(TDR-3R) DATA EVALUATION RECORD PAGE 1 OF

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 00074625 CONTENT CAT 01

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 (Unpublished study received May 21, 1981 under 241-257; prepared by Wildlife International Ltd., submitted by American Cyanamid Co., Princeton, N.J.; CDL:245263-D)

SUBST. CLASS = S.

DIRECT RVW TIME = | (MH) START-DATE 5/23/83 END DATE 5/23/83

REVIEWED BY: Ann Stavola
TITLE: Aquatic Biologist
ORG: HED/EEB
LOC/TEL: CM2 11Jf 557939
SIGNATURE: Ann Stavola DATE: 5/23/83

APPROVED BY:
TITLE:
ORG:
LOC/TEL:
SIGNATURE: DATE:

Data Evaluation Record

1. Chemical: Phorate
(O,O-diethyl) S-[(ethylthio) methyl]
phosphoredithioate

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 Final Report, Project number: 130-131 C. Unpublished report by Wildlife International Ltd., for American Cyanamid, Princeton, N.J. In support of Registration #241-257.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 3.

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

5. Date Reviewed: July 9th, 1981

6. Test Type: Avian simulated field study

a. Test Species: Bobwhite quail, Colinus virginianus

7. Reported Results: " Based on the results of the 14-Day..... study, Thimet 20G broadcast over the corn whorls at rates of 6 oz. per 1000 row ft., and 12 oz. per 1000 row ft. does not represent a significant threat to Bobwhite quail survival.

'Exposure related brain cholinesterase depression will increase on Days 1 through 5 following.... application, ... recovery ... by Day 14 following..... application.

'Mortalities experienced in the 6 oz. per 1000 ft. row..... and the 12 oz per 1000 ft row test plot were close to normal background mortality levels as occured in the control test plot, and were probably not Thimet 20 G related."

8. Reviewer's Conclusions: The study is scientifically sound and does ~~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.~~ However, the investigator's conclusions are contradicted by the data. Appropriate residue data was submitted and is acceptable - JJB 1/25/82

The study demonstrates that acute field mortality will result from a broadcast application over 38" corn plants at label rates, (6 oz./1000 'row). The 6 oz/1000' row rate resulted in acute mortality at 2x the "background" or control mortality. The pattern of mortality and brain ChE levels clearly indicate organophosphate poisoning leading to death in both treatment groups but not in the controls.

A

Since no risk assessment was performed in this study, the investigator's conclusions concerning "significance" of the threat to birds are spurious.

No evidence indicates that the brain cholinesterase depression "peaks" at Day 5 as implied by the investigators. On the contrary, because of observed mortality patterns, brain AChE inhibition is presumed to increase to about Day 9 post application, and then gradually return to control levels by Day 14.

9. Materials/Methods

A. Test Procedures: birds were hatched at the testing facility and reared in flight pens. The wing-cipped quail were approximately eight(8) months old when tested. They were indistinguishable from wild quail.

Design

Plots	Exposure	#Birds male	#Birds Female	Plot size
#1 Control	none	30	30	15,000 ft.^{2}
#2 - Thimet 20G	6 oz. per 1000' row (non-irrigated) following 12 oz. per 1000' row (irrigated)	30	30	15,000 ft.^{2}
#3 - Thimet 20G	12 oz. per 1000' row (non-irrigated) Following 12 oz per 1000' row (non-irigated).	30	30	15,000 ft.^{2}

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 toothing immediately prior to planting.

The site was not subjected to the stardard pre-emergence teatment 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, the 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 20G 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). 12 oz / 1000' row was delivered to Plots 2 and 3 (Thimet trated).

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

When the corn was approximately 38" heigh a Gandee Granular Applicator was used to broadcast a second application of Thimet 20G in a 7-inch band over the whorls of the corn plants. Six (6) oz./1000' row was delivered to plot #2 and twelve (12) oz./1000' row to plot #3. No irrigation followed these applications.

No soil incorporation can be used for this treatment.

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.

Cover

Control and Thimet 20G test plots measured 50' x 300' (15,000 ft^2) 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

One-hundered eighty (180) quail (30 cocks and 30 hens in each test plot) were introduced into the control and each treated plot at 3:30 pm. on September 12, 1980.

