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PRODUCT MANAGER NO. W. Miller (16)

PRODUCT NAME(S) Terbufos

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SUBMISSION PURPOSE Avian field study protocol

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FIELD STUDY PROTOCOL REVIEW

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

2. Study Type: Level II terrestrial field study using the following sequence of tests:

   a. Brain cholinesterase depression and recovery study in bobwhite quail and Peromyscus.
   b. Quantification of numbers and COUNTER-content of exposed COUNTER granules on the soil surface after application.
   c. Preliminary semi-controlled field study to assess impact on small passerines and bobwhite quail.
   d. Preliminary avian, mammalian, and reptilian population survey for site selection and method validation for the full-scale field study.
   e. Full-scale field study for population effects.
   f. Ancillary laboratory or simulated field studies to answer questions from previous studies.

3. Pesticide Use: Field corn, popcorn, sweet corn, sugar beets, and grain sorghum.

4. Study Purpose: This study protocol (EPA Record No. 203351; American Cyanamid Protocol No. 981-86-101.1) has been submitted as a result of EEB data requirements in the Terbufos Registration Standard and the Data Call-In letter of June 3, 1987. The Agency required an advanced (Level II) field study to quantify the hazard to wildlife populations identified in a previous (Level I) field study (Dingledine 1985). This protocol represents a further revision of the original Level II field study protocol dated February 24, 1986. A previous revision was submitted March 18, 1987.

5. Site Description (preliminary and full-scale field studies):

The study area is located in Lucas and Warren counties, Iowa. Nine study fields have been identified, divided into 3 blocks of 3 study fields each. Each block contains 2 treatment fields (1 field receiving banded treatment, 1 field receiving in-

furrow treatment) and a control (no terbufos treatment) field. Each study site consists of a 40 acre "core experimental unit" embedded within a 160 acre corn field and bisected by a hedgerow. Each block of fields is considered to be ecologically dissimilar from each of the other blocks; the 3 fields within a given block are considered to be ecologically similar.

6. Exposure Regime:

a. Brain cholinesterase study in bobwhite and Peromyscus-

Location: Institute of Wildlife Toxicology, Huxley College, Western Washington University.
Dose: Oral, by intubation; 3 treatments (acute oral LD_{50}, 1/2 LD_{50}, 1/4 LD_{50}) and control.
Duration: Bobwhite- 48 hours (at intervals); Peromyscus- 96 hours (at intervals).

b. Quantification of exposed COUNTER granules-

Location: Lake Terrell Wildlife Management Area, Ferndale, Washington.
Application: 8 oz. per 1,000 ft. of row with rows 30 inches apart resulting in 1.3 lb ai/acre for both banded and in-furrow treatments.
Duration: One application for each treatment type; granules sampled up to 63 days after application.

c. Preliminary semi-controlled field study (pen study)-

Location: As per 6b.
Application (dose): As per 6b.
Duration: 63 days.

d. Preliminary population survey-

Exposure regime not applicable.

e. Full-scale field study-

Location: As per 5.
Application: As per 6b with treatment/control blocks as per 5.
Duration: 2 seasons; each season to monitor 14 days prior to and 63 days after COUNTER application.

7. Study Methods (quoted in part from protocol text; section numbers referenced from protocol):

a. Brain cholinesterase study in bobwhite and Peromyscus (Section 10)-

Dose series; highest dose = acute oral LD_{50} (15.0 mg/kg in

bobwhite, to be determined in Peromyscus), other doses at 1/2 and 1/4 acute oral LD_{50}. Control included. At least 3 bobwhite sacrificed from each treatment at 1, 2, 4, 8, 16, 24, and 48 hours after treatment. At least 3 Peromyscus sacrificed from each treatment at 2, 4, 8, 16, 24, 48, and 96 hours after treatment. Brain cholinesterase levels and brain residues of COUNTER measured and correlated. Observations of behavior and recovery included.

b. Quantification of exposed COUNTER granules (Section 11)-

COUNTER 15G applied using typical equipment at rates described in 6b. Granules coated with fluorescent dye and counted in quadrat (25cm X 25cm) using blacklight. Ten quadrat samples (50 cm intervals) at each of 3 locations (on row, between rows, turn rows). Subsampling of counted granules to determine terbufos content. Sampling at 1, 2, 4, 8, 16, 32, and 63 days after application. Weather and soil conditions monitored. Mean number and percentage granules exposed estimated.

c. Preliminary semi-controlled field study (pen study) (Section 12)-

COUNTER application as per 7b. House sparrows and bobwhite separately confined in cages over treated areas. At least 3 cages with 20 quail or sparrows each. Birds observed to assess behavior/intoxication. Brain cholinesterase and brain residue levels determined for dead birds during study and for all birds at end of study.

d. Preliminary population survey (Section 13)-

Fields indicated by aerial and ground surveys. Primary feature searched for was hedgerow (660-880 yards long) surrounded by 60-80 acres of tillable land. Nine fields located; differences in habitat complexity of sites dictated division into 3 blocks of 3 fields each: A-type most complex with maximum amount of edge, B-type less complex, and C-type with little edge. Line-transect technique to census avian numbers; nesting species determined. Live-trapping and mark-recapture to sample small mammals. Bobwhite quail and ring-necked pheasants captured on nests and radio-tagged. Residue analysis on mammals, earthworms, and insects. COUNTER quantification study also proposed.

e. Full-scale field study (Section 14)-

14.3. Treatment and control fields- Study fields as described in 5 and 7d; application rate to be 1.3 lb ai/acre for each treatment type (banded, in-furrow).

14.4.1. Bird census, nest location, and monitoring- Line-transect census technique to monitor occurrence, distribution, activity and habitat use of birds 14 days prior to and 63 days after COUNTER application. Transect parallel to and 20 m off hedgerow on each side; 70 m corridor on each side of hedgerow censused. Daily censuses between 0500 hours (sunrise) and 0730 hours to identify species by sight or song.

Nest-sight searching to begin 14 days prior to treatment; nest monitoring to begin after nests are located. Common nesters along hedgerow include robin, red-winged blackbird, brown thrasher, grackle, and mourning dove. Data to be collected: a) onset of egglaying; b) number of eggs produced; c) hatchability of eggs; d) survival of hatchlings; e) mortality in embryos, hatchlings, or adult birds; f) nest abandonment rates.

Pilot study (1987) monitored 30-40 nests (species not identified in protocol) on 2 study areas.

14.4.2. Estimation of avian activity- Fifty starling nest boxes to be established in each treated and control field on untilled portion of hedgerow (10 m off fence line). Approximately 25 boxes at each site to be sampled for reproductive success. Remaining boxes with fledgings will be sampled to determine fledging brain cholinesterase levels if differences in reproductive variables are noted between treatment and control.

14.4.3. Radio-telemetry- Radio-telemetry monitoring to be performed on great horned owls, red-tailed hawks, and American kestrel; up to 36 individuals to be used. LD_{50} testing performed with 1 of species listed. Up to 10 pheasants and 10 rabbits or quail also to be monitored (an incident of apparent COUNTER intoxication was identified in a cottontail rabbit in 1987 pilot study).

14.4.4. Small mammal trapping- Deer mice mark-recapture studies (spring 1988) using 100 Sherman traps (10 X 19 station grid, 10 m trap interval) monitored 1 month prior to and 1 month after COUNTER application on 6 fields. Population parameters to be documented include: a) mortality (carcass searching plus trap mortality not attributable to researcher); b) minimum number known alive; c) habitat use; d) population movement; e) sublethal effects (recovery inferred from capture frequency data; weight of male mice determined); f) reproduction (proportion of sexually active males, pregnant and lactating females, examination of newborns if occurring in traps). Mice collected before and after application; tissues analyzed for AChE activity, pesticide residues, and metabolic products.

Pitfall traps at 50 ft intervals within each hedgerow to collect shrews. Residue analysis on trapped individuals.

14.4.5. Carcass search and carcass search efficiency testing- Daily census transects within avian survey corridors in a "back-and-forth, zig-zag pattern". Carcasses and feather spots collected, location recorded, and analyzed for residues.

