PN-ABA-036

Pesticide Testing 87/07/02 ARE

TECHNICAL PLAN
FOR
TESTING PESTICIDES AGAINST O. SENEGALENSIS
FOR BOTH ENVIRONMENTAL IMPACT
AND EFFICACY
IN THE REPUBLIC OF MALI

PREPARED BY:

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ROCKVILLE, MARYLAND

FOR:

USAID
BUREAU FOR AFRICA
OFFICE OF EMERGENCY OPERATIONS

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This report outlines a technical plan for testing the efficacy of eight pesticides on the Senegalese grasshopper (Odaelus Senegalensis) in Nara-Dilly area of Mali, and for monitoring the effects on beneficial flora and fauna, determining pesticide residuals, and assessing possible effects of bioaccumulation and magnification.

The plan is organized as follows:

I. Purpose
II. Selection of Test Chemicals
III. Schedule
IV. Efficacy Testing
V. Impacts on Beneficial Insects and Arthropods
VI. Impacts on Fauna and Flora
VII. Residuals
VIII. Safety Procedures
IX. Staffing
X. Reporting

I. PURPOSE

The project proposed for Mali is part of a larger USAID scientific program to test the efficacy and environmental effects of certain pesticides used, or being considered for use by the U.S., other international donors, and African countries to combat the threat of grasshoppers and locusts. The program is being coordinated with other pesticide testing programs through the Food and Agriculture Organization, Rome. The findings are expected to contribute to decisions concerning the pesticides to be used in future AID-funded control programs, and will also contribute information for use by other donors and African countries.

The program will consist of projects in three zones of Africa: West Africa, East Africa, and Southern Africa. The Republic of Mali is being proposed as the site of the West African project.

The program will work on four species of locusts and the Senegalese grasshopper. It will:

A. Test the efficacy of eight pesticides,

B. Monitor the effects of the pesticides on beneficials, including grasshopper/locust predators and parasites, and other beneficial fauna and flora.

C. Monitor and estimate any impact of residues of the pesticides in the environment.

II. SELECTION OF TEST CHEMICALS

A. AID identified the following pesticides as candidates for the testing program following discussion with the U.S. Environmental Protection Agency, the Food and Drug Organization, and others:

Pesticide	Class
Carbaryl (Sevin)	Carbamate
Carbosulfan (Marshal, Advantage)	Carbamate
Chlorpyrifos (Dursban, Lorsban)	Organic Phosphate
Lambda-cyhalothrin (Karate)	Pyrethroid
*Acephate (Orthene)	Organic Phosphate
*Bendiocarb (Ficam)	Carbamate
*Diazinon (Diazinon)	Organic Phosphate
*Propoxur (Baygon)	Carbamate
Fenitrothion (Sumithion)	Organic Phosphate
Malathion (Malathion)	Organic Phosphate

*Candidates for two open slots.

B. The following additional pyrethroid pesticides were proposed for consideration by their manufacturers:

Pesticide	Manufacturer	Date
Cyfluthrin (Baythroid)	Mobay	April 7, 1987
Alpha-cypermethrin (FASTAC)	Shell	May 21, 1987
Tralomethrin (Scout/Tralate)	DuPont	June 4, 1987

C. Based on a preliminary review of available information, the general characteristics of each pesticide class are presented below:

- Carbamates - The carbamates are residual insecticides of moderate to low mammalian toxicity (toxicity category II). Atropine (but not 2-PAM) is the antidote. The LD_{50} (male rat, acute oral) for bendiocarb is 40-156 mg/kg; for carbaryl is 283 mg/kg; and for propoxur is 95 mg/kg. The dermal LD_{50} for these compounds will run 500 to 2000 mg/kg (toxicity category III). They are generally not oncogenic but are suspect teratogens and weak mutagens. They are rapidly excreted by animals, disappear from plants by mechanical attribution, volatilization, and uptake into the plant. They do not seem to be appreciably photodegraded, but are degraded by soil organisms through hydroxylation of the side chain and ring structures. The carbamates are of low toxicity to birds, moderate toxicity to fish, and high toxicity to honeybees, aquatic invertebrates and estuarine organisms.

- Organic Phosphates - These pesticides are broad-spectrum contact insecticides of high to moderate acute toxicity to mammals. Of those in the above list, the LD_{50} (male rat, acute oral, mg/kg) values are: acephate, 866-945; chlorpyrifos, 97-270; diazinon, 300-400; fenitrothion, 800; and malathion, 1000-1375. Some of the most highly toxic pesticides known are organic phosphates, and yet compounds such as malathion are relatively safe to use. The organophosphates are cholinesterase inhibitors: atropine and 2-PAM (pralidoxime chloride, iodide or methanesulfonate) are the common antidotes. This group of pesticides is so broad that few

generalizations are valid for all member compounds; however, the organic phosphates tend to show little or no oncogenicity, teratogenicity or mutagenicity. Environmental toxicity effects vary depending upon the compound. Diazinon, for example is highly toxic to wildlife (birds, fish, aquatic invertebrates); its use has caused serious bird kills after applications to golf courses. In contrast, malathion has been applied by aircraft over broad areas of the United States for control of imported fruitfly outbreaks with little or no evident environmental problems (except for damage to automobile paint). Chlorpyrifos is highly toxic to wildlife and honeybees. The organic phosphates are photodegradable, but can be leached into the soil where they tend to have rather long half-lives.

