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PRODUCT MANAGER NO. R. Rubis (50)

PRODUCT NAME(S) Terbufos

COMPANY NAME American Cyanamid Company

SUBMISSION PURPOSE Submission of Revised Level II Terrestrial Field Study Protocol for corn use

Shaughnessey Code	Chemical and Formulation	% A.I.

10 pages

105001
Shaughnessey Number

FIELD STUDY PROTOCOL REVIEW

1. Pesticide Name : Terbufos (Counter 15G insecticide/nematicide). Manufactured by American Cyanamid Company.

2. Study Type : Terrestrial Field Study

3. Pesticidal Use: Terbufos is registered for use on sugar beets,grain sorghum, sweet corn, popcorn, and field corn. The protocol under review is for field corn.

4. Study Purpose : To quantify the effect to wildlife species identified in a previous field study (Dingledine 1985). The present protocol is for the third year of a multi-year study. It should be noted that EEB has raised substantial concerns with this study in previous protocol reviews.

5. Site Description : The study area is located in Lucas and Warren counties, Iowa. Each study site consists of a 40 acre "core experimental unit" embedded within a 160 acre corn field and bisected by a hedgerow. Nine areas are being studied, which had been classified into three types, based on habitat complexity. However, the statistical strength they hoped to gain by the blocking did not materialize, and therefore, blocking will not be used in 1989.

6. Chemical Application : Each treatment site will receive counter 15-G at planting time. Two application methods are being evaluated, banded and infurrow. The banded treatment sites will receive Counter 15G applied in an 18 cm (7 in.) band on the soil surface center on each row. Infurrow applications will apply the counter 15G directly with the corn seed in the furrow in front of the press wheel. The control sites will not receive any insecticides, but will be treated identically to treatment sites in all other agricultural practices. The Counter 15G application rate will be at 1.45 kg/ha (1.3 lbs ai/ A) for both banded and infurrow treatment sites. The protocol indicates this is the maximum label rate for planting time application of Counter 15G, however, unless the label has been modified since Oct. 1984, this appears to be in error. The counter 15G label (EPA Reg. No. 241-238) allows a maximum use rate for at planting for the banded treatment of 16 oz./1000 ft. of row for any row spacing greater than 30-inches for corn, 2.6 lbs. a.i./A. This label, also, allows a post emergence application to corn at similar rates, as well as other uses at even higher rates.

7. Study Objectives : The following are the objectives of the study as listed in the protocol, page 7.

1. To measure the amount of Counter 15G parent material and degradation products in the soil for 60 days following application.

2. To measure the amount of terbufos and terbufos degradation products in earth worms and corn shoots following application of counter 15 G to soil.

3. To monitor the amount of terbufos and its degradation products in invertebrates selected as food by European starlings.

4. To measure reproductive effects in starlings through nest box monitoring on Counter treated sites, and to monitor survival of these animals.

5. To monitor northern bobwhites and eastern cottontails using radio telemetry to determine the amount of time spent on the treated sites, and to monitor survival of these animals.

6. To monitor blood ChE activities of radio-tagged northern bobwhites and eastern cottontails before and after exposure to counter 15 G on treated cornfields and concurrently on control cornfields.

7. To monitor blood ChE activities of red-winged black birds, brown-headed cowbirds and other selected passerine species before and after exposure to counter 15G treated cornfields and concurrently on control cornfields.

8. To monitor movement and hunting activities of great horned owls and kestrels to determine how often they hunt on Counter 15G treated fields and to document home range and habitat use of these species in the study region as it relates to application of Counter 15G on study sites.

9. To monitor blood ChE activities, fecal terbufos residues and owl pellet terbufos residues from radio-tagged great horned owls and American kestrels before and after application of Counter 15 G on the study sites.

10. To determine brain ChE activities and gastrointestinal tract residues of avian and mammalian species found dead on nine study sites.

Note: This may be a typographical error in the protocol. This appears to be more of a list of methods than objectives.

