US EPA ARCHIVE DOCUMENT
To: Barbara Briscoe  
Product Manager 50  
Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief  
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 239-2471  
Chemical Name : Orthene Technical  
Type Product : Insecticide  
Product Name : Acephate  
Company Name : Valent USA Corporation  
Purpose : Registrant response to upgrade residue  
monitoring study previously reviewed by EEB  
for tobacco.

Action Code : 660  
Date Due : 08/30/89  
Reviewer : R. Felthousen Date In : 04/12/89

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Y=Acceptable (Study satisfied Guideline)/Concur  
P=Partial (Study partially fulfilled Guideline but  
additional information is needed)  
S=Supplemental (Study provided useful information but Guideline was  
not satisfied)  
N=Unacceptable (Study was rejected)/Nonconcur
ECOLOGICAL EFFECTS BRANCH

Chemical: Acephate

100.0 Purpose of Submission

The Registrant (Chevron) has submitted rebuttal comments relative to EEB's review (See review by R. Felthousen dated June 20, 1988) of a study entitled: "A Residue Monitoring Study in Tobacco to Access Exposure to Avian Species Under Standard Agricultural Use Condition in North Carolina" (MRID# 41023505). The study was required in order to satisfy the Subdivision E 158.145 data requirements for wildlife as per the Acephate Reregistration Standard issued September 22, 1987.

101.0 Discussion

In the June 20, 1988 review, the EEB identified 10 issues, relative to either the design or conduct of the study, that made the study "Invalid" to satisfy the data requirement. The following discussion includes EEB's original comment, the Registrant's rebuttal comments and EEB's response to the rebuttal for each of these issues:

1. PRESENCE OF TARGET (PEST) SPECIES

EEB Comment

The EEB commented that "No mention was made as to whether the target species (i.e., flea beetles, aphids, budworms or hornworms) were present at infestation levels at the initiation of the study?" The EEB believes that the presence of the pest species is fundamental to the conduct of an acceptable study—especially a residue monitoring study where the primary objective is to determine likely levels of exposure to non-target organisms.

Registrant Rebuttal

The Registrant argues that in a "Screening Study", as they conducted in tobacco, (they) do not believe that the study should be delayed until the target pest species appear in epidemic proportions for the following reasons:

1. Orthene Tobacco Insect Spray is applied for remedial control of pest infestations. However, it is regularly applied as a preventative treatment on a schedule determined by an individual growers past experiences. The application schedule used in this study was designed to satisfy requirements specified in the Standard. These requirements necessitated applications at the maximum
permissible use rate repeated at the minimum permissible interval. Such a schedule is preventative in nature and cannot be timed to coincide with the "appearance of the pest species". Therefore, the study was initiated before the "appearance of the pest species".

2. There is no evidence that the targeted pest species would be a major avian food item or that residues on target pest species would be different from residues on other insects that may be avian food items.

3. If spraying were withheld until the appearance of pests, it is possible that (a) spraying might not occur or (b) pests might not occur until later stages of crop growth in which case the maximum number of sprays might not be applied. In scenario (a), no insecticide would have been applied and (b), it's possible that spraying might have been limited to a single application.

4. Justification why we conducted the study under worst case conditions (before appearance of the pest) can be supported by reference to the "Guidance Document for Conducting Terrestrial Field Studies, E. C. Fite, et. al."

5. In no place does the Guidance Document state that pest species must be present before application occurs.

EEB Response

The Registrant claims that the study was a "Screening Study" - as defined by the Guidance Document. However, the study in question is really a residue monitoring study designed to determine potential exposure to non-target species rather than a "Screening study" which is specifically designed to look for effects vs. non effects under actual use conditions (Fite, personal communication).

1. The EEB telephoned Dr. Ross Liedy, Director of the Pesticide Research Laboratory at North Carolina State University to determine what actually constitutes the typical or standard use of Orthene on tobacco (See telephone conversation record Attachment 1). Dr. Liedy referenced a brochure entitled: "Flue-cured Tobacco. 1992 Information" which showed that acephate was primarily used in North Carolina as a remedial treatment to control aphids, cutworms, budworms, flea beetles,
grasshoppers and hornworms on tobacco. Based upon his personal experience, as well as other available information, Dr. Liedy believes that most tobacco growers apply Orthene as needed to control infestations (i.e., remedial treatment) and not as a preventative treatment. Dr. Leidy suggested that I talk with Dr. Sterling Southern for additional information.

The EEB telephoned Dr. Sterling Southern, Head-Entomology Extension for North Carolina State University for information on the use of Orthene on tobacco in North Carolina (See telephone conversation record-Attachment 2). Dr. Southern stated that, "... the major use of Orthene to control insects on flue-cured tobacco in North Carolina is as a foliar spray for remedial control". He supported this statement by referencing 1989 agricultural statistics that showed 80% of the tobacco growers in North Carolina used Orthene in this manner while only 25% used it as a preventative treatment (does not equal 100 percent because some growers use it both ways). It's important to note that nearly all the preventative use occurs when transplant water is used. These same statistics showed that, on average, 3 applications were made per growing season when applied for remedial control. In addition, Dr. Southern also referenced data that showed a majority of tobacco farmers used published "threshold" levels as guidance for when to initiate application.

Based upon this information the EEB must conclude that remedial treatment represents the majority of use of Orthene in North Carolina and, contrary to the Registrant's claim, sufficient information (i.e., scouting reports, threshold levels, extension training) is available to time the use of the chemical to coincide with the infestation of the pest(s) species with this treatment practice.