- Pyrethroids - These compounds are based on "pyrethrum," a naturally-occurring insecticidal complex derived from certain species of chrysanthemum. Pyrethrum consists of pyrethrins (esters of pyrethrolone and chrysanthemic acid and pyrethroic acid), cinerins (esters of cinerolone and chrysanthemic acid and pyrethroic acid), and jasmolines (jasmoline and chrysanthemic acid and pyrethroic acid). They are characterized as being resistant to photodegradation, and are applied at extremely low active ingredient dose rates (in the range of 0.05 to 0.3 lb a.i./acre). They generally have low mammalian toxicities. Like the organophosphates, many pyrethroid products are classified by the EPA as Restricted Use Pesticides for some or all uses.

D. Of the chemicals listed above, six were identified for testing in the AID Scope of Work: Carbaryl, Carbosulfan, Chlorpyrifos, Lambda-cyhalothrin, Fenitrothion, and Malathion. Four chemicals were identified as candidates to fill the remaining two testing positions: Diazinon, Bendiocarb, Propoxur, and Acephate. Three additional chemicals nominated by manufacturers did not appear in the AID Scope of Work: Cyfluthrin, Alpha-cypermethrin, and Tralomethrin, .

A preliminary literature review and evaluation was conducted on all candidate chemicals as a basis for designing the field program and for selection of the eight pesticides to be used in the West Africa testing program.

It is the Africa Bureau Policy "that any AID-financed pesticide for locust/grasshopper control be registered with the EPA and have a tolerance established for at least one food use, or meet the established acceptable daily intake and maximum residue level recommended by the Joint Meeting on Pesticide Residues to the FAO/WHO Codex Committee on Pesticide Residues." The EPA registration and Codex status of the candidate pesticides are presented below:

Candidate Pesticide	EPA Registration	Food Crop Tolerance (40 CFR 180)		FAO/WHO Codex ADI/MRL
		yes	no	
Carbaryl*	✓	✓		✓
Carbosulfan	pending**		✓	✓
Chlorpyrifos*	✓	✓		✓
Lambda-cyhalothrin*	pending**		✓	✓
Acephate	✓	✓		✓
Bendiocarb*	✓		✓	✓
Diazinon*	✓	✓		✓
Propoxur	✓		✓	✓
Fenitrothion*	✓		✓	✓
Malathion*	✓	✓		✓
Alpha-cypermethrin			✓	
Cyfluthrin	pending**		✓	
Tralomethrin*	✓	✓		

*Proposed for West Africa testing program.
**Section 3 application submitted; data under review.

Manufacturers of these products were contacted to determine their continued interest in the testing program and identify any logistical problems that would prohibit their product from being available within the appropriate timeframe for testing in West Africa. On the basis of product availability, satisfaction of the Africa Bureau technical criteria, general balance among the three classes of pesticides to be evaluated, and consideration of potential environmental impacts and efficacy as determined by the initial literature review, the following compounds were selected for field testing in West Africa:

Pyrethroids	Carbamates	Organophosphates
Lambda-cyhalothrin (Karate)	Carbaryl (Sevin)	Chlorpyrifos (Dursban)
Tralomethrin (Scout/Tralate)	Bendiocarb (Ficam)	Diazinon (Diazinon)

Test Standards

Fenitrothion (Sumithion) - organophosphate
Malathion (Malathion) - organophosphate

The five candidate pesticides not selected for field testing in West Africa, Carbosulfan, Acephate, Propoxur, Alpha-cypermethrin, and Cyfluthrin, will be placed on a backup list. Additional information on these chemicals will be requested from EPA and the manufacturers of the compounds. The rationales for the elimination of these pesticides from the West Africa field testing program follow:

- Carbosulfan - This compound does not appear to completely satisfy the technical criteria. Although Codex ADI and proposed temporary MRL are established, EPA registration is still pending. The other members of the carbamate class selected for West Africa, Carbaryl and Bendiocarb, more fully satisfy the technical criteria in that both have Codex ADIs/MRLs established and both have EPA registrations; Carbaryl also satisfies the domestic food crop tolerance criterion. In addition, there is concern for the potential heath and environmental toxicity of the major degradate of carbosulfan, carbofuran.