8. Study Design

The proposed study is indicated to be a stratified random, fixed effects (model I) design. Past protocols proposed site blocking based on habitat complexity. The key factors in the block assignments were the ratio of total linear edge between crop and non-crop vegetation to the surface area of non-crop vegetation on each site, plus the maturity and structure of the central hedgerow on each site. However, when survival and site use rate for selected wildlife species and starling reproduction data were analyzed, the blocking had no influence. They concluded from these results that the particular response variables they were monitoring were not significantly effected by the habitat complexity factors they chose for guiding the study site blocking. Hence, the increased statistical strength they hoped to gain by the blocking did not materialize, and blocking will not be used in 1989.

The proposal states some of the parameters measured in the study will produce nominal data (binomial distribution). These include survival and site use rates, and these data sets will require non parametric procedures other than ANOVA.

The proposal goes on to suggest that studies have shown that type of application method influences the availability of terbufos in the environment. Therefore, the study design is to group sites into threes, infurrow , banded, and control. This will results in three control repetitions, three banded applications repetitions, and three infurrow applications repetitions.

Study Methods

The following methods are proposed to be used in this study to monitor the effects of terbufos on wildlife species:

* Environmental Sampling - Soil, corn shoots, earth worms, mice, and invertebrates will be collected at specified intervals before and after applications to asses the environmental fate of Terbufos.

* Starling Nest Box Monitoring - Twenty-five starling nest boxes have been placed within the 40 acre core area of each of the study areas to attract adequate numbers of starlings for use as a indicator species in evaluating the impacts of terbufos on avian reproduction.

* Blood Sampling for Cholinesterase Analysis - To monitor the duration and degree of starling nestling exposure to terbufos, blood samples will be drawn from those birds that will be involved in the crop sampling at specific time intervals.

* Residue Analysis and Brain Cholinesterase Analysis - All adult, juvenile, or nestling starlings found dead during monitoring will be collected. GI tract, liver, and brain tissue will be excised from each bird.All GI tissues will be analyzed for terbufos and terbufos metabolites residues If GI tract tissues residues are high, liver tissue will also be analyzed, plus, total brain ChE activity will also be determined.

* Photographic Monitoring of Adult Starlings Feeding Young - Concern that crop sampling procedures could influence the selection of food items fed to the nestling by the adults by inducing changes in nestlings begging behavior. To evaluate this potential its believed that photographic nest monitoring will provide some insight into this potential influence of data collected.

* Banding - Nestlings will again be banded during this year study to evaluate first year survival in starlings from terbufos. Nestlings (418 total) , as well as adults (85 total) were banded in 1988. Its suggested that 25 to 50 % of the birds banded as juveniles may return to the study sites as breeding adults the next year. The protocol goes on and suggests that if no differentiated post-fledgling mortality is associated with terbufos application on the study sites, the return rate should be equal on all sites.

* Passerine Blood Cholinesterase Monitoring - A variety of bird species will be captured and blood samples taken for plasma ChE activity analyses to help evaluate if species are being exposed to terbufos at sublethal levels.

* Upland Animal Monitoring - Radio telemetry techniques will be used to monitor bobwhite quail and eastern cottontails on each study site to evaluate use of the area by these species and survival of these animals.

* Raptor Monitoring - American Kestrels and Great horned owls will be the target raptor species this year. The redtailed hawk was found to be less than satisfactory for use as a indicator species in the pilot study due to its relative large home range. Therefore, the above species will be used this next year.

* Weather Data Collection - Weather information will be collected throughout the study using two field weather stations. The stations will be located near study sites 1 and 3. These locations were indidated to be selected to provide coverage for both the north and south end of the study area.

Statistical Analysis

Starling Nest Box Study : All reproductive data sets will be subjected to Bartlett's test of homogeneity (Zar 1974). Values for variables comprising percentages data will be arc-sine transformed prior to analysis. Means of variables which fail to meet the assumption of homogeneity will be compared between treatments using the Kruskal-Wallis one-way ANOVA.