Observations and Measurements

Mortality, Toxic Symptoms, Behavior - quail were observed daily for signs of toxicity, symptoms of cholinesterase inhibition and abnormal behavior. Transects of each plot were walked daily. Records of quail and wild bird mortality were made daily.

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

Body weight - Individual body weights were recorded on all quail at the beginning of the study, and on each quail removed for cholinesterase determinations.

Brain cholinesterase - on Days 1, 3, 5 and 14 ten quail (5 males, 5 females) from each of the three plots were sacrificed for brain cholinesterase activity determinations (used modified Ellman method - see EEB's file).

Residues - the following samples were taken:

Corn Plants - (whole) on Days 0, 7, and 14
Soil - Three 3-inch cores to 4". From the center of row on Days 0, 7, and 14.

Bird Tissue - ten (10) quail carcasses from each day of brain cholinesterase determinations (1, 3, 5, and 14) were frozen for residue analysis.

Bird Maintenance

Five (5) gallon, vacum 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 "statisticl 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 such as foraging, dusting, territoriality and aggression.

Climate - average high temperature was 83°F and the average low was 65° F. There was 2.99" of total rainfall for the study, with 1.48" occurring on Day 6 (first rainfall); 0.24" on Day 12; 1.25" on Day 13; 0.02" on Day 14.

Quail Mortalitly - # Dead/ # alive
(and cholinesterase activity)

Day	Control (0)	Plot 2 (12 + 6 oz.)	Plot 2 (12 + 12 oz.)
0	0/60	0/60	0/60
1	0/50	0/50 (20-24%)*	0/50 (39-31%)
2	0/50	0/50	0/50
3	0/40	0/40 (46-42%)	0/40 (63-55%)
4	0/40	0/40	1/39 (f)
5	0/30	0/30 (54-49%)	0/29 (63-63%)
6	0/30	1/29 (f)	0/29
7	0/30	0/29	2/27 (m, f)
8	0/30	0/29	0/27
9	0/30	0/29	0/27
10	1/29 (f)	0/29	0/27
11	1/28 (m)	3/26 (m, f, f)	0/27
12	0/28	0/26	0/27
13	0/28	0/26	0/27
14	0/28	0/26 (24-30%)	0/27 (32-25%)

* indicates brain cholinesterase activity - percent depression as compared to control birds - (males - females). Except for males on Day 1, all observed cholinesterase impairment is statistically significant as compared to controls at $p<.001$. For males on Day 1, impairment is statistically signigicant, as compared to controls, at $p < .01$.

Behavior and signs of Toxicity -

Subtle lethargy was observed for Days 3 through 7 in addition to a decrease in response to external stimuli. By Day 8 no discernable differences between treated groups and the control, with regard to lethargy and response to stimuli, were observed.

Body weights - control quail showed an average loss of 26 grams as compared to an average loss of 11 grams in Plot 2 and a 9 gram loss in Plot 3. Losses are considered normal for test and were not biologically significant or treatment related.

Levels of Cholinesterase (Brain)

Statistically significant brain cholinesterase activity depression existed on Days 1, 3, 5, and 14 (see Quail Mortality TABLE above).

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 III 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 or the number of granules left on the surface by the planting and application methods (with soil incorporation). Results, (quantified in a seperate 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. Determinations should have been made on Day 7 and 9 since the "peak" had not been reached and birds were still dying.

The method of analyses of bird tissue samples for Phorate was not submitted (method M-0163 was referred to in a seperate 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.

B. Statistical Analysis - since no statistics were submitted, no validation was performed.

C. Results/Discussion

The data generated by this field study demonstrate two aspects of phorate's effects on birds under actual use in corn fields. First, it is clearly shown that acute mortality to birds resulted from label recommended rates of 6 oz./1000' row in broadcast treatment to corn plants. This acute mortality was double the observed "control mortality", or that normally expected in field. The "control mortality" showed no relationship to organophosphate poisoning (no demonstration of

depressed cholinesterse levels for brain tissue)*. Second, the pattern of the acute treatment mortalities is clearly indicative of OP poisoning, as demonstrated by the timing of these acute mortalities, i.e., they followed "peaks" in brain ChE inhibition. When brain ChE dropped to at least a 50% depressed level (as compared to control's brain ChE), death occurred.