2. Although there is some evidence that certain pest species (i.e., budworms and hornworms) may not be primary food sources for avian species, there is evidence that many birds will consume aphids, flea beetles and grasshoppers. The important point to remember is that, because they are opportunistic in nature, certain avian species will consume whatever insects are available at any given time. The EEB maintains that this is an important consideration in hazard assessment in that pest infestations are usually indicative of ecological conditions that may or may not enhance avian activity (and
subsequent exposure) on the treated crop. The burden of proof in this matter lies entirely with the Registrant. The EEB has not received any rationale or justification as to why this issue should not be addressed.

Based on the study report there appears to have been a paucity of invertebrates on the study area prior to treatment (e.g., report states,..." "Following the first collection period, the number of (pitfall) traps was expanded to fifteen (from 10) to increase sample masses" ..." Earthworm sampling was never found to be successful due to low earthworm densities in the upper soil layers and..."If sweeping for fifteen minutes proved unsuccessful, (which was usually the case at field interior stations) efforts were terminated. Because, as the document so aptly states..., "Invertebrates are important food items for wildlife", (See attachment 3) the EEB can only ask how could adequate residue data, for this primary source of exposure to non-target species, be obtained without having sufficient numbers of target pest species present to sample?

3, 4. There are any number of "might have been" or "might occurs" that one can argue could happen during the course of any study. Such are the difficulties and uncertainties when conducting field studies. It's important to remember that the purpose of this particular study was to generate residue data that are indicative of "worst case" situations under standard (typical) agricultural practice. Therefore, given what has been discussed in the above paragraphs, it is imperative for the conduct of a valid study that the chemical should have been applied according to standard practice (i.e. as a remedial treatment when the pest species have exceeded "threshold levels") using the maximum application rate and number of applications allowed by the label, to be representative of a "worst case" scenario for hazard assessment purposes. Contrary to the Registrant's response the "Guidance Document for Conducting Terrestrial Field Studies" does mention that this should be considered in the conduct of a definitive field study.

In addition, according to Dr. Southern, on average, 3 applications of Orthene are typically applied for remedial treatment. In so much as label rates allow for up to 5 applications per season, it is very likely that, for remedial treatment anyway, there is a good possibility that at least 3 applications would be required to get control of the pest(s) and
that 5 applications are not that unreasonable (Why else would the label allow for this use if it wasn't determined to be necessary and/or efficacious under certain conditions). Therefore, the Registrants' argument is not based on the data but on conjecture as to what "might happen". This is insufficient rationale for not conducting the test when there was no infestation of the pest(s) species.

5. The purpose of the Guidance Document is to provide guidance for the conduct of field studies. It is not a "cook book" for how to conduct the study (See attachment 4). That is, the Guidance Document does not specifically mention every technique, method, process or statistical measurement that could or should be used. This allows a certain amount of flexibility to the testing outfit to "tailor" the study to fit the particular field circumstances. It is the responsibility of the Registrant to explain the rationale for the conduct of the study.

The Registrant argues that the Guidance Document makes no specific mention relative to the presence of the pest species during the conduct of a "Screening Study". This is correct as far as it goes. However, it must be remembered that there are a lot of factors relative to the conduct of an adequate study that are not specifically mentioned in this document. This does not diminish their importance or at least warrant their consideration. Scientific rationale should be provided by the Registrant to justify their position on the presence of the pest species.

It is also important to note that under the section for conducting a "Definitive Study", the guidance document specifically mentions that, "... "consideration needs to be given to whether the target pest species will be present (See attachment 5). If it is not, one must consider what influence its absence may have on potential results." No such discussion was provided relative to this issue by the Registrant in the study report. The fact that this issue is not specifically mentioned under the "Screening Study" section does not mean that it does not have to be a consideration. This is especially true in this case where the primary purpose of the study was to monitor field residues.

The EEB admits that, under many circumstances and conditions, wildlife utilization of tobacco fields is low. However, there are instances where
utilization may increase greatly (e.g., such as in infestation of grasshoppers or other "attractive" pest species). The EEB simply does not have any "hard" data that shows whether or not utilization increases or not due to pest infestations on tobacco (See discussion for # 2). If the Registrant has data relative to this issue it should provide it to the Agency. The salient point is that, since data for a major route of exposure (i.e., invertebrates) it was extremely difficult to collect sufficient invertebrate samples to begin with, are the data sufficient to adequately determine the residues for major route of exposure to non-target organisms? There was no formal discussion of this in the study report.

2. TANK MIX SAMPLES/DEPOSITION CARDS

EEB Comment

Tank mix samples were taken and analyzed, however, the results of the analysis were not reported. In addition, spray deposition cards should have been placed in the field to verify application rates.

Registrant Rebuttal

Tank Mix Sampling:

The Registrant stated that tank mix samples were not analyzed because (1) "wide variation in analytical results normally observed in such measurements" and (2) they believe that "GLP record keeping is sufficient to verify application rates." The Registrant, however, agreed to analyze the tank mix samples and has submitted the data.

Spray Deposition Cards:

The Registrant believes that deposition cards are not needed because, "GLP records and residue data are adequate to verify application rates."

EEB Response

Because there are many factors (both biotic and abiotic) that can affect the amount of pesticide that actually "hits" the ground, the EEB believes the placement of spray deposition cards on and off the target site is a requisite for the conduct of an acceptable field residue monitoring study. The EEB is aware of instances where, even though the pesticide was properly tank mixed and agitated, spray equipment properly calibrated, and wind
conditions favorable, virtually none of the application reached the ground because of the highly volatile nature of the chemical.

The EEB asked Dr. Liedy to comment on this issue. Dr. Leidy mentioned that he has data that shows there is tremendous variation in the amount of pesticide that actually gets applied to the crop even under ideal climatic conditions. He believes that ground level wind currents, relative humidity, and temperature are primary factors influencing this variation. He further pointed out that the North Carolina State University Pesticide Research Lab routinely requires the use of spray deposition cards for the conduct of any study and that this practice is part of the training curriculum for extension personnel (See attachment 1).

As mentioned earlier, the Registrant agreed to analyze the tank mixes and has submitted these data (See Table 1 - Attachment 6). Table 1 shows that samples taken from the TW-1 tank mix ranged from 1,026 to 5,369 ppm of acephate with the average of the five samples being 3,482 ppms. Samples taken from the TW-2 tank mix ranged from 1,678 to 7,696 ppms of acephate with the average of 5 samples being 4,304 ppms. Samples taken from the JB-1 tank mix ranged from 2,685 to 3,929 ppms with an average of 9 samples being 3,067 ppms. These tank mix data indicate that there was great variation both within and between tanks. Such variation in the tanks could cause wide variation in what actually is applied. Deposition cards could have been used to detect this variation.

Therefore, even though the study was conducted in accordance with GLP and accurate records were kept, unless deposition cards are used as a "yardstick", there is no assurance that what was applied actually "hit" the ground or that residue levels are indicative of what was applied. (Note: The Registrant argues that those residues found on the tobacco plants are reflective of the application rate, however, as mentioned in the study report itself, plant phenology played a major role in the variation observed between fields (the report states..."At the time of first foliar application tobacco plants on TW02 were considerably larger than TW01 or JB01, thus the same amount of product was applied to larger plants. This could have resulted in the lower observed residues on TW02) and therefore may not be reflective of pesticide application (i.e., what was actually applied.)" (See attachment 7).
3. **Sample Collection - Number of Samples**

**EEB Comment:**

Three on-site stations (field interior) may not be sufficient to establish a typical residue profile for soil, water and invertebrates, especially since these samples were composites. Compositing samples tends to mask the typical residue variation. The report makes no mention as to how many samples were collected per station, to form the composite, or whether the same number were collected for each collection period.

**Registrant Rebuttal**

"The report specifies the minimum amount of biomass or volume to be collect per sample. The same minimum biomass/volume was used for each sample period." It further states that..."equal sample effort was used to collect material from each station to form the composite (i.e., 1/3 sample per station). In this manner a single representative sample was obtained. Tobacco foliage was an exception with one complete sample being collected per station... material."

"While the Guidance Document states that ".. samples from different locations within a site should not be pooled" and "... separate analysis of samples can provide data on the range and variability of exposure as well as mean levels," we believe that individual sample separation and analysis are not necessary ... We believe that EEB should consider HED/EAB's dietary risk assessment approach in terrestrial risk assessments and use "mean anticipated residues" rather than "worst case" assumptions."

**EEB Response**

First of all, the report does not state that "equal sample effort was used to collect material from each station to form the composite". What it does state is..."whenever possible, samples to be composites were collected from stations in equal proportions." (See attachment 8) The EEB does not know what is meant by "whenever possible"? When wasn't it possible to collect the material-(i.e., when the required amount couldn't be collected)? How can this equate to "equal effort"? Did this result in sample bias?
Secondly, in effect, the sampling for soil, water and invertebrates actually resulted in only one sample/field. How can this provide any information on the variation for these substrates within each field?

Thirdly, the averaging of the samples does not provide the EEB with an idea of what is the range or spectrum of residues likely to occur in the environment. For instance, water samples taken up-slope would probably contain fewer residues than samples taken down-slope (or in a depression) because of runoff. Compositing the up-slope samples with the down-slope sample dilutes those residues found down-slope. This practice does not satisfy the purpose or intent of the residue study.

It is Agency policy to use "worst case" situations/scenarios/residues to develop hazard assessments for ecological effects just as it is Agency policy to use mean anticipated residues for setting food tolerances. As such the EEB requires these data for hazard assessment purposes. The EEB suggests that if the Registrant wants the Agency to change current policy it should formally present a position document on the matter to an appropriate scientific forum (i.e., the Science Advisory Panel) for discussion and resolution.

Sample Collection 2- Residue Variation

EEB Comment

"In addition, ... the EEB questions whether three samples are sufficient to develop a typical residue profile even for the target crop."

Registrant's Rebuttal

"Although it is true that there is some variability in tobacco samples on field JB01, the results are reasonable and are not inconsistent with values typically found in field residue evaluations."..." It is believed that the variation observed both within and between fields reflects variation which occurs under typical field conditions."..." Considering the maximum application rates and frequencies employed in this study the larger residue values observed may reflect the extremes one would expect to encounter from applications that are consistent with the product label."

EEB Response
The EEB can only ask, given the data, what makes the results of this test reasonable and consistent with values typically found under field conditions—sample size? study design?, or comparison with other data sets? The point is that the Registrant has failed to provide the scientific rationale for their opinion. What is their rationale for why the samples regime may reflect the "extremes one would expect to encounter...label?" For example, although it is theoretically possible that only two samples could provide data on the extremes of residues likely to occur, it is highly improbable that such a sample size would be adequate. What is the Registrants' rationale for why three samples are adequate?

The Registrant argues that the variation observed both within and between fields is typical. Why? What is the basis for their argument? The EEB notes that on field JB01, residues ranged from 17 to 146 ppm one day prior to the first foliar application of the pesticide (See attachment 9). Data also show that field TW02 had residues that were less than or equal to only 0.14 ppm one day prior to the first foliar application. Which one of these fields have residues that are typical of field conditions? Do these residues reflect an agricultural practice (i.e., transplant water which contained acephate) which occurred on JB01 but did not occur on TW02? If so, why wasn't this issue discussed and/or explained?

Sample Collection 3. Day 0 Samples

EEB Comment

Sample collection should have been conducted immediately following application and on the same dates. In some cases samples were taken up to 254-hours post-treatment and on different days.