Upland Wildlife Monitoring : Daily telemetry locations of northern bobwhites and eastern cottontails will be used to test for the independence of the rate of use of the study sites by radio-tagged animals from treatment using Pearson's chi-square. Activity rates of bobwhites among treated sites and control sites will be compared using a two-way ANOVA. If the data fails tests for homogeneity of variance, a Kruskal-Wallis non-parametic ANOVA will be used. Survival of radio-tagged bobwhites and cottontails will be monitored for survival. A modification of the Mayfield (1975) method, which incorporates chi-square testing, will be used to test survival rates among radio-tagged animals on study sites for independence from effects of treatment. The number of observation periods (each 24 hour period) without mortality and the number of observation periods with mortality will be tested against treatments and controls in 2x3 contingency table analysis.

Raptor Home Range Analysis : Owl and kestrel location data will be analyzed using a program called Microcomputer Programs for the analysis of Animal Locations (MCPAAL), version 1.2. This program calculates home range areas using the harmonic mean method (Dixion and Chapman 1980, Kenward 1987) and generates contoured plotes of the calculated home ranges.

A 2x3 chi-square test will be used to compare the number of relocations on study sites to the number of relocations off site by preapplication and postapplication periods for each radio-tagged bird. The factors will include off and on site against treatment methods.

Blood Cholinesterase Reactivation : T-test analysis will be used to compare mean ChE activities of triplicate blood plasma subsamples incudated with 2-pam with triplicate subsamples incubated without 2-pam. If the 2-pam group mean is at least 5 % greater than the control group mean, and the differce is sigificant with $P < 0.05$, they will consider the reactivation to be a positive, significant response, which would indicate blood ChE inhibition by an OP insecticide.

Brain Cholinesterase Activity : Mean brain ChE activity of bobwhites, cottontails and selected passerine species found dead will be compared among treatments and between preapplication and postapplicatoion time periods using a 2-way ANOVA to identify difference associated with treatment effects. The age dependent

brain ChE activity of nestling starlings found dead in the nest boxes will be analyzed using a linear regression of ChE activity against nestling age. Mean blood ChE activities (total ChE, AChE, and BChE) of bobwhites, cottontails, selected passerine species and raptor species will be compared using nested ANOVA to identify differences among treatments and preapplication and post application time periods. Also, plasma ChE activities of animals trapped repeatedly over time will be illustrated to depict the direction and magnitude of temporal changes. Blood plasma ChE activities (total ChE, AChE and BChE) of nestling starlings will bee analyzed for differences among treatments using multiple linear regression of ChE activities against nestling age.

Counter Residues in Earthworms : Counter residue levels in earth worms will be reported in a histogram in time units for each treatment method (infurrow and banded) . Graphical representation of these results will be supplemented with a three-way ANOVA of the data in which treatment, worm location (infurrow vs. between furrow), and time after application are the factors.

Transect Counts of Dead Earthworms : The transect counts of dead earthworms will be compared using a one-way ANOVA for differences in the mean counts among treatments.

9. Protocol Evaluation : As indicated above, EEB in previous reviews, has raised substantial questions with the adequacy of this study to support registration. This revised protocol does not greatly reduce our concerns about the adequacy of this study to address the effects of Terbufos to non-target species. Since, most of our major concerns with this study have been raised in previous reviews, we'll limit our comments in this evaluation to a summary of our major questions with this study. For an indepth discussion the reader is referred to previous reviews of study protocols.

* A major concern with this study is the application rate. The initial study which triggered this study was conducted at 16 ozs./1,000 ft of row (2.61 lbs ai/ acre). This study is being conducted at 1.3 lbs ai/ acre, half that used in the previous study. The protocol claims the maximum EPA approved label rate at planting is 1.3 lbs ai/ acre. This does not appear to be correct. The label, EPA Reg. No. 241-238, allows a maximum of 2.61 lbs ai/ acre on corn; therefore this point alone renders the study inadequate to support the current label.