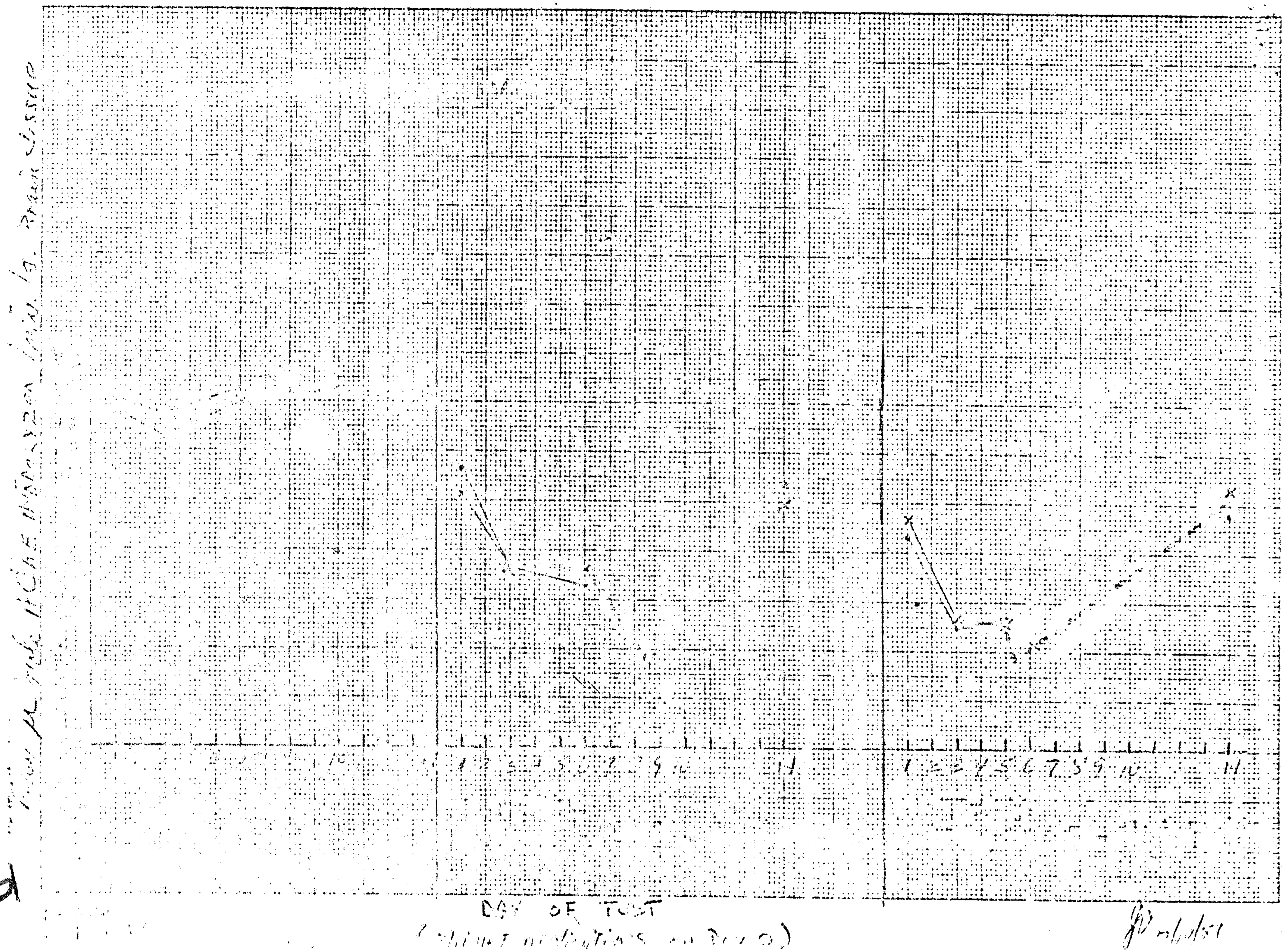
Note that three(3) acute mortalities occurred in Plot #2 (6 oz/ 1000' row) on the same day, Day #11. Although brain ChE levels were heading to "peak depressions" as indicated by percent ChE inhibition, the ChE determinations were abruptly stopped, so that the "true peak" of depression could not be shown. This data should have been determined as death was still occurring. This is a major problem with this test. Because of the observed mortality pattern EEB can only assume that ChE depression "peaked" somewhere around Day 9 or 10 at some level above 63% inhibition, since the trend on Day 5 was increasing ChE inhibition at 50 - 60% for both treatments (see graph).