Registrants' Rebuttal

"Due to weather and planting schedules of growers it is frequently impossible to guarantee planting dates and times. Furthermore,...it is not always practical to treat all fields on the same day. In addition... some fields are sprayed late in the afternoon making it impractical to collect Day 0 samples."

"Note that tobacco foliage consistently had the highest acephate residues. Comparisons of residue values observed in tobacco foliage collected
immediately after application and those collected one day after application show no differences that could be ascribed to the time of collection. Therefore, these data suggest that samples collected one day after treatment are comparable with those collected immediately after treatment."

**EEB Response**

The EEB realizes that there are a great many difficulties in the conduct of a field study. However, it is the responsibility of the testing facility to insure that study design is such that the data required by the Agency can be collected. As such, sufficient control over the conduct of the study must be maintained to ensure that grower cooperation on such issues as planting dates and application timing are agreed upon before hand in order to ensure that the study will satisfy the data requirement.

The Registrant argues that no differences could be ascribed to time of collection and that residues collected one-day post treatment are comparable to residues collected immediately after treatment. The EEB wonders what the Registrant means by comparable. For instance, mean residues for TW01 at +1 day was 139.7 and 51 ppms for the first and third foliar applications, respectively (See attachment 9). Is this comparable to 79 and 40.7 ppms for day 0 residues on the fourth and fifth application on TW02? These data suggest +1 day residues are higher than residues immediately following application?

**Sample Collection 4. Transplant Equipment/Variability**

**EEB Comment**

The report mentioned that Orthene was applied in the transplant water at the same rate with the same equipment on TW01 and TW02 but that different equipment was used to treat field JB01. The great variation in residue levels both within and between fields suggest that the type of equipment used and the operator can greatly influence the amount of exposure.

**Registrant Rebuttal**

"We concur with the EEB that equipment and operators can contribute to variability in residues. However, that does not appear to be the
case in this study. Table 1 of this discussion shows that greater variability occurred between TW01 and TW02 following the first foliar spray than is shown between TW01 and JB01. Thus the greatest variability was observed between fields having the same equipment and the same operator (refer to report pages 13 and 14). Variability of residue data both within and between fields may also result from other factors such as soil moisture and texture, vitality of transplants, root system development and vigor, and plant adsorption kinetics.

Even though variability occurs, the density of wildlife food items are extremely low during the transplant stage of tobacco culture. While we don't disagree that it might be desirable to use a single piece of equipment, we believe that it is sometimes impractical or unnecessary to do so. Therefore, we see no problem from using two kinds of transplant equipment as was done in this study.

EEB Response

The greatest variation observed between fields did not occur between TW01 and TW02, after the first foliar spray, but, surprisingly, between JB01 (mean = 60.3 ppms) and TW02 (mean = 0.11 ppms) one day prior to the first foliar application (See attachment 9). The EEB speculates this was the result of different equipment/ agricultural practice/ and/or operator technique used to transplant the plants. The point is that there is tremendous variation that is inherent in the application of pesticides, that can occur even when precautions are taken to reduce it as much as possible, without introducing additional variation by using different pieces of equipment and/or agricultural practice.

Sample Collection-Crop Residue Profile

EEB Comment

A comparison of crop residue data, collected on 5/31 after the first foliar application, shows that there was a wide range in residue levels between the treated fields. These data show that the highest residues occurred after the first application when there was apparently residues remaining from the previous transplant treatments. What concerns EEB is that the residues on TW02 were extremely low as compared to TW01 and JB01.
Registrant Rebuttal

We agree with EEBs' assessment that higher residues occurred following the first foliar spray. However, we do not feel that it can be assumed that the higher values were due to transplant application. The first foliar application was made using a smaller spray volume than later applications. Tobacco plants were also much smaller at the first spray than at later sprays. The combination of a more concentrated spray mix and the smaller plants could have caused the higher observed residues following the first foliar spray.

The lower residues values on TW02 may be due to crop phenology. It was noted in the report that TW02 was planted prior to arrival of study personnel. What may not have been evident from the report was that there was a different crop phenology for TW02 than for the other two fields. At the time of first foliar application tobacco plants on TW02 were considerably larger than TW01 and Jb01, thus the same amount of product was applied to larger plants. This could have resulted in the lower observed residues on TW02.

EEB Response

The EEB agrees that crop phenology and spray volume are factors that can account for the variation in the level of residues likely to occur, however, it does not appear that either of these factors accounted for the variation in this study. According to the data table, Jb01 had residues ranging from 17 to 146 ppm, as compared to residues on TW02 which were 0.14 ppm or less, one day prior to the first foliar application (See attachment 9). How could spray volume or crop phenology be a factor prior to treatment? Therefore, the EEB can only assume that this difference was the result of acephate being in the transplant water and that the different agricultural practice/equipment used on each field caused the variation. The critical question is, given the tremendous variation that can be introduced from such factors, why was the study conducted under these conditions in the first place?

Sample Collection 6. Off-Site Noncrop Vegetation

The Registrant has adequately addressed this issue.

Sample Collection 7. Soil Samples
EEB Comment

Soil samples should have been collected from the top 1/10 inch of soil rather than 5 cm deep. Collecting soil cores sampled at a depth of 5 cm is not representative of residues that birds are likely to be exposed to.

Registrant Response

We question EEB's assumption that birds are not exposed to residues in soils at depths greater than 1/10 inch, particularly if birds were foraging on invertebrates. However, even though samples were not collected from the top 1/10 inch of soil, the amount of residue that might occur in the top 1/10 inch of soil can be calculated from the data provided.