- Acephate - This compound was not recommended for field testing in West Africa because: (1) there is no documented record of the manufacturer's expression of interest in participating in the field testing program; and (2) there are concerns for the potential health effects of acephate (i.e., the compound is a suspected human oncogen) and its metabolite methamidophos is highly toxic to birds.

- Propoxur - Propoxur was not recommended for inclusion in the West Africa field testing program because there is no documented record of the manufacturer's expression of interest in participating in tne program. This chemical has been widely used in Africa for locust control; it is a good candidate for the East and Southern Africa field testing programs pending expression of interest by the manufacturer. EPA has expressed some concern for the potential oncogenic activity of the compound that needs to be addressed pending receipt of additional information.

- Alpha-cypermethrin - This compound was not selected because it does not appear to satisfy the technical criteria (i.e., no EPA registration or Codex tolerances). Concern has also been expressed about the mammalian toxicity of the compound. More data will be requested from the manufacturer.

- Cyfluthrin - Cyfluthrin does not satisfy the technical criteria as completely as the pyrethroids selected for testing. For example, Tralomethrin has an EPA registration and a domestic food crop tolerance established. Lambda-cyhalothrin, like Cyfluthrin, has an EPA registration pending, but unlike Cyfluthrin, also satisfies the Codex tolerance criterion. Lambda-cyhalothrin has also been field tested in Africa for locust control. Cyfluthrin appears to be a good candidate for the East and Southern Africa field testing programs. By the time these programs are underway, the compound may more fully satisfy the AID technical criteria; e.g., the manufacturer has stated that pending receipt of domestic registration, an application will be made for domestic food crop tolerances. The manufacturer has also stated that EPA registration is expected to be approved in the next several months. We will continue discussions with the manufacturer of Cyfluthrin in order to update the status of this candidate for future use.

III. SCHEDULE

A. The test mission would break down into two phases involving small (16ha) and large (256ha) plot trials. Each phase will involve site survey and delineation, spray activities and the actual efficacy and environmental sampling. The timeframe of the Phase 1 (small plot) trials is 10 days for survey and pretest sampling, 12 days for spray and 40 days for posttest sampling. Since spraying and sampling occur simultaneously, approximately 50 days will be spent on this trial series. The Phase 2 (large plot) trials will take approximately 36 days to complete. These numbers are approximations and could vary either way by as much as 20 percent. We believe though, that the Dynamac field team would be in the country on the order of 95 days.

B. The field testing schedule, which is governed by rainfall patterns and subsequent grasshopper outbreaks is expected to be approximately as follows:

- Small Plot Trials: Mid-July - Early September
- Large Plot Trials: Mid-September - Mid-October

However, the presence of testable populations may in actuality require departing from the above schedule. Flexibility will be required to account for this.

C. For the Phase 1 tests in the Nara-Dilly area, plots will be selected to the extent possible that have a significant percent under agricultural cultivation. This will permit testing under the same conditions that are expected to be encountered in the control campaign.

For the Phase 2 tests, it is anticipated that a major percent of the test plots will be grassland, because of the larger areas involved.

IV. EFFICACY TESTING

A. The efficacy testing protocols outlined below reflect discussions with USDA/APHIS or USDA/ARS personnel.

1. Experimental Design

The preliminary design allows for the evaluation of at least eight of the most promising materials under "Small Plot" conditions. Plot size should be 16 ha. (39.5 acres). Treatments will total 16 (8 insecticide x 2 dosage rates) each to be replicated 4 times in a randomized complete block design.

Those materials offering greatest promise on the basis of small plot tests will be retested in "Large Plots." Should the Phase 1 tests warrant, at least three of the "experimental" pesticides and one "test standard" will be included in Phase 2. Plot size in this instance will be 256 ha. (633 acres), and each treatment will be replicated three times. One dosage rate will be tested. This will be the lowest rate to prove effective (80% kill or better) in the Phase 1 trials. As above, a complete block design will be utilized, but there will be subsampling within each plot.

2. Data Analysis

As with plot design, analysis of the data will concur with the protocol and recommendations of USDA/ARS and USDA/APHIS. This will include an analysis of variance with a mean separation using the Duncan Multiple Range Test and a probability level of 0.05.

3. Application Parameters

a. Application Rates - Application rates will be in accordance with the manufacturer's recommendations for the maximum ULV dosage rate. Where the manufacturer has also recommended an alternative, lower rate for testing, this will serve as the

second application rate. Where this has not been done, we will use 50 percent of the recommended dosage for the alternate rate. Application will be with micronair nozzles to achieve uniform droplet size.

b. Serial Treatments - The series of treatments for each pesticide will be made in early morning or late afternoon to minimize spray drift caused by inversions and midday winds.

c. The application will be done in accordance with U.S. standards concerning narrow swath width, precision spraying. However, modifications may be necessary to take into account wind speed.