Also note that no data for AChE, was provided for days 6 through 13. The investigators imply that AChE inhibition "peaks" at Day 5 and then returns to control levels. The data on observed mortality patterns, however, indicate that "peak" brain AChE inhibition due to Thimet 20G treatments probably occurs somewhere around Day 9-10, and then returns to control levels (see attached graphs).

In any case, the treatment mortalities (both the high and low application rates) do <u>not</u> show a similar pattern to the control, and certainly are significantly more than the "background" level of mortality. Clearly, the mortalities on the phorate plots <u>are</u> treatment related.

The "significance" of the acute mortality in the treatment groups is referred to by the investigator as "not..... a significant threat". However, it should be pointed out that this conclusion cannot be made by this study alone, since a full risk assessment must be made to draw a conclusion of that kind. Since no such assessment was made in the report, such a conclusion is spurious.

*<u>N.B.</u> - Although brain ChE levels were considered "depressed" only as compared to controls, it should be noted that the control birds' ChE values did <u>not</u> significantly change during the course of the study as did the treatment group's brain ChE levels. Brain ChE levels for control birds remained in the range of 11.88 - 16.10 micromoles AChE hydrolyzed/min/g. brain tissue, with a mean value of about 14 micromoles AChE hydrolyzed/min/g. tissue in the first 5 days. Treatment groups Brain ChE levels ranged from 4.18 - 13.49 micromoles AChE hydrolyzed/min./g. tissue, with a mean value of 7.33 micromoles AChE hydrolyzed/min/g. tissue in the first 5 days.


DAY OF TEST

D. Conclusions

"core"

1. Category: ~~Supplemental~~ Study

upgrade 12/5/82
appropriate residue data was submitted and is acceptable. - JS

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. Inlcude 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?)

Addendum:

Since 30 quail were removed from each plot over the course of the study the percentages for the mortalities are:

Control 2/30 - 7%
6g. ~~irrigated~~ 4/30 - 13%
12g. ~~non-irrigated~~ 3/30 - 10%

Ann Stavola - 10

REPORT OF TELEPHONE CALL OR VISIT

X	INCOMING CALL: 2 calls related OUTGOING CALL	VISIT CONFERENCE	DATE: 7/1/81 -7/2/81 TIME OF CALL: both approx. 9 AM

NAME AND ADDRESS OF CALLER OR VISITOR

Mr. Ray Barron
American Cyanamid
Princeton, N.J.

PHONE NO. (Include Area Code or IDS No.): (609) 799-0400

REGISTRATION, ID NO. OR FILE SYMBOL: 241-257

DATE OF LATEST SUBMISSION: 5/20/81 (Acc. #245263)

BRIEF SUMMARY OF CONVERSATION:

I had originally called AM. Cyanamid to ask questions about the Wildlife International field studies performed on bobwhite quail and submitted on 5/20/81 in support of the above registration. He was not in, so he returned my call on 7/1/81. I asked the following questions. He answered these questions when he called me back on 7/2/81

Q. What was the number of phorate treatments each plot received per exhibit?
A. Treatment plots #2 & #3 of Exhibit #3 received two treatments each - once during exhibit 2 and then again during exhibit 3. Treatment plots of Exhibits #1, 2, and 4 had only received one (1) treatment at the time results were recorded.

ACTION TAKEN OR RECOMMENDED

Q. How were individual birds identified during these studies?
A. Individual birds were not identified during these studies.

Q. Why was spraying of the test plots done prior to introduction of the birds?
He doesn't know.

Q. Where are the results of the vegetation and soil sample residue analyses?
A. American Cyanamid has these samples in their possession in storage but has not performed the required residue analyses because they do not want to pay for this. They will perform the analyses only if the Agency insists on it.

(I said that without this information the studies could never fulfill the requirements of the Proposed Guidelines, but that I could use the rest of the information contained in the studies to perform an incremental risk assessment. Chronic risks cannot be addressed without the residue data. It is particularly important to get this information since there is no existing environmental chemistry information on phorate as of this date.).

RECORDED BY (Name): John E. Rinehart

REFERRED TO (Name):

EPA Form 8500-5 (8-77) PREVIOUS EDITION OF FORM 1-5 WHICH MAY BE USED UNTIL EXHAUSTED