EEB Response

Using the Registrants' own argument, the EEB can only ask why didn't they collect 10 cm or even 1 foot deep soil samples (i.e., Certain avian species may be exposed to invertebrates that are going as deep as 1 foot in the soil (i.e., earthworms)? Why did they select only 5 cm? Is this really representative of what non-target wildlife would be exposed to in tobacco fields?

Soil surface residues (i.e., top 1/10 inch of soil) are not only important from the standpoint of oral exposure (i.e., non-target wildlife consuming soil invertebrates) but also for identifying potential dermal exposure that could occur from "dusting" activities and/or through contact with wet soils. The EEB believes that collecting samples at various depths (including the soil surface) would have provided a broader spectrum of environmental concentrations likely to occur in soils that have been treated with acephate.

Sample Collection 8. Bird Brain Cholinesterase

EEB's Comment

Because typical cholinesterase levels in healthy birds can fluctuate by 10 to 20% at any given time, the EEB questions what value there is in collecting these data from apparently healthy birds to determine if there are differences in brain cholinesterase levels between treated and untreated areas? The EEB believes that birds impaired from brain cholinesterase depression would
probably be somewhat incompacitated and definitely not "flying around" the study area.

Registrant's response

Based on the data collected during this study, the Registrant concluded that Orthene did not have any effect on avian brain cholinesterase levels. The Registrant further provided a lengthy response which generally concluded that Orthene does not effect avian behavior even when such exposure causes cholinesterase inhibition at greater than 50%. In fact, the Registrant cited data for the bobwhite quail and mallard duck that shows no adverse behavioral activity in birds with brain cholinesterase activity levels depressed as much as 75%.

EEB Response

Brain cholinesterase samples were collected within a very narrow period of time near the end of the study (i.e., three days after the final spray). Based on the field residue data, sampling occurred when field residues were at their lowest. The EEB believes that it would have been more appropriate to collect samples throughout the course of the study, especially after each application.

The report further states, .."only birds known to be in physical contact with soil or vegetation within 50 meters of the field perimeter were collected for the treatment group. Based on this information, the EEB does not know whether or not the birds collected for analysis actually utilized the treated area? Was there any attempt to mark the birds in the area (i.e., patagial tags and or radio transmitters) to verify their utilization of the treated fields?

The EEB also questions the timing of the sample collection. According to the data table, the highest reported residues occurred one day after the first foliar application. Wouldn't it have been more appropriate to collect samples at this time rather than when field residues were at there lowest? Sampling should also have been conducted as soon after application as possible rather than waiting three days. As reported, the data, at best, are only indicative of cholinesterase levels three days post-treatment, after the final application, for birds that may or may not have actually utilized the treated area.
The EEB telephoned Dr. Elwood Hill, a wildlife toxicologist with the U.S. Fish and Wildlife Service at the Patuxent Wildlife Research Center for his opinion of our assessment (See attachment 10). Dr. Hill is a noted expert on brain cholinesterase inhibition and has extensively researched brain cholinesterase depression in avian species. The EEB described the sample collection procedure and the Registrant's position regarding the effects of acephate on avian cholinesterase levels.

Dr. Hill explained it has been his experience, as well as others, that birds suffering from cholinesterase inhibition generally are not very active and tend not to be moving about. He also noted that such birds are very difficult to locate and that unless one made..."an honest attempt to specifically look for dead or dying or otherwise debilitated birds, and not just look for any birds, that the data are not representative of a "worst case" scenario.

Dr. Hill also noted that, because birds are very mobile, ... " a simple collection of live birds from around the field perimeter doesn't really tell you a lot unless you know the birds history." As such, Dr. Hill felt that baseline data, on avian activity patterns, should have been gathered to insure that the animals collected for analysis had, in fact, utilized the treated area prior to collection. Unless these measures were taken there is no way of knowing whether the birds actually used the treated fields or were just "passing through" the study area at the time of collection. He reiterated his opinion that a lot of time should have been spent conducting formal carcass searches in and around the treated fields.

The EEB made its initial comment in terms of trying to determine the usefulness of the brain cholinesterase data for hazard assessment purposes. The Registrant's rebuttal argument has not convinced the EEB that simply collecting live birds "flying around" the treated area is sufficient for determining if exposure is causing brain cholinesterase inhibition.

Based upon the available data and the conditions under which it was collected, the EEB can only conclude, from this study, that brain cholinesterase levels in birds, that may or may not have actually utilized the treated fields, within three days following the final foliar treatment,
were not effected. This does not really address the "worst case" scenario (i.e., whether application of Orthene to tobacco fields causes brian cholinesterase depression to birds that actually utilized the fields immediately following application.) as required for the conduct of a hazard assessment.

The EEB believes that since there were no attempts to formally conduct carcass searches and/or make behavioral observations, there is no way of knowing whether or not mortality occurred. Likewise, since there were no formal attempts made to observe for any behavioral effects the EEB cannot conclude, based on the data provided, that such effects did not occur. Therefore, the EEB must conclude that the these data do not adequately address the issue.

102.0 SUMMARY

The EEB has completed a review of the Registrant's rebuttal arguments to our initial review of a field monitoring study designed to collect field residue data on the use of Orthene in tobacco. In conducting this review the EEB has contacted various "experts" on tobacco agricultural practices as well as some noted ecotoxicologists for their opinions and comments relative to the design and conduct of the study. In addition, the EEB has spent considerable time revisiting the Acenaphthylene Standard and EEB file, rereading the terrestrial field study Guidance Document, and internally discussing the issues with the terrestrial field study team.

Based upon these discussions and rereview of the entire study, the EEB must again conclude that the study was poorly designed and that the Registrant has failed to adequately rebut the EEBs original comments (See Section 101.0 for discussion).