4. Evaluation Procedures

a. Sample Unit - The sample unit consists of a 0.25 m^2 ring. Forty such rings will be placed at random in a 50-meter diameter circle, i.e., about three meters apart on center.

b. Sample Interval - Target species will be counted at four intervals, including a pretreatment count, i.e., one to two days before treatment as well as at one, seven, and 14 days following treatment.

c. Controls (untreated checks) - Eight sample sites will be randomly located in the proximity of the test block. Using the 40-ring system, these sites will be sampled at the intervals discussed above. Statistical corrections will be used to account for normal population variations.

d. Developmental Stage, Species, Density - One hundred standard samples will be taken in the control areas at each sampling interval. Total grasshopper/locust counts will be made and tabulated according to life stage and species. Depending on population characteristics, the sample units may require modification.

e. Deposition and Droplet Size - To ensure a reasonable basis of comparison, it is necessary to validate spray deposition and droplet size. A suitable technique involving oil- or water-sensitive cards will be used. Ten cards per plot will be distributed just prior to application. Data will be reported as droplets per cm^2, volume median diameter, and droplet size spectrum in 50 micrometer-size classes.

f. Large Plots - Testing of large plots will begin approximately 14 days after completion of small plot testing. The above procedures will be duplicated in the large-plot tests. However, each large plot will be subdivided into quadrants, and each quadrant will be sampled as above.

VI. IMPACTS ON BENEFICIAL INSECTS AND ARTHROPODS

A. We will also evaluate the impact of the candidate pesticides on beneficial insects and related arthropod species. The beneficials will include grasshopper parasites and predators as well as parasites and predators contributing to the balance of other potential arthropod pest species.

B. In the absence of reliable information on the life histories and role of beneficials, the initial sampling plan is a general one. This will involve sampling closely linked to the efficacy testing as follows:

1. Baseline data for the control or check sites will be developed in collaboration with efficacy studies. Utilizing sweep samples (100 sweeps per sample area), pitfall traps ten meters apart in a 4 x 4 inch grid, and visual transects over 25m length by 1m wide, eight sites will be sampled in proximity to the test block. Sample intervals will be the same as those used in the efficacy tests.

2. Identical procedures will be followed in the pesticide test sites. Samples will be drawn from the center of the test plots at intervals of 1 to 2 days pretreatment and at 1, 7, and 14 days post-treatment. As in the efficacy tests, subplots will be sampled in the large plot tests.

3. The monitoring will also include an evaluation of each pesticide's impact on indicator species or other arthropod predators and parasites (i.e., those affecting species other than grasshoppers and locusts) and non-predatory or parasitic insect species (e.g., indicator food chain species).

C. Although the initial plan for monitoring impacts on beneficials is a generalized one, this will be paralleled by literature searches and consultations to identify important key or indicator species, vulnerable life stages, and other specific information. This review may lead to modifications in the monitoring program, or to recommendations concerning protection of beneficials in spraying programs.

VII. IMPACTS ON FAUNA AND FLORA

A. The following design for impacts on fauna and flora and for residual sampling (Section VII) is based on an initial review of available environmental effects and fate data for the candidate pesticides. The design outlined here is conservative; i.e., in the absence of sufficient information, worst-case assumptions were used, leading to sampling of all significant parameters and potential effects on all organisms (see Table 1). However, it should be noted that, although all the pesticides are potentially toxic to fish and aquatic invertebrates, we do not expect to encounter significant aquatic habitats in the Nara-Dilly test area.

It should be noted that additional information is expected to be available from EPA and the pesticide manufacturers. This information has been requested. The design will be modified appropriately upon review of these additional data. The design will also have to be modified when site-specific information becomes available, i.e., during the baseline evaluations of the selected test sites.

Table 1

Potential Sampling Parameters for Pesticides Selected for Mali

Candidate pesticide	Non-target Effects on						Residue Samples				
	Fish	Aquatic invertebrates	Non-target insects	Birds	Mammals & livestock	Plants & algae	Soil	Water*	Crops/ forage	Sediment	Milk
Bendiocarb[a]	X	X	X	X							
Carbaryl[b]	X	X	X			X		X	X		
Chlopyrifos[c]	X	X	X	X						X	X
Diazinon[d]	X	X	X	X	X				X		
Fenitrothion[e]	X	X	X	X	X	X			X	X	
Lambda-cyhalothrin[f]	X	X	X		X			X	X		X
Malathion[g]	X	X	X								
Tralomethrin[h]	X	X	X		X		X	X	X	X	

*Surface water and ground water, as appropriate.

Footnotes continued on next page.

[a]Environmental fate: Mobile; low bioaccumulation potential; rapid field dissipation. Environmