

CASE GS0103

PHORATE

PM 300 08/27/82

CHEM 057201

Phorate ( O,O-diethyl S-(ethylthio)met

BRANCH EEB

DISC 40 TOPIC 05103542

GUIDELINE 40 CFR 163,71-5

FORMULATION 04 - GRANULAR

FICHE/MASTER ID 00074624

CONTENT CAT 01

Fink, R.; Beskid, J.C. (1981) Final Report: Simulated Field Study--  
Bobwhite quail; Project No. 130-131B. (Unpublished study re-  
ceived May 21, 1981 under 241-257; prepared by Wildlife Inter-  
national Ltd., submitted by American Cyanamid Co., Princeton,  
N.J.; CDL:245263-C)

SUBST. CLASS = S.

DIRECT RVW TIME = 1 (MH)

START-DATE

5/23/83

END DATE

5/23/83

REVIEWED BY:

Ann Stauda

TITLE:

Aquatic Biologist

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5/23/83

APPROVED BY:

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DATE:

Data Evaluation Record

1. Chemical: Phorate

(0,0-diethyl) S-[(ethylthio) methyl]  
phosphorodithioate

2. Formulation: Phorate 20% a.i. granule  
(Thimet - 20 G)

3. Citation: Beskid, J.C. and R. Fink. 1981. Simulated  
field study - Bobwhite Quail. Final Report.  
Project number: 130-131 B. Unpublished report  
by Wildlife International LTD., for American  
Cyanamid, Princeton, N.J. In support of  
Registration #241-257. Under Accession #245263,  
received 5/21/81. Exhibit 2.

4. Reviewed By: John J. Bascietto  
Wildlife Biologist  
EEB/HED

5. Date Reviewed: July 6, 1981

6. Test Type: Avian simulated field study

a. Test Species: Bobwhite quail, Colinus virginianus

7. Reported Results: "Based on the results of this 14 day ...study,  
Thimet 20G used at a rate of 12 oz. per 1000 row ft. at cultivation  
may result in quail mortality. Irrigation following application may  
reduce the mortality, but due to extremely hot and arid conditions  
existing during the study, the effect of irrigation could not be  
thoroughly evaluated."

8. Reviewer's Conclusions: The study is scientifically sound and <sup>does</sup> could  
fulfill the Proposed Guidelines requirement for simulated acute avian  
field testing only [as per 163.71-5 (a) (1)] if residue data for  
vegetation and soils are submitted. <sup>Residue data submitted and accepted  
(no. # 245263)</sup>

Irrigated and non-irrigated corn fields treated with phorate at 2x  
the label rate at cultivation pose an acute hazard (mortality) for  
representative game bird species utilizing them for feeding and cover.  
Reproductive hazards are unknown.

Irrigation immediately following application does not affect a  
significant reduction in the acute hazard for the "at cultivation"  
treatment (18" height of crop).

## 9. Materials/Methods

### A. Test Procedures:

Quail used were hatched at the testing facility, (Wildlife International Ltd., St. Michaels, Md.) and reared in flight pens. The birds were approximately 8 months old when tested and indistinguishable from wild quail. Birds were wing-clipped to prevent escape.

<u>Design</u>	<u>Exposure</u>	<u>#of Birds</u>		<u>Plot size</u>
		<u>Male</u>	<u>Female</u>	
Plot 1 (Control)	none	15	15	15,000 ft <sup>2</sup>
Plot 2 - Thimet 20 G (Irrigated)	12 oz. per 1000' row	15	15	15,000 ft <sup>2</sup>
Plot 3 - Thimet 20G (Non-Irrigated)	12 oz. per 1000' row	15	15	15,000 ft <sup>2</sup>

### Site

The test was conducted on 3 plots on the border of a 56 - acre field used for soybean production in 1979. It had not been previously used for investigation. Site was prepared for corn by discing, chisel plowing to 12" and spring tothing immediately prior to planting.

The site was not subjected to the standard pre-emergence treatment for Maryland (for control of corn cutworms, and for weed control) to avoid complications with interpreting results of multi-chemical exposure.

Planter - a John Deere 7000 six - row corn planter, was used for planting. Corn was planted 1" deep; in-row spacing was 7"; seed population was 30,800 per acre., rows were on 30" centers.

### Planting -

Site was planted in Dekalb 43-A seed corn (Captan treated) on July 30, 1980 at 10:30 a.m.. Turn rows were planted first, then long rows.

### Cultivation and application -

The cultivation of test plots 1, 2, and 3 was completed on August 27, 1980 by 10:30 AM. Corn was approximately 18" high. A six row cultivator-mounted Gandee Granular applicator applied Thimet 20 G in a 2" band at the base of the corn plants on both sides of the row. Soil incorporation was accomplished by a trailing shovel (1" incorporation).

Irrigation - Immediately followed cultivation and Thimet 20G application on Plot 2 only. Used 0.5" of water from an overhead sprinkler system.

