105001
SHAUGHNESSY NO.

REVIEW NO.

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TYPE PRODUCT(S): I, D, H, F, N, R, S Insecticide/Nematicide

DATA ACCESSION NO(S).

PRODUCT MANAGER NO. William Miller (16)

PRODUCT NAME(S) Counter

COMPANY NAME American Cyanamid Company

SUBMISSION PURPOSE Submission of terrestrial field protocol for review

SHAUGHNESSY NO.	CHEMICAL & FORMULATION	% AI
105001	Terbufos	15

12 pages

Field Study Protocol Review

1. Pesticide Name: Counter 15G (terbufos systemic granular insecticide/nematicide)

2. Study Type: Avian pen study (cholinesterase)
Avian field study (population impact)
Mammal field study (mesocosm)

3. Pesticide Use: Insecticide/corn rootworm plus other crops as per label.

4. Study Purpose: To quantify the acute hazard to birds, mammals, and other terrestrial wildlife (reptiles) observed in small-scale carcass search studies in corn.

5. Site Description: (Avian field studies only) - All in southern Iowa. Four blocks of two similar corn fields, each about 160 acres. Eight sites in sets of two. "Experimental units": 1/4-mi length of hedgerow and the adjacent 40 A of corn-field, known as the "core"; plus the adjacent 120 A of corn (figure 1). A total of 1/2 mi of hedgerow will be in each "experimental unit."

6. Exposure Regime

a. Brain Cholinesterase on Bobwhite and Peromyscus -

Oral intubation; laboratory.

b. Granule Exposure Estimate - (1.3 lb ai/A)

Location: unspecified
Dose: "worst-case"; corn - 8 oz/1000-ft row--banded on 30" centers.
"realistic best-case"; 8 oz/1000-ft row, in seed furrow on 30" centers.
Duration: one application to soil each.

c. Penned Bobwhite and Passerine/Soil Treatment Study

Dose: Contingent on study "6.b" above,
Duration: Contingent on results of "6.b"
Method: As per 6.b
Location: Unspecified

d. Earthworm Exposure & Toxicity

Location: Laboratory
Dose: As per mammal mesocosm field study below.

e. Full-scale Field Study for Population Effect

Location: To be determined by "preliminary site selection" field study.

1 Block = 2 similar units = 1 control unit (untreated) + 1 treated unit.

Exposure: Standard commercial corn production. All fields planted to soybeans in the previous year. Standard herbicides used in corn will be applied 2 to 4 weeks before planting. Both study fields will be cultivated at "layby" to reduce weeds. Each treatment field to receive 7-in banded application at planting at a rate of 1.3 lb ai/A (8 oz/1000-ft row on 30-in centers).

f. Mammal Mesocosm

(8) 1/4 A each-
4 at 1.3 lb ai/A in 7-in band;
4 untreated

7. Study Methods

a. Cholinesterase Study -

Dose series; highest dose = acute oral LD_{50} (28.6 mg/kg) for quail; to be determined for Peromyscus: Ellman method (1961) for cholinesterase as reported by Kendall (1985b) and others. Brain residues of terbufos to be determined. Observations include behavior and recovery time.

b. Granule Exposure Estimate -

Sampled by 25 cm^2 quadrants; three locations-on row, between row, and at turns. 30 samples per quadrant - 50 cm intervals between samples. Fluorescent dye - blacklight to visualize granules. Samples are analyzed for terbufos content. Sampling on days 1, 2, 4, 8, 16, 32, and 64. Statistical analysis of mean number and percentage granules by regression analysis. "Standard compartment model" to predict fate of compound may be used.

c. Penned Bobwhite and Passerine/Soil Treatment

Penned over bare soil--cages as per Galind et al. (1985). At least three cages with 20 birds for each species. No fresh food or water for first 24 hours. Observations

at "specified intervals." Brain AChE and brain residues done for dead birds and for all at study termination.

d. Earthworm Toxicity and Exposure

Lab - O.E.C.D. Guidelines for artificial soil test.

Field - Earthworms from the mesocosm study described for mammals. Sampled at two randomly selected sites on days 1, 2, 4, 8, 16, 32, and 64 post-treatment. Protocol for sampling will be that of Kruse and Barrett (1985); 1000 cc block of soil, 10 cm deep. Subsample at each site will be tested in a learning paradigm (Rattner and Gardner, 1975) and for locomotor activity in "open field" by electronic quantification. Residue analysis done on untested specimens.

e. Full-scale Field Study for Population Effects

(1) Bird Census, Nest Location, and Monitoring -

Minimum census 14 days pre- and post-treatment. Post-treatment monitoring period to be determined after cholinesterase recovery study. Line transect technique (Kendall et al. 1985a) 10 m off hedgerow and parallel to it. Each transect includes 50 ft to each side and 50 ft above. Bird surveys daily between 5 and 7:30 a.m.

Nest monitoring begins 14 days pre-treatment. Daily monitoring after nests are located. Not all species will be monitored--only robin nests (to reduce impact to hedgerow).

Observe: a) onset of laying; b) number of eggs produced; c) hatchability of eggs; d) survival of hatchlings; e) mortality in embryos, hatchlings, adults.

(2) Estimation of Avian Productivity -

Fifty starling nest-boxes to be established (Kendall et al. 1985b and 1985; and Berry-Robinson et al. 1985) in each treated and control field. They will be at equidistant points parallel to and both sides of hedgerow, 50 m out into fields. Only less than 1/2 of the boxes will be sampled for reproductive parameters (discussed above for robins). If a difference between treated and controls is seen, then others will be sampled for fledgling success. Cholinesterase analysis on brains of these fledglings.

(3) Small Mammal Trapping -

Sherman live traps, transect sampling and mark-recapture techniques to be used. Traps to be established outside the hedgerow of the core experimental unit. One transect in the hedgerow; other transect outside of the hedgerow. Twenty-five stations at 50-ft intervals on each line (100 live traps per field). Trapping to start 14 days prior to treatments; to be conducted on the same night in both study fields in a block. Continue for at least 64 days.

Pitfall traps placed at 50-ft intervals within each hedgerow outside of the core experimental unit to collect shrews. Residue analysis on these.

(4) Carcass Search and Carcass Search Efficiency -

Standard Search - Daily census of transects following the bird census. Carried out within the avian survey corridors and adjacent to hedgerow. No collection of intoxicated carcasses. "Zig-zag, back and forth" search pattern.

Search Efficiency - 60 bobwhite quail per study area of various ages, placed randomly on unannounced days. Survey areas noted on scaled grids. Samples placed in the evening and retrieved the following morning.

Birds will also be left in open fields and along hedgerows to estimate scavenging. This will not be done in study or buffer fields.

(5) Sampling and Residue Analysis -

i. In each core unit -

Treated soil, earthworms, vegetation, insects, shrews. Sampling on days 1, 2, 4, 8, and 16 after application.

ii. Gastrointestinal tracts of birds found dead or moribund will be examined and analyzed.

(6) Statistical Analysis -

ANOVA for given response variables on randomized complete block with two fields per block. Non-parametric techniques reserved for response variables

that cannot be transformed to fulfill assumptions of ANOVA.

"Repeated measures ANOVA" - for time comparisons. Mean differences between treated and control fields will be estimated; c.i.'s to be reported.

f. Mesocosm Study for Small Mammal Populations

Seminatural ecosystems ca. 1/4 A; described in Barrett (1968), Bular and Barrett (1971), Streck and Barrett (1978), Stuttman and Barrett (1979), Maly and Barrett (1984). Eight mesocosms will be built--four treated at 1.3 lb ai/A--7" band at planting--4 controls, untreated, 2-year treatments.

Small mammals - Five pair Mus or Peromyscus released in May. Parameters measured during growing season = population density, growth rate, natality, survivors, sex ratios, weight, trapping efficiency, recapture efficiency, home range, dispersal pattern changes. Mortality will be monitored during each trapping period. 25 livetraps in each--at least three trappings per week.

Earthworms - Subsampled on days 1, 2, 4, 8, 16, 32, and 64 posttreatment--to be used in previously discussed earthworm study. Also subsamples to be analyzed for residues.

8. Protocol Evaluation

It is very difficult to evaluate this protocol without the referenced material. In my opinion, a "protocol" includes a step-by-step road map of the experiment. I do not believe it is intended for Ecological Effects Branch to do the library research on these protocol reviews. Accordingly, I am suggesting that a revised document provide specific details such as residue methodologies, mesocosm constructions, statistical models, earthworm learning tests, etc., or provide "hard copy" of referenced material.

Generally, I am not agreeable to the idea of hypothesis testing for this type of study--i.e., one in which acute hazard must be quantified. (The registrants have already failed to negate a presumption of acute hazard [see Dingledine, (1984) and my reviews and DER's on this study (terbufos file)]. The mammal mesocosm study is interesting but I am not sure about the methods or of how to use the results. I do not think much of the earthworm "learning" study; how will this be used in a hazard assessment?

In the large-scale field study it seems that only 40 A of each field will be sampled. They have already done this size study (Dingledine, 1984). It seems that the transect techniques and ANOVA analysis are designed for bird density work and hypothesis testing. I do not believe bird density estimates are of use to us unless estimated on thousands of acres. The carcass searches should be more intensive and cover more than just two transects, particularly at planting and even for a month after planting. Duration of search should not be determined by cholinesterase studies, but should continue until no more birds are found.

Should all the fields to be in southern Iowa? Why not do multiple, smaller, more intensive carcass searches in many areas of the country, or in different crops?

Specific Comments

2.2.3a Bobwhite quail are known to be less sensitive to terbufos than other species--we rejected the use of quail in previous small-pen proposals; however, they may be useful for cholinesterase work.

2.2.3b These studies have already been done for terbufos (Erbach and Tollefson, 1983) and for phorate by Wildlife International (small-pen study with granule exposure estimate; exposure is not at issue here). The interpretations can be drawn with whole-body residues.

2.2.3c Again, since bobwhite quail are not the most sensitive species, this is not a "worst case"--also, the application rate is not "worst case" (see below).