* Why Iowa? The protocol suggest Iowa strikes a suitable balance between two sets of conflicting needs in study site location. One attempting to bias the sites towards the greatest risk to wildlife versus site selection toward a representative risk to wildlife. The second set of conflicting needs in study site location, that they identify, is extensive study versus intensive study. The protocol indicates, that the

7

first set of conflicting needs, at present, cannot be defined. If correct, then the first statement, the Iowa area strikes a suitable balance would seem difficult to determine. Further, we are not sure where this first set of conflicting needs is derived. The idea of biasing toward areas likely to present the greatest risk was to reduce the number of areas where test would have to be conducted. That is if hazards appear to be low under worst case conditions , it can be reasonably concluded that impacts under less severe conditions would be minor. If these conditions cannot be identified, then multiple sites are needed. However, the Guidance Document suggests that in some circumstances preliminary monitoring of several areas may be appropriate to determine which should be selected for detailed study. This would have been particularly important in this case, since the original screening study which triggered this study was done at twice the application rate as this study is proposed and in a different area. In fact, further screening studies in several areas may have been more appropriate given the change in use rate and area. Also, it should be noted the implication in the protocol that the Guidance Document suggest that there is a intensive vs a extensive approach to a definitive study, is incorrect. An intensive approach is all that is suggested, which may be needed in more than one area.

* Sample sizes - The protocol gives very limited attention to this extremely important aspect of this study. Nine sites are proposed, three infurrow, three banded and three controls. The protocol attempts to justify this with:

> A larger number of study sites have been suggested for definitive (level II) studies (Fite et al. 1988) to assure statistical strength. However, radio telemetry monitoring, plasma cholinesterase, feces residues, and reproductive success of the above mentioned animal populations will quantify both mortality and sublethal biochemical responses of the selected wildlife to the test chemical. We believe that the intensity and level of refinement of the techniques described herein justifies the use of nine study sites, which encompasses a total area of approximately 583 ha (1,440 acres). We believe that data sets resulting from monitoring procedures presented herein will provide ample statistical sensitivity and power.

As they indicate we have suggested that a larger number of replicates are required to provide "ample" statistical sensitivity and power for designs of this type. In fact, to achieve "ample" statistical sensitivity and power for this type of design, 64 replicates for each treatment and control are required assuming a relative conservative coefficient of variation. Without further information on what additional

refinements of the techniques they are referring to are, we still believe sample size may be inadequate. In fact, we would be surprized if the refinements are adequate to give "ample" statistical sensitivity and power with the proposed replications.

* Species Selected - The protocol gives three general characteristics that a suitable species must have. The first is through its feeding habits and behavior, it can reasonably be expected to be exposed to the test chemical. Second, it must be tractable experimentally so that it can be studied over time, and sampled to detect temporal changes in exposure. Third, it must occur in sufficient numbers on the study sites to permit stsistical separation of inherent variability from chemically-induced variability of the measured endpoints. While we do not disagree totally with these characteristics, further qualification is required. But, for most of the species listed which are to be monitored, they do not indicate how they meet all three criteria, particularly the last characteristics of sufficient numbers. Also, sensitivity to the chemical needs to be considered, which appears to have been over looked for most of the species listed. For the starling, they do give some attention to this point, however we are not completely clear on how they reach thier conclusion that the starling, relative to other passerine species, best exhibits the key traits needed for an appropriate passerine model species for investigating the effects of a pesticide. They refer to preliminary investigations into the sensitivity of adult starlings to various pesticides and said reference suggests that they were not highly sensitive relative to other passerine species. (No citation is given.) They then go on and reference work with two organophosphates that has shown significant reproductive effects from exposure. They then reference preliminary studies with starling nestlings that indicate 1 to 3 day old nestlings are highly sensitive to pesticide exposure. Based on this, they concluded that relative to other passerine species the starling best exhibits the key traits needed for an appropriate passerine model species for investigating the effects of pesticides. This is somewhat surprising in that the only comparison to other passerine species was in sensitivity, and it showed the other species may be better indicators.

10. Summary

American Cyanamid Company has submitted a revised protocol for a field study for turbufos. Since, as in the past the protocol disregards most of EEB's previous comments on this study, and since EEB has for the most part reviewed this protocol indepth previously and the study is well into its third year, we have limited our comments to summarizing our major concerns with this study. These include: rate of application is half maximum label rates,

replication appears to be extremely limited to provide adequate statistical sensitivity or power, and justifications for geographical area, site selection and species are limited. Given these questions, EEB has severe concerns if this study will be adequate to support the registration of Turbufos.

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