Search efficiency testing with at least 3 pretreatment and 3 posttreatment searches for varied age (2-, 4-, 12-weeks) bobwhite quail (minimum of 60 per study area) within survey corridors. Carcasses placed in evening (identified by legband) and retrieved following morning. Efficiency based on "return ratio after correction for predator removal".

14.4.6. Sampling and residue analysis to assess exposure- Within each core experimental unit, treated soil (10 standard core samples to depth of 10 cm at 2 cm increments), earthworms (2 locations to obtain a minimum 10-worm sample), vegetation (2 random weed samples), insects (removed from 2 sets of 10 soil samples; sweep net sampling along hedgerow), and shrews (as described above) will be collected 1, 2, 4, 8, 16, 32, and 63 days after application and analyzed for residues of COUNTER.

Gastrointestinal tracts of birds found dead or moribund to be examined and analyzed for residues of COUNTER.

14.4.7. Weather data- Standard weather station established at a representative location for each set of 3 fields. Temperature, rainfall, relative humidity, and wind speed and direction recorded.

14.5. Statistical analysis and limitations on inference- ANOVA (2-Way), Pure Model I, Without Replication design. Treatment and habitat variability accounted for by ANOVA linear model. Species richness, minimum number known alive, number of nests, number of fledgings analyzed via contigency tables or higher level log-linear models. Inferences to other sites limited by habitat factor.

8. Protocol Evaluation:

Introduction

This protocol disregards modifications, suggestions, comments, and criticisms provided by previous EEB reviews and the recommendations found in the EPA Guidance Document For Conducting Terrestrial Field Studies. This evaluation provides further review comments where appropriate; several sections could be addressed only with questions due to lack of information provided to EEB. These questions reflect EEB's concern of

whether study design and methodology are sufficient to address study objectives, which according to the EEB review of the 24 February 1986 protocol, are to: 1) determine amount of acute mortality caused by terbufos to non-target 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.

Studies a, b, and c were each referred to in the protocol as "this work is complete". These studies were therefore completed without prior EEB final concurrence.

Specific Comments (Section numbers referenced from protocol)

2.2.2. Sequence of studies-

Full-scale field study identified in protocol as being initiated in "Year 2" and completed in "Year 3". The registrant is reminded that the field study must include a minimum of 2 consecutive years (seasons) of population effects monitoring (this does not include the pilot study). Depending on the results, and in consultation with the registrant, the study may be terminated or may need to be extended after the second year. Each study (section) identified as being part of the full-scale field study must be repeated each season.

8. Schedule-

What is the correlation between this schedule and the sequence of studies (Sec. 2.2.2)? There needs to be clarification as to exactly what each annual report is to contain. EEB recommends that all field data for each season be reported in the annual report (December 31) of that year (e.g. spring 1988 data reported in the annual report due December 31, 1988).

10. Brain cholinesterase depression and recovery study-

EEB's previous (and current) concern was that bobwhite quail are known to be less sensitive to terbufos than other species. The registrant responded (protocol cover letter) that acute oral LD_{50} studies with passerines are also planned to compare susceptibilities to that of quail. These further passerine studies are not included in current protocol. If such passerine studies have been completed, results should be submitted in support of this cholinesterase depression/recovery study. If passerine studies have not been completed, what is justification for proposed study?

While this particular study may provide information of cholinesterase inhibition and recovery under laboratory conditions,

extrapolations to field situation are limited. If the registrant is concerned about the detectable relationship between dose and risk in the field, it is advisable to follow the recommendations and conclusions of Hill and Fleming (1982).

11. Quantification of numbers and COUNTER-content of exposed COUNTER granules-

This study should be done only as part of the full-scale field study (repeated each year with the full-scale study). No clear correlations can be made as to non-target exposure in the full-scale study from an independent study conducted the previous year on a different site.