2.2.3f Can a mammal mesocosm quantify the known mammalian hazards? (See Dingledine's 1984 study for mammal carcasses found).

10 (p. 7) Bobwhite quail are not most sensitive to terbufos.

11.2 (p. 8) This is not the worst-case application rate--worst-case corn is 16 oz/1000 ft on 30" centers. Sorghum can be 16 oz/1000-ft row on 20" centers.

11.3 Shouldn't the granule counts be done in the large-scale test?

11.5 Why do this? What is the standard compartment model?

12 Pen study--bobwhite quail not most sensitive bird. Dose protocol is rather tentative. No assessment of secondary poisoning potential. Application rate is unacceptable (see 11.2 above).

12.5 Why not have food and water in pens prior to applications? How will the results be used, specifically?

14.6
(p. 12) 40 A studies have already been performed.

15.2 Exactly how many years will Counter treatments be applied?

The study must last at least 60 days.

15.3.1
(p. 14) - How far apart are treatments and controls? Why is it crucial to have identical fields? (Are we doing hypothesis testing or hazard quantification?)

- Other agricultural in-use chemicals must be identified prior to use.

- Application rate is too low.

- Will the control fields receive any chemical treatments?

(p. 15) - What happens if the study is terminated after the first treatment year? Is the "second year" really the "first year" of the carcass searches?

15.3.2 In the past we have considered contact with variable habitat to be a good point in a field study.

15.4.1 The monitoring period must be at least 60 days.

The carcass searches should not be limited to two transects or 40 acres. What about perimeter searches?

Section on 50-ft corridors conflicts with transects being 10 ft off the hedgerow. We will need diagrams.

(p. 17) - How will they monitor embryo mortality in nest surveys?

15.4.2 Need diagram; need references or actual protocol.

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15.4.3 Same comment as 15.4.2. Need more details on the trapping.

15.4.5 b and c - looks like sampling number is insufficient.

Also add days 32 and 64 to schedule.

16
(p. 22) - How will effects of predators be controlled?

- We need much more detail on this protocol (submit references or actual protocols).

- Application rate is too low.

- Peterjohn, et al. (1981) does not appear in bibliography.

9. Suggested Modifications

The Ecological Effects Branch protocol review committee suggests the following guidance for the development of this protocol:

Issues:

1. Determine the amount of acute mortality caused by terbufos to nontarget species in and around areas of use.

2. Determine if reproduction or growth of young are reduced in non-target species due to the use of terbufos and to what extent.

3. Determine if survival is influenced in non-targets by the use of terbufos and to what extent.

Approach:

Appropriate approaches to answering these questions include a multiple site study for a minimum of 2 years. The first year would provide baseline data on species that frequent the crop areas treated with terbufos. In the second season an intense monitoring program designed to quantify the above parameters sould be initiated using appropriate methods for the species present, such as carcass searches, radio telemetry, multiple mark-recapture, nest monitoring, nest boxes, etc. An outline of suggested components of a field study protocol is attached for convenience.

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10. Conclusions

This protocol is basically acceptable but will require certain modifications and clarifications prior to final acceptance. The questions posed in this review and EEB's suggestions for modifying the study should be discussed at a meeting between EEB, RD, and American Cyanamid Co. prior to final acceptance of this protocol. The purpose of such a meeting will be to prepare for modifications to protocol to be submitted by company for approval.

Through probably no fault on the part of the company, this study will be delayed one (1) year. EEB suggests that a one (1) year extension of time indicated in 3(c)2(b) be granted to company.

Protocol accepted _____

Protocol accepted with modifications (to be determined at meeting indicated in No. 10 above) X

Protocol rejected _____

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Suggested Components of a Field Study Protocol
Submitted to EEB for Review

(Adapted from Giles, R.H., ed. (1971) Wildlife Management Techniques. The Wildlife Society, Washington, D.C.)

I. Title

II. Problem Definition

A. A review and summary of the available information on the pesticide in relation to nontarget hazard.

B. A precise statement of the goals and purpose of the study(ies) (objective(s)).

C. A brief statement of the problem and the context in which it exists, specifying the limits of the proposed work (scope).

D. Precise statements of the major hypotheses to be tested.

III. Methods and Materials

A. A brief discussion of various methods and procedures that have been or could be used to evaluate the problem. This discussion should identify the strengths and weaknesses of each method or procedure discussed.

B. Descriptions

1. Identify the study area(s) selected and their general suitability for achieving the objectives of the study.

2. Identify the species present in the study area(s), discussing characteristics pertinent to the problem being evaluated.

3. State the research procedures, designs, and sampling plans to be used.

a. Specify the kind and amount of data needed and to be sought.

b. Describe in detail how all data are to be obtained, including details of instrumentation, equipment, sampling procedures, etc.

4. Describe how the data are to be treated, including specifying what statistics are to be calculated, what models will be used, what tests of data will be used, etc.

5. Describe in detail the methods to be used to check the sensitivity and accuracy of the procedures used.

6. Briefly describe the resources (people, facilities, etc.) to be applied to the study.