Pens - 20 gauge galvanized wire fencing strung between 8' x 4" x 4" wooden corner posts sunk 3' into ground. Wire was supported, 20' by 6' steel "U lug" fence posts. 12" at the bottom of each side was turned out 90° and covered with 6" of dirt so as to discourage predators from burrowing under the pens. At the top of the fence (36" high) was a single strand of electrified wire to discourage climbing predators.

Cover -

Control and Thimet 20G test plots measured 50' x 300' (15,000 ft<sup>2</sup>) and contained a cover area along one border to provide a natural quail cover. This border measured 50' x 20' and cover was alfalfa and natural grasses (18" high at beginning of test). Rest of plot was planted in corn on 30" centers. The twelve turn rows bordered on the cover area.

Birds

Ninety (90) quail (15 cocks and 15 hens in each test plot were introduced into the control, irrigated Thimet 20 G and non-irrigated Thimet 20 G test plots at 5:45 p.m. on August 27, 1980.

Observations and Measurements

Mortality, Toxic Symptoms, Behavior - quail were observed daily for signs of toxicity, symptoms of cholinesterase inhibition, abnormal behavior, and foraging activity. Transects of each plot were walked daily. Records of quail and wild bird mortality were made daily. Foraging activity was quantitatively evaluated five(s) times per day on tilled portions of plot only.

Climate - climatological conditions, including high and low temperatures, sky conditions, and precipitation were recorded daily.

Body weight - individuals body weight's were recorded at initiation and on Day 14 (but apparently no identification of individual birds was made).

Brain Cholinesterase - determinations (by a modified Ellman Method) were made on twenty (20) animals (10 male, 10 female) from the control and each test plot on Day 14. (Ellman method in files).

Residues - Samples of whole corn plants were taken on Days 0, 7, and 14. Samples of soil to 4" depth on row centers were taken on Days 0, 7, and 14. Bird tissue was taken on Day 14 from the twenty (20) carcasses used in cholinesterase determinations. Corn and soil samples were frozen and sent to the Registrant for storage. Bird tissue residues were analyzed by the Registrant by method M-0163, (not submitted for review). Soil samples were stored frozen but were not analyzed (pers. comm., R. Barron, American Cyanamid).

## Bird Maintenance

Five - gallon, vacuum fed, galvanized waterers were used in the "cover" areas. Birds had access to water ad libitum. Birds were fed exclusively off naturally occurring foods in plots through Day 3. On Day 4 a mixed grain supplement (sunflower seed, wheat, cracked corn, millets, and sorghums) was provided in turn rows as needed to insure nutritional character of birds.

B. Statistics - none presented although arithmetic "means" were calculated on daily foraging indices and "statistical significance" was referred to in the Brain cholinesterase section. No statistical analysis of mortality was performed.

## C. Results and Discussion

Adaptation to test Plots - birds adapted satisfactorily exhibiting normal wild bird behaviors including foraging, dusting, territoriality and aggression.

Climate - average high temperature was 90°F, average low was 69°F. 1.76" of rain fell during the study period with the most occurring on Day 9 when 1.42" was recorded.

## Quail Mortality

Control - 1 male and 1 female found dead on Day 6.

Thimet (irrigated) - one male, day 1; one female, Day 4; one male, Day 6; one male (skeleton) Day 9; two birds (decomposed-dead for 3 days), Day 11; totals 6/30 dead.

Thimet (non-irrigated) - one female, Day 2; one female, Day 3; one male, Day 5; 4 males plus 1 female, Day 6; one male, Day 9; Total = 9/30 dead.

At the end of the study four (4) for control birds, four (4) (irrigated) treatment birds and eight (8) (non-irrigated) treatment birds were unaccounted for.

## Behavior -

Depression was observed on Day 2 through 5 among both treatment plots.

Foraging - no adverse effects observed.

Body weight - normal values observed. More weight loss observed for controls than for for treatment groups (mean weights).

## Brain Cholinesterase

Depression on treated areas was not "statistically significant" (no statistics submitted) with depression levels on Day 14 as follows (as compared to controls)

	% Depression of ACHE	
	Males	Females
Thimet 20 G (irrigated)	4	1
Thimet 20G (non-irrigated)	5	1

#### 10. Reviewers Evaluation

- A. Test Procedures - The protocol used is a substantial departure from that outlined by the Proposed Guidelines FR 43, No. 132 July 10, 1978, for "small" or "large pen" field studies. The protocol used however, was in substantial conformity to that presented by the Registrant to EEB on 6/25/80 (see memo in EEB file from J. Leitzke to J. Edwards, dated 7/2/80) but was slightly modified from that originally required by EEB in conditional concurrence with the field corn registration, (memo from Leitzke to Edwards, 4/4/80). This test corresponds to Phase II of the proposed protocol of 6/25/80.

Procedures are in substantial agreement with the proposed protocol and are acceptable to EEB, as being scientifically sound.