Therefore, the EEB maintains that the study is "Invalid" and that it cannot be used to satisfy the data requirement as specified in the Standard.

Richard W. Feltbusken, Wildlife Biologist
EFED/EEB

Norm Cook, Head Section 2
EFED/EEB

Antony F. Maciorowski, Chief
EFED/EEB
Telephone Record

Dr. Bob Liedy, Director (919) 515-3391
N.C. State University
Pesticide Research Lab

I asked Dr. Liedy what major use of lanniate on tobacco. He cited a brochure (Flue-cured tobacco, 1992 Information by the North Carolina Cooperative Ext. Service) that stated that lanniate was primarily used as remedial control against aphids, cutworm, budworm, flea beetles, grasshoppers and hornworms.

2. Deposition Cards

Bob asked Dr. Liedy his position on use of spray deposition cards. As Director of The Lab he specified that deposition cards are routinely used in the conduct of any field study and that extension personnel are instructed to do this in training sessions. He pointed out that studies show there is a tremendous amount (less) that never gets on the plant (about 80% or less) than what was applied.
Director, 
Paul Ross Liddy  NCS University (919) 515-3391 
Pesticide Research Lab.

Timing of applications —

Remedial use vs. preventive —

1992 Information on flue cured tobacco.

Brochure: Title:

Remedial control — Acephate
Aphids, cutworm, seedworm, flea
louse, grasshoppers, thorn worms.

Extension People - Training Session —

Dr. Sterling Sue Farmers, Head Entomology Extension (919) 515-2703 (2831) N.C. St.

Tremendous losses on plants, 80% or less. Very seldom get on plant what.
Telephone Record

Dr. Sterling Southam, Head
Entomology Extension
North Carolina State University

D. Remedial vs. Preventative Use

EDS asked Dr. Southam what was the major use of acephate on tobacco in N.C. He cited 1987 agricultural statistics which showed that 70% of growers used Orthene foliar spray an average of 2-3 # applications, while about 25% used 1 application in transplant water. He further pointed out that almost all the growers use published "threshold" levels for determining the timing and number of applications. He concluded that the majority of use was as a foliar spray (remedial) with very little use as preventative.
Dr. Sterling Southern: (919) 555-2703

Not very much use as preventative use (approach) by far its minority.

Tobacco aphid - treat at low threshold.
An early spray is effective.

True Prevents use
Transplant water

* Majority used as foliar spray in reduced fashion.
1989 Statistics
25% used 1 app. in transp. (preventive)
80% of growers used Orthene at least in foliar. Average # 2 to 2.5.
Almost all growers using published thresholds.
T.c.i. Typically only effect. for 4 weeks after plant. Shorty after control of yellow beetles (4 weeks)
five more (further south) more preventive.

* Georgia - 50-60,000
275,000 N, C
30,000 Va
Invertebrates

Invertebrates are important food items for wildlife. Due to their association with treated soils and vegetation, invertebrates are potential sources of exposure of wildlife to Orthene® Tobacco Insect Spray. Two types of groups were sampled: those collected by sweep netting, and those collected in pitfall traps.

At each station, ten pitfall traps were set at approximately one meter intervals centered around the station flag in a line parallel to the field boundary. Following the first collection period, the number of traps was expanded to fifteen per station to increase sample masses. Each set consisted of a plastic cup set in the ground (flush to or slightly below the soil surface) with a second liner cup and funnel collar.

Attempts also were made to collect earthworms by soil sieving. At each station, soil was sifted through a one-quarter inch wire mesh. Earthworm sampling was never found to be successful due to low earthworm densities in the upper soil layers.

Sweep net samples tended to be rather small. Sampling at perimeter stations was considerably more successful than at field stations. If sweeping for fifteen minutes proved unsuccessful, (which was usually the case at field interior stations) efforts were terminated. If efforts proved successful sweep netting was conducted for 20 minutes at each station. In addition to insects collected with sweep nets, any observed on foliage (particularly larvae) which could be collected with forceps were added to the sample. Vegetation which had accumulated in samples was later removed (to the extent possible) after the sample had been frozen.
PREFACE

This document is a technical paper intended to provide guidance on how to perform terrestrial field studies, those studies designed to address the potential adverse effects of proposed pesticide use(s) to nontarget wildlife. These studies are presented as outlined in § 71-5 of the Pesticide Assessment Guidelines, Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, EPA-540/9-82-024, October 1982. Such studies represent Tier IV, the most complex of the terrestrial tests presented in Subdivision E. They are required to support those pesticide uses the Agency determines are likely to result in adverse effects to nontarget terrestrial wildlife. Such studies consist of testing performed in the field under actual pesticide use conditions and, generally, they address the potential acute, subacute and/or chronic adverse effects of pesticide residues to nontarget mammals and birds. The effects to birds and mammals are emphasized because the lower-tier Subdivision E tests usually employ these organisms, but effects to other terrestrial organisms, such as amphibians and reptiles are also examined and considered. Terrestrial field studies, as discussed in this paper, are typically multiyear/multisite studies and consist of two levels of tests: a level 1 or screening study, which essentially determines if adverse impacts occur to nontarget wildlife under actual pesticide use conditions and a level 2 or definitive study, that quantifies those adverse effects identified in the screening study or from other information. Also, the Agency requires that these tests be performed only with nonendangered organisms and only in areas where impacts to endangered or threatened species will not occur.