Proposed methodology is not consistent: Section 11.3 describes a granule count method utilizing fluorescent dye marked granules while the cited protocol in the Appendix ("Semi-controlled Field-Pen Study to Assess Availability and Persistence of COUNTER-15G Granules and Their Toxicity to House Sparrows (Passer domesticus) and Bobwhite Quail (Colinus virginianus)") describes a binocular scope method. Regardless of method used, EEB requires that the granule quantification study address the following: 1) each field should have a quantification study performed; 2) on band/in-furrow concentration should be determined independently of between row areas and results should be expressed as number of granules per square foot; 3) sample size within each field must be adequate to account for typical in-field variations (e.g., tractor maneuvering at end rows, contour, traction, effects of crop residues from previous years), therefore, samples should be randomly selected rather than taken at random 50 cm intervals; 4) sampling should also be done at Day "0", immediately after application; 5) application equipment type and use should be described in detail (see Sec. 14.3.1); and 6) report should include a table of number of granules/quadrat/site.

Terbufos application rates are not sufficient. Refer to review of full-scale field study (Sec. 14.3.1) for explanation.

12. Preliminary semi-controlled field study (pen study)-

This type of study is not adequate to address the concerns of a Level II study. Registrant justifies study in cover letter (p. 8) to protocol by stating: "The pen study aims to determine if exposure occurs in a worst case exposure scenario." First, exposure has already been documented in the Level I study (Dingledine 1985); and second, this study does not represent a worst case scenario (application rates are not sufficient- refer to Sec. 14.3.1).

13. Preliminary avian, mammalian, and reptilian population survey for site selection-

13.1. What is justification for restricting study at this stage to 3 blocks (replicates)? The Guidance Document For Conducting Terrestrial Field Studies outlines a rationale for determining the number of blocks necessary for a Level II study.

13.4b. Based on sensitivity, quail and pheasant do not represent species of greatest concern. The registrant should also include ground feeding granivorous avian species utilizing (feeding, nesting) proposed study sites and which are also known to be more sensitive to terbufos than quail (e.g. red-winged blackbirds).

13.6. Outline study plan ("Response of Selected Wildlife to Planting Time Application of COUNTER 15-G: A Pilot Study", Appendix 1) is both confusing and incomplete. Line-transect avian census technique is only referenced, not described. There is no proposed method to census reptilian populations included. EEB cannot concur that this study is likely to demonstrate adequate study sites with sufficient vertebrate populations given this incomplete methodology description.

What is the "preliminary study" identified in this plan? Pilot study objectives/methods are not clearly defined in this outline study plan; full-scale study plans are also discussed. For example, granule counts and residue studies are not appropriate for a pilot study.

13.7. Pilot study is identified as completed. Discussion is limited to site (habitat) description only. No other objectives are addressed. EEB cannot comment on validity of proposed full-scale study without complete results from other preliminary efforts.

14. Full-scale field study for population effects.

14.2. Baseline information should be provided.

14.3.1. What will be used to "lightly incorporate" granules in banded treatment? Are the various tillage techniques commonly used for corn being represented?

Terbufos application rate (1.3 lb ai/acre) is inadequate. The registrant is reminded that field studies must be conducted using methods which maximize exposure to non-target species. Current accepted labeling for COUNTER application in corn permits 16 oz of product per 1,000 ft of row with minimum 30-inch row spacing (equivalent to 2.6 lb ai/acre) banded or in-

furrow. Further, the label states this rate may be applied using 16 oz per 1,000 ft of row at planting or 8 oz per 1,000 ft at planting plus 8 oz per 1,000 ft at cultivation. Study design should address potential risk of each application method (i.e., one treatment at planting vs. split treatments at planting and cultivation). Also, it is noted that current labeling for grain sorghum permits at planting application (banded, no incorporation) rates of 26 lb of product/acre which is equivalent to 3.9 lb ai/acre; sugar beet at planting application (knifed-in) may be as high as 29 lb product/acre which is equivalent to 4.4 lb ai/acre. Given this significantly greater exposure potential in sorghum and sugar beets, further field studies are needed. (N.B.- Ecological Effects Chapter of the Terbufos (FRSTR) Registration Standard identified that field testing must also be done to support terbufos use on sorghum; no protocol for such field studies has been submitted to date).