Note that the method of planting (considered representative of that used in 75% of American corn agriculture), was specifically observed to leave many granules on the surface. Observations required by protocol were made on the number of granules left on the surface by the planting and application methods (with soil incorporation). Results, (quantified in a separate exhibit - Exhibit 4 Accession #245263) showed that even with soil incorporation, exposed granules were plentiful, with as many as 350 granules per sq. ft. with the 12 oz. rate.

The "modified Ellman method" for brain cholinesterase determinations, is available in EEB's files, but was not validated by EEB.

The method of analyses of bird tissue samples for phorate was not submitted (method M-0163 was referred to in a separate Exhibit, but was only superficially described as employing "a 3% ov-210 column .... With a flame photometric detector on a Trace Model 550 gas Chromatograph" (P. 2 of Exhibit 5, Acc. #245263).

Since individual birds were not tagged for identification, the body weights of dead and/or sick birds are unknown.

#### Brain cholinesterase

Samples were taken on day 14 only so that the information is not useful in drawing conclusions about mortality. Investigator's claim that observed depression is "not statistically significant" is not supported by statistical analysis.

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## Residues

Only residues in quail remains were reported. Other samples were stored for future reference but not analyzed (pers. comm., Mr. R. Barron, American Cyanamid). No residues above the limits of detection (.05 ppm Phorate) were observed.

- B. Statistical Analysis - Since no statistical data was presented, no validation of statistics was performed. However, EEB's "Contingency Table" program was used to test differences between controls and treatment group mortalities. The lack of "statistical significance" attributed to the cholinesterase result is invalid since no statistics were shown.

## C. Discussion/Results

The study demonstrated that Thimet 20 G applied to corn at 12 oz per 1000' row, at cultivation (18" height) will result in a very substantial amount of acute mortality. The acute mortality may be very slightly reduced by an irrigation at the time of application (0.5" water).

The significance of the acute mortality is difficult to assess without first applying a population dynamics model to a specific use pattern. A single application to corn may not cause a significant population effect, but multiple applications are authorized and many agricultural crops are eligible for treatment, thus population exposure could be quite significant. Since mortality is demonstrated, it follows that the "significance" or extent of the population effect would be directly correlated with the exposure.

Much of the observed mortality was delayed, occurring near the middle of the test (end of first week) suggesting the possibility of dietary accumulation, rather than acute granular poisoning (many birds died on Day 6 suggesting this) - however, two (2) control mortalities occurred on Day 6. Predation must be considered here, so that the possibility of subacute dietary effects is less likely in this case.

Reproductive and other chronic hazards were not addressed by this field study.

## D. Conclusions

1. Category: <sup>"Core"</sup> Supplemental Study

2. Rationale: The vegetation and soil samples taken during this study have not been analyzed (pers. comm., Ray Barron, American Cyanamid Co.) for Phorate residues as required by the Proposed Guidelines and as intended by EEB reviewers when they agreed to the modified protocol as evidenced by their requirements for sampling of vegetation and soil.

*reproducible 1/25/82  
residue data submitted  
and is acceptable. - JPB*

3. Repair: Analyze vegetation and soil samples and submit results to EEB. Submit also, a detailed description of the methods of sampling, transportation of samples, storage of samples, and analysis of samples. Include a rationale concerning the acceptability and scientific validity of analyzing said samples at the time of analysis (specifically, how much if any degradation has occurred as a result of the delay in analysis?)

### Addendum:

If the numbers of missing quail are subtracted from the original numbers placed on the plots the percentages of dead birds are:

$$\text{control } 2/26 = 8\%$$

$$\text{irrigated } 6/26 = 23\%$$

$$\text{non-irrigated } = 9/22 = 41\%$$

Although the birds were noticed as missing at the end of the study it is unknown when they actually disappeared (probably due to predation) since daily head counts were not made. These values illustrate the not only the toxic effects of phosphate, but also the mitigating effect of irrigation.

Ann Starvo

WILDLIFE INTERNATIONAL LTD.

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TABLE II  
 QUAIL MORTALITY INCIDENCE  
 THIMET 206

DATE	CONTROL PLOT 1	IRRIGATED PLOT 2	NON-IRRIGATED PLOT 3
8-27-80 - Day 0	0/30	0/30	0/30
8-28-80 - Day 1	0/30	1/30	0/30
8-29-80 - Day 2	0/30	0/29	1/30
8-30-80 - Day 3	0/30	0/29	1/29
8-31-80 - Day 4	0/30	1/29	0/28
9-01-80 - Day 5	0/30	0/28	1/28
9-02-80 - Day 6	2/30	1/28	5/27
9-03-80 - Day 7	0/28	0/27	0/22
9-04-80 - Day 8	0/28	0/27	0/22
9-05-80 - Day 9	0/28	1/27	1/22
9-06-80 - Day 10	0/28	0/26	0/21
9-07-80 - Day 11	0/28	2/26	0/21
9-08-80 - Day 12	0/28	0/24	0/21
9-09-80 - Day 13	0/28	0/24	0/21
9-10-80 - Day 14	0/28	0/24	0/21