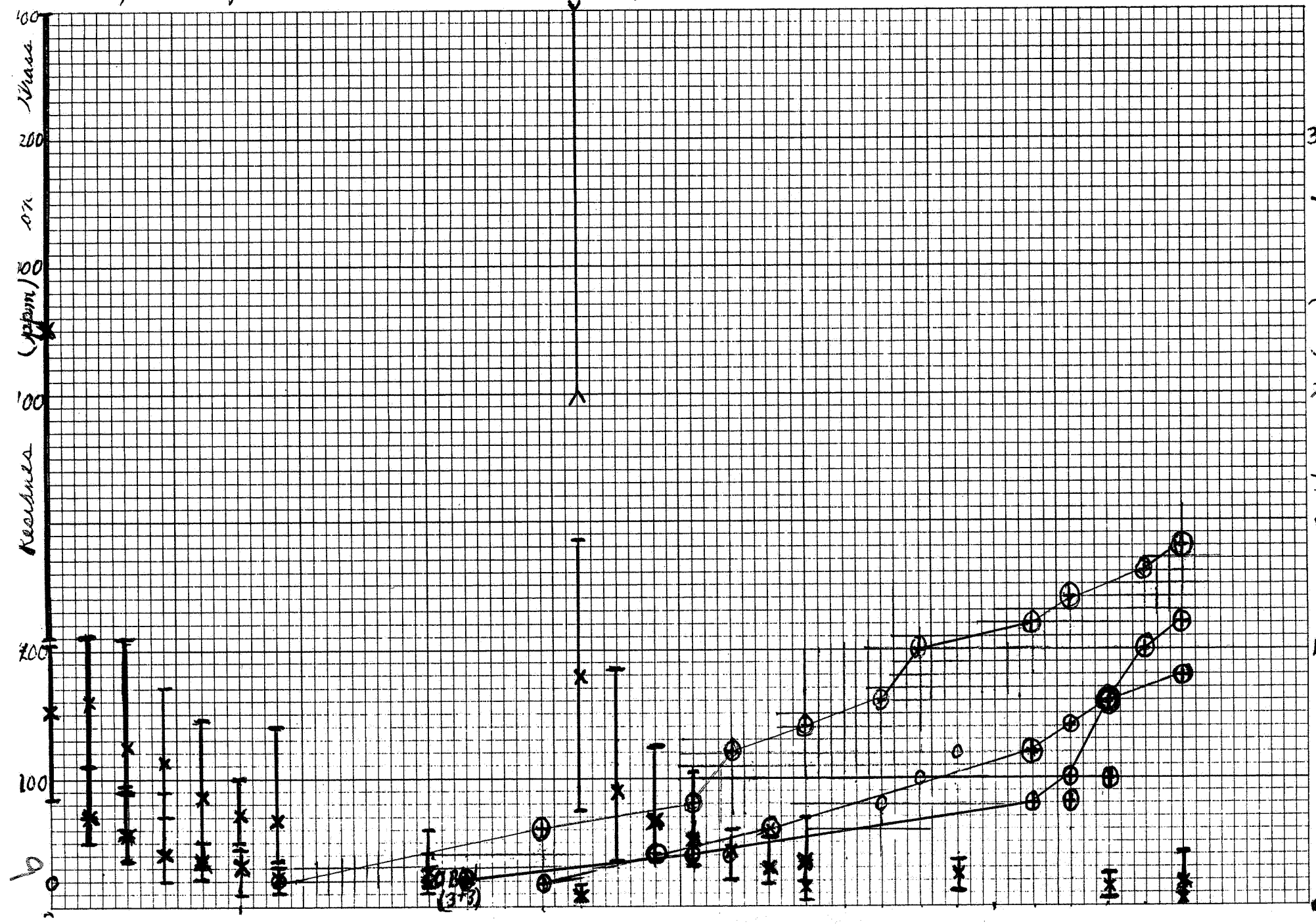
(3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER:** N/A

Controls
6-lb ai/A
3+3-lb ai/A
× Mean; |×| Range of values

FPI-MI -10 X 10 TO 1 INCH
10TH LINE HEAVY

Chlorpyrifos – Bobwhite Quail
Large Pen – Simulated Terrestrial Field Study
Second Application



Page____ is not included in this copy.

Pages 10 through 17 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.