As an amplification of § 71-5 Subdivision E, this paper discusses a variety of basic biological research techniques and wildlife investigative methods for use in assessing the effects of pesticides in the field. These methods and techniques are not new, for the majority of them have been used by wildlife biologists, fisheries biologists and game managers for decades. They are presented here, along with adequate references, in order to assist scientists planning to undertake terrestrial field studies. This document is intended to provide guidance (it is not a cookbook or checklist) and will be updated by the Agency as the state of the art for performing these studies advances.
planning field studies, one must be careful to consider the power of the study design to
determine the limitations of the study. Studies with adequate replication are highly
preferred to support registration; the use of less replication will not necessarily render the
study inadequate. However, what is objectionable is to use a study with low power to
imply no biological damage, when the study was not capable of detecting it if it occurred.
In cases where large numbers of replicates are impractical, subjective and biological
knowledge should be used in a decision process to decide if there was a treatment effect.
In most instances, it is highly advisable to involve statisticians or biometricians who are
familiar with this kind of field study in the planning and analysis phase of the field work
to avoid costly technical errors.

STUDY AREA AND SITE SELECTION

Selection of geographical areas and study sites within the areas for the definitive test
generally requires the same considerations as for a screening study. For the definitive
study, however, the selected areas and study sites must have adequate populations of the
species of concern. Obviously, the crop of concern must be grown on a representative
portion of the area. Also, consideration needs to be given to whether the target pest
species will be present. If it is not, one must consider what influence its absence may
have on potential results. For example, if the pest is a major food source for nontarget
species, its absence could significantly influence results. Finally, the potential variation in
populations of concern over the geographical area(s) selected should be considered. It
may be difficult to find sites that are sufficiently similar to provide paired plots, which
limits the coefficient of variation so that the desired sensitivity can be achieved.

NUMBER AND SIZE OF SITES

As suggested in the section on study design for the definitive test, the number of sites
will depend upon the species density on sites and the sensitivity required. Ideally, sample
size should be large enough so there will be an 80 percent probability of being sure to
detect a 20 percent difference when it exists. The size of the study site must be large
enough to provide adequate samples. The size depends on the survey methods used, sen-
sitivity required, and the density and range of the species of concern. For a paired plot
design the number of sites required is a function of the average density of the species.

In general, the breeding density of the species of concern can be used to provide a
rough estimate of the size of area needed to provide adequate samples. However,
preliminary sampling most likely will be required to verify the estimates.

METHODS

Essentially, the methods used in a definitive study are a means to quantitate
reproductive and mortality rates of animals on treatment and control areas. There are
many texts and monographs available on methods of sampling to estimate these para-
ters (see Appendix A). Anyone not familiar with the theory and principles of the
various techniques should review these references in depth. The objective of this section
is to provide a general guide to the various methods that could be used in a definitive
field study. In addition, these methods can be applicable to some screening studies.

The methods to be used in an individual field study will depend on the nature of the
identified concerns. Some methods are useful for investigating several types of concerns;
and most types of concerns can be studied by several methods. When the concern
becomes more specific (e.g., secondary hazards to raptors) or the use pattern and/or
<table>
<thead>
<tr>
<th>E #</th>
<th>Field</th>
<th>Date of Collection</th>
<th>Study Day</th>
<th>Mix No.</th>
<th>Date Received</th>
<th>Date Extracted</th>
<th>Date Analyzed</th>
<th>ppp (ug/ml) of Acephate Found</th>
</tr>
</thead>
<tbody>
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<td>TW-1</td>
<td>5/29/87</td>
<td>F1</td>
<td>1</td>
<td>6/04/87</td>
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<td>F5</td>
<td>8</td>
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<td>3/01/88</td>
<td>3/11/88</td>
<td>5369</td>
</tr>
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<td>6/30/87</td>
<td>F5</td>
<td>4</td>
<td>7/09/87</td>
<td>3/01/88</td>
<td>3/11/88</td>
<td>3187</td>
</tr>
</tbody>
</table>

**Tw-1**

| 1026 | 7,696 | 3,292 |
| 3,705 | 1,178 | 2,485 |
| 5,369 | 3,298 | 3,878 |
| 3,571 | 3,529 | 2,833 |
| 3,742 | 3,454 | 2,978 |
| 3,482 | 4,305 | 3,146 |

**Tw-2**

| 31 |

**JB-1**

| 7,696 | 2,833 |
| 3,292 | 2,875 |
| 3,292 | 2,978 |
| 3,125 | 2,849 |

9069
Response

The residue of 0.10 ppm on TW02 for 5/31 is a pre-spray value for the day prior to the first foliar application (Day -1) and not postapplication. The correct maximum value observed one day postapplication (first spray) for TW02 was 49 ppm not 0.10 ppm (attached Table 1.)

We agree with the EEB's assessment that higher residues occurred following the first foliar spray. However, we do not feel that it can be assumed that the higher values were due to the transplant application. The first foliar application was made using a smaller spray volume than later applications. Tobacco plants were also much smaller at the first spray than at later sprays. The combination of a more concentrated spray mix and smaller plants could have caused the higher observed residues following the first foliar spray.

The lower residue values on TW02 following the first foliar spray may also be attributable to crop phenology. It was noted in the report that TW02 was planted prior to arrival of study personnel. What may not have been evident from the report was that there was a resulting different crop phenology for TW02 than for the other two fields. At the time of first foliar application tobacco plants on TW02 were considerably larger than on TW01 or JB01, thus the same amount of product was applied to larger plants. This could have resulted in the lower observed residues on TW02.

Sample Collection 6. Off-Site Noncrop Vegetation

The EEB questions why off-site noncrop vegetation was sampled for residues. These samples would only provide data on how much of the chemical drifted and/or possible translocated from runoff or some other indirect route, from the treatment site, but would not provide data on residues that could occur if noncrop vegetation was sprayed directly. In this case, contamination from drift would be expected to be minimal because the pesticide was applied with ground equipment.

A much better method for determining where the highest levels of noncrop contamination would likely occur would have been to sample the vegetation growing in those rows left vacant for crop maintenance and access and/or that vegetation growing between the tobacco rows. Although wildlife utilization of tobacco fields is relatively low, the greatest amount of use would likely occur on these areas. In addition, these areas could harbor residual pest or other invertebrate populations that could be utilized by wildlife.
Sample Collection

Residue samples were collected from tobacco fields and their surrounding habitats at the designated sample collection stations. Samples of soil, nontarget vegetation, tobacco, invertebrates and water were collected because they represent potential sources of exposure to wildlife.

Soil and plant foliage (crop and noncrop) samples were collected within approximately three meters of sample stations except for JB01. As vegetation invaded the bare soil between the crop boundary and the adjacent habitat on JB01, sampling progressed towards the crop boundary except for pitfall cup samples which were collected on the originally established trap line. When it was not possible to collect the required amount of invertebrates, plant reproductive tissues, or water within three meters of sample stations, the radius was increased as necessary. Samples were labelled and immediately placed on wet ice until they could be transferred to a freezer. Vegetation was collected and stored in Ziploc® bags, while other materials were placed in Nalgene jars or bottles. Samples were shipped on dry ice via air express to EN-CAS Analytical Laboratories, Inc. for residue analysis.

Table 1 lists samples collected during the study. Whenever possible, samples to be composited were collected from stations in equal proportions. All samples collected were composites with the exception of crop vegetation. Material collected from stations 1 and T1 on JB01 were placed in separate containers due to the changes in station locations. Descriptions of sampling techniques are given below.

Soil

In adjacent habitat, seven soil cores, approximately 5 cm in depth, were taken at each station using a 2 cm diameter stainless steel hand corer. Soil cores were composited to form a minimum sample mass of 500 g.
Table 1. Acephate Residue Values

Acephate Residues (ppm) in Tobacco

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<th>SPRAY APPLICATION</th>
<th>SAMPLE DAY</th>
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<th>FIELD</th>
<th></th>
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<td></td>
<td></td>
<td></td>
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<td>TW01</td>
<td>TW02</td>
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<tr>
<td>1</td>
<td>-1</td>
<td>4</td>
<td>17</td>
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<td>&lt;0.10</td>
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</tr>
<tr>
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<td>-1</td>
<td>5</td>
<td>18</td>
<td>59</td>
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<td>146</td>
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<td></td>
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<td>0.98</td>
<td>3.26</td>
<td>2.6</td>
<td>0.89</td>
</tr>
</tbody>
</table>

(-) indicates sample not collected, sample not analyzed or calculation inappropriate.
Dr. Elwood Hill, Wildlife Toxicologist
PWRC

3 Brain Cholinesterase Levels

The DBS asked Dr. Hill what he thought about collecting line hikes that were "flying around." The treatment area for collecting information on brain cholinesterase levels in birds. Dr. Hill believes that "a simple collection of line hikes in a field doesn't really tell you a lot unless you know the bird's history." He pointed out that unless this was done there is no way of knowing whether or not the birds collected actually used the treated field. They could have "just moved into the area."

2 Effect

Dr. Hill pointed out that birds suffering brain Chol. Tabh. are not moving around very much and are difficult to find, and that unless an honest attempt was made to specifically look for dead or debilitated birds, collection of line hikes is not very informative. He said that the researchers should have spent a lot of time carcass searches in and around fields. Former carcass searches should
have been conducted.

3 How much Depression (Behavior)?

The CBO asked Dr. Hill about Chevron's claim that certain lab tests show that at 75% inhibition caused no abnormal behavior. Dr. Hill said he "would not expect "on average" to see 75% inhibition under field conditions, and see no abnormal behavior.

4 Summary

Dr. Hill concluded that in his opinion, unless the researchers "looked specifically for less mobile or debilitated fluid", the study does not reflect "worst case" scenario.
Wandy Hill - Patient
Mary Kings -
Brain Cholinesterase Levels

Somewhat Suspect:

Control vs. Treatment

If nothing else finds become subdued,
(Muiran + Green)
""Want once are hungered down or not moving much. Hard to find the kind that are the most effect.

We haven’t attempt made to look for delirium or just took any of the kinds."

Question: Would not expect on average to see 75-80 initial + no abnormal behavior.

Check Henry: (503) 757-4840 Corpsis
Ken Stromberg:

"Can Oxtelum Kill bugs? Even with 75-80 Inhib. They’re saying that Oxtelum does not kill bug. 
Just an idea.
Compensation for Inhibition

Is it possible that a bees system can "acclimate" to 85% inhibition.

Acclimation not specific to Orthene.

"None appeared to look for death with this liquid."

"Look specifically for less mobile and deliriated kinds, unless they die. This is not "worst case" scenario.

CT: Less in ones that are flying.

"Under what circumstances, can a kind have 75% inhibition & be normal?"

"Kill Data"

Wooly will.

"Just moved into an area.

Plant - Methamidophos from Orthene.

Sprayed - animals come + go. Long time after.

If Civil, Inti is rather gradual then they can coexist."
Other meth.
Animal can coexist.

Died shortly after exposure

Ex: 60% Chl. Inhib. in animal

Immediate post exposure marked Inhib.
Once exposure stops

Quest: is Chl. going up or down?

Recovery phase
Dropping phase

No way of knowing when collecting live animals. Can confuse the issue simply don't know.

Mice: small territory
Birds: very mobile.

Live animals also dead animal.
Day of application \( \rightarrow \) potential recovery.

Must have intensive carcass searches

Physiological override to handle neurological
Should have spent a lot of time scanning searches in and around leads. Should have been required to conduct formal searches.

A simple collect of leads:

The field doesn't really feel your alot unless you know this history.

Dr. Elwood Hill - 410