Applicator equipment should be set by the landowner (operator) using normal operating procedure. Verification of calibration accuracy should be performed at each site using standard USDA (Extension Service) techniques; however, researcher verification procedure should not influence normal operating application procedure in any way. Calibration trials should adequately represent the various field situations (slope, soil condition, etc.) and results should be submitted in raw data form. Applicator equipment manufacturer and model should also be specified.

14.3.2. Given the variability of each site (i.e., types A, B, and C), detailed habitat maps should have been provided. These will be required in annual and final reports (discussed below). To support the block type division, results of the various criteria inventories (pesticide use and cropping histories, topography, specific hedgerow compositions, and bird and mammal surveys) should be included. Also, what tillage techniques are utilized at each site? It is assumed by the reviewer that study sites are the same as those described in Section 13; if so, what is the justification for restricting study to 3 replicates? EEB questions if this design has the sensitivity to detect changes at levels low enough to provide meaningful inferences.

14.4.1. Section outlines a basically adequate avian study; however, methodology needs to be discussed and justified in greater detail. How will avian census data be used- to determine density, species composition or both? How will data be analyzed to determine population impacts? Red-winged blackbird, robin, and mourning dove represent 3 possible species warranting further investigation (provided density is adequate). LD_{50}/LC_{50} data should be obtained for all proposed species to document sensitivity.

14.4.2. Estimation of avian productivity using artificial starling nest boxes is not acceptable. Starlings are almost exclusively insectivorous during spring; exposure to COUNTER granules would be minimal. Video-recording starling feeding behavior (as proposed) is not likely to replace 50+ years of well documented food habit studies performed for this species. A major objective of the pilot study should be to identify those species likely to be at greatest risk; full-scale studies are then to document population parameters of these species. Efforts should emphasize those outlined in Section 14.4.1.

14.4.3. Methods for this section are described only as "radio-telemetry monitoring". It is not possible to comment on/review a proposed study so deficient in discussion of methodology as this. What hypothesis is being tested? What data will be collected? How will data be collected? What is justification for proposed methods? It is unlikely that radio-telemetry monitoring of raptors will yield meaningful results in this study. EEB recommends 1) analyzing residue content of raptor prey items on site to indicate hazard to raptors; and 2) determine LC_{50} values for representative raptor species to obtain baseline toxicity data for possible future study.

Given that "an incident of apparent COUNTER 15-G intoxication was identified in a cottontail rabbit in a treatment field" (protocol p. 16), there appears to be an opportunity and basis to study population effects on cottontails as well as the extent to which cottontails represent a source of secondary poisoning to raptors.

14.4.4. Methods for small mammal study inadequately described and justified. Have preliminary efforts indicated sufficient numbers of deer mice on all fields to target this species? Monitoring schedule is insufficiently described. EEB recommends trapping schedule to coincide with other faunal surveys; i.e., 14 days prior to and 63 days after COUNTER treatment with trapping efforts of 3-4 consecutive trapnights at 3-4 day intervals. Trapping must be repeated each season. What is the justification for trapping on only 6 of the 9 fields? How will differential habitat use be detected? What is the proposed method of marking? Have carcass searches demonstrated an ability to detect deer mouse mortalities? Discrepancy in protocol: 10 X 19 station grid proposed, but 100 live traps identified per study field. Trapping grid is located outside of core study area (Figure 5)- why? EEB suggests splitting the trapping grid into 2 smaller grids- one associated with hedgerow (as proposed) and one remote from hedgerow incorporating other habitat types within core study area.

Specimen collection (proposed) is strongly recommended; however, methodology insufficiently described. Exactly when will specimens be collected? How will specimens be collected? How many specimens will be necessary to ensure that the sample size is able to detect residues? What methods will be used to analyze for AChE activity, pesticide residues, and metabollic products? How will specimen collection affect livetrap studies?

Recommend that pitfall trapping (shrews) be conducted along hedgerow/field edge instead of within each hedgerow. Also, investigators should not disregard fauna other than shrews (e.g., toads) captured in pitfall traps.

