CASE GS0103  PHORATE  PM 300  08/27/82

CHEM 057201 Phorate (O,O-diethyl S-((ethylthio)methyl)

BRANCH EEB  DISC 46 TOPIC 05103542  GUIDELINE 40 CFR 163,71-5

FORMULATION 94 - GRANULAR

FICHE/MASTER ID 00974623  CONTENT CAT 01

Bobwhite Quail: Project No. 130-131A. (Unpublished study re-
ceived May 21, 1981 under 241-2571 prepared by Wildlife Inter-
national Ltd., submitted by American Cyanamid Co., Princeton,
N.J. # CDL:245263-8)

SUBST. CLASS = 8

DIRECT HWV TIME = 1 (MH) START-DATE 5/23/83 END DATE 5/23/83

REVIEWED BY: Ann Hovdland
TITLE: Animal Biologist
ORG: CMA 1977 377 780

SIG/TEL: Ann Hovdland
DATE: 5/23/83

APPROVED BY:

SIG/TEL:

DATE:
DATA EVALUATION RECORD

1. Chemical: Phorate (0,0-diethyl) S-[(ethylthio)methyl] phosphorodithioate

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


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

5. Date Reviewed: July 1st, 1981

6. Test Type: Avian simulated field study (acute)
   a. Test Species: Bobwhite quail, Colinus virginianus

7. Report Results: "Based on the results of the 14-day simulated field study, Thimet 20 G used at a rate of 12 oz. per 1000 ft. row at corn planting may cause quail mortality. The mortality incidence appears to be directly related to the amount of precipitation following the Thimet 20G application."

8. Reviewer's Conclusions: The study is scientifically sound and 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. Residue data submitted are acceptable (Acc # 245263).

   Non-irrigated Corn fields treated with Phorate at 2x the label rate appear to pose an acute hazard (mortality) for representative game bird species utilizing them for feeding and cover. Reproductive hazards are unknown.

   Immediate irrigation (or rainfall) rather than "amount of precipitation following the......application", as stated in the report) maybe a mitigating factor for quail.
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.

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<th>Plot size</th>
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<td>Female 15</td>
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<tr>
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<tr>
<td>Plot 2 - Thimet 20G</td>
<td>12 oz. per 1000' row</td>
<td>15</td>
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<td>(Irrigated)</td>
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<tr>
<td>Plot 3 - Thimet 20G</td>
<td>12 oz. per 1000' row</td>
<td>15</td>
<td>15</td>
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<tr>
<td>(non-Irrigated)</td>
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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 tooting immediately prior to planting.

Planter - a John Deere 7000 six row corn planter, was used for simultaneous planting and application of Thimet 20G. Corn was planted 1" deep; in-row spacing was 7"; seed population was 30,800 per acre., rows were on 30" centers. Distance between the opening disc and the closing wheel was 18". A 7" insecticide band spreader was suspended between them. 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.

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. Control plot was planted first to avoid contamination.

Irrigation - Immediately followed planting and Thimet 20 G 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 each 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.
Control and Thimet 20G test plots measured 50' x 300' (15,000 ft²) 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 an 30" centers. The twelve turn rows bordered the cover area.

Birds - Ninety (90) quail (15 cocks and 15 hens in each plot) were introduced into each plot at 6:40 pm on July 30, 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 (5) times per day on tilled portions of plots only.

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

Body weight - individual body weights 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.

Residues - Samples of whole corn plants were taken on Day 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. No statistical analysis of mortality was performed.
C. Results and Discussion

Adaptation to test plots

Birds adapted satisfactorily exhibiting normal wild bird behavior such as (1) periodic foraging on both cover and tilled portion of the test plots (2) individual and communal dusting sites (3) territoriality and aggression between cocks. The investigators felt that the plots realistically simulated quail habitat in regions of corn agriculture.

Climate - The average high temperature was 91°F; the average low = 72.8°F. 2.84" of rain fell total, with 1.16" falling on Day 2; 0.50" on Day 5; 0.19" on Day 7; 0.21" on Day 10; and 0.78" on Day 12.

Foraging Activity - The foraging index is % of control; by number of birds foraging on tilled areas only. The investigators showed that Thimet exposed quail equalled or exceeded the control quail in daily foraging for 12 of 14 days on the irrigated plot, and 9 of 14 days on the non-irrigated plot. After supplemental feeding began (Day 3) wild birds frequented the plots in large numbers. These included:

Field Sparrow
Brown Headed Cowbird
Red-Winged Blackbird
Mourning Dove
Robin
Rock Dove

Quail Mortality Incidence

No control mortality was observed for the entire test (14 days). No significant mortality occurred on the irrigated plot (1 death on Day 12 was considered incidental and not relative to Thimet 20G treatment. The non-irrigated plot had several mortalities including two (2) on Day 1 - one (1) of these (a female) was found decapitated, the other was a male. On Day 2, a male which was observed to be severely affected on Day 1 (prostrate), died at 3 pm. On Day 4 two (2) females died. On Day 5 one (1) male died in the turn area. These six (6) mortalities occurred early in the exposure period and are considered Thimet 20G related, but also relevant here was the lack of irrigation. (N.B. However it did rain 1.66" through day 5 on these plots also, indicating that early (or immediate) irrigation with >1.66" of water may be necessary to mitigate acute hazards to bobwhite quail).