14.4.5. The protocol fails to provide data on what percentage of the study area will be searched for carcasses. If mortality occurs, how will the number of dead animals per acre be extrapolated? Carcass searching should also be done along field/hedgerow edge and within the hedgerow in addition to in-field searches. What "residue analyses" will be done? What are the analyses methods? Cholinesterase assays should be performed in addition to residue analysis.

Carcass search efficiency testing description is poorly written and confusing; "Kendall et al. 1985a" citation should have been provided with protocol to clarify and detail proposed methods. How is "correction for predator removal" accomplished?

14.4.6. Sampling and residue analysis methods poorly defined. Sampling should also be done at Day "0" (i.e., immediately after application). What are study objectives? What data will be collected? What is the justification for using each method? What will the data indicate? Specifically:

a. Treated soil- Is adequate representation of each field (turn rows, soil types, topography, tillage techniques, etc.) accomplished by only 10 samples per field? How will samples be analyzed for COUNTER? Samples must not be composited.

b. Earthworms- There is no "13.3b" as described for sampling procedures. What are the methods of collection? Why are 2 locations and 10 worms per study field sufficient? How will earthworms be analyzed for residues?

c. Vegetation- Why are 2 vegetation samples per treatment field adequate? What species will be collected? How will vegetation be analyzed for residues? Plant parts (seeds, leaves/shoots, roots) should be examined individually. What about crop (previous years) residues? Crop residues containing unincorporated COUNTER gran-

ules from band application are likely to represent a significant route of exposure.

d. Insects- How will soil insects be taken? Other soil arthropods should also be collected/analyzed. How will insects be analyzed for COUNTER residues? What is the justification for sampling intensity? Above-ground soil invertebrates (beetles, grasshoppers, etc.) should be included.

What methods will be used to analyze avian gastrointestinal tracts for COUNTER residues?

14.4.7. Meteorlogical data must be collected daily during the study.

14.5.1. Given the inadequately described and justified field testing methods, such a detailed discussion of statistical analysis seems premature. Although the registrant believes that "because of the intensive nature of the measurements, it should be possible to gather an almost total sample of the response variable on each experimental unit" (protocol p. 23), there is little support for this within the protocol.

What is a "Pure Model I"? Given the proposed study methods and results from preliminary studies, what level of change in each measured variable does the registrant estimate would have to occur for statistical differences to be considered significant?

14.5.2. EEB agrees with the protocol statement: "Inferences to other habitat in Iowa or in other states can only be drawn if it is assumed that habitat in this study is representative of these other areas of interest." Given the large degree of variation that exists between corn growing regions in the U.S. (soil types, climate, irrigated vs. non-irrigated, tillage techniques, seasons, etc.), it is the registrant's responsibility to select and justify study sites so as to alleviate EEB concerns regarding the ecological effects of terbufos within its use range.

16. Final report.

This section should address annual as well as final report requirements.

In addition to those items listed in the protocol, EEB will require in each report, first generation detailed maps drafted from current aerial photos and/or USGS maps denoting topography, individual study sites (including fencelines, waterways, etc.), study site locations, soil types, adjacent habitat

types, and all sampling locations for each study method. Black and white orginal photographs (ground level perspective) of study sites and adjacent habitat types as well as photo-documentation of all field study methods are strongly recommended.

9. Summary:

American Cyanamid Company has submitted a revised protocol (Protocol No. 981-86-101.1) for a Level II field study for terbufos as part of Registration Standard requirements. Objectives (as identified in the EEB review of the February 24, 1986 protocol) are to: 1) determine amount of acute mortality caused by terbufos to non-target 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.

EEB's previously noted concerns remain largely unresolved. Further comments, suggestions, criticisms, and questions have been included in this review to provide guidance to the registrant in conducting the study. Most methods proposed will require amendment (as described within protocol evaluation) in order to meet study objectives; others may require little more than clarification and justification due to deficiencies in protocol detail and organization. This review represents EEB's final evaluation; it is now the registrant's responsibility to initiate an acceptable Level II study according to the schedule previously identified. Should the registrant have further questions as to study objectives and methods, a meeting between EEB, RD, and American Cyanamid Company is suggested.

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Literature Cited

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