Wild Bird Mortality

Transects revealed two (2) field sparrows found dead, one (1) on Day 4 in the control plot, and one (1) on Day 6 in Plot 3 (non-irrigated). Both were thought to be due to electrification by wire encircling the pens.
Behavior and Signs of Toxicity

All behavior was "normal" and comparable among the three plots. No signs of toxicity were observed in the surviving quail.

Body Weights

Normal test weight loss was observed (34-47g) in all plots, due to transition from ad libitum game bird diet to the foraging conditions. N.B. Since individual birds were not tagged (pers comm., Barron) the body weight conditions of the dead birds could not be addressed.

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 I 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-o 163 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 Tracer 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

Levels of cholinesterase depression of Thimet 20G exposed quail on Day 14 were not "statistically significant" (N.B. but no statistics were described).

<table>
<thead>
<tr>
<th>Plot</th>
<th>% depression</th>
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<tbody>
<tr>
<td></td>
<td>male</td>
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<tr>
<td>2 (irrigated)</td>
<td>3</td>
</tr>
<tr>
<td>3 (non-irrigated)</td>
<td>4</td>
</tr>
</tbody>
</table>

No conclusions were drawn from the above data since this parameter was not measured at the beginning of the study when mortalities were observed.

Residues

Only residues in quail remains were reported. Other samples were stored for future reference but not analyzed (pers. comm., R. Barron). 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 20G applied to corn at 12 oz. per 1000' row at planting will cause acute mortality to representative upland game birds.

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 substantial. Since mortality is demonstrated, it follows that the "significance" or extent of the population effect would be directly correlated with the exposure.

The study turned up two(2) dead field sparrows whose death was attributed to electrocution by the fence wire. However the investigators based that conclusion on "the placement of the bodies relative to the electrified fence".

The effects of immediate irrigation followed by rainfall may be mitigating, but rainfall alone (occurring after 48 hrs) is not effective in eliminating acute mortality. Potential additional acute mortalities on both irrigated and non-irrigated plots may have been masked by the significant amount of rainfall recorded on Day 2 (1.16"), 48 hrs after the applications.
Apparently non-irrigated plantings need a 1-2" rainfall immediately after application in order to avoid significant acute mortality. Some delayed mortality may still occur, however, as evidenced by the mortality in Plot 2 on Day 12. The likelihood of a 1-2" rainfall occurring immediately after application should be considered before concluding that the application of Thimet 20G to corn will not cause acute mortality.

No sublethal acute effects were observed. Absence of such effects, unfortunately cannot be correlated with brain tissue levels, since sampling was not done at the beginning of the test. However, other field trials during this testing (see Exhibit 4 under this submission - Accession #245263) have demonstrated significant ACHE depression in the first few days of the tests.

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

D. Conclusions

1. Category: 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.

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 actual analysis (specifically, how much if any degradation has occurred as a result of the delay in analysis?)
This sheet summarizes my discussions with Mr. Lignowski which originated out of my inquiries to Mr. Ray Barron of the same firm, concerning the avian field studies on phorate submitted on 5/20/81.

7/8/81 - Mr. Lignowski identified himself as the project director on these field studies. He indicated that the birds had not been in the test plots when spraying commenced because in order to do the spraying, tractors had to be used, so that the fences could not be put in place around the plots until after the job was done. I made note in the Data Evaluation record:

He called to explain why the field sparrows found dead during Exhibit 1 were thought to be electrocuted rather than poisoned. Basically there is no evidence of electrocution, but that the investigators felt, through their "experience" that the position of the dead birds near the fence indicated that they had been electrocuted.

I indicated that this was not evidence in the sense that there is no physical parameter, such as burned or mutilated carcass, to measure this. I would consider that both mortalities are part of the overall test mortalities. Also, the possibility exists that the sparrow found dead in the control plot could have picked up a lethal dose in a test plot and then flew over to the control plot where it ultimately died (my speculation). This was possible because the plots are large and uncovered from above. Mr. Lignowski did not offer an alternative hypothesis.

7/4/81 - Also, Lignowski declined to give info on granular weight but gave this info on size, which I could not interpret:

- Coarse particles: 
  - #10 Tyler screen: 1.67 mm pore size
  - #20 Tyler screen: 840.0
  - #60 Tyler screen: 250.0
  - #325 Tyler screen: 0.89
  - >840.0 >250.0
  - 7.5% <250.0 >44.0
  - 0.5% <44.0
  - 3% <3.0

(Handwritten notes on the page.)
ATTACHMENT III

THIMET 20G TEST PLOT DESIGN

PLOT CODES
1. Control
2. Irrigated
3. Non-irrigated
<table>
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<tr>
<th>Date</th>
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<th>Non-Irrigated Plot 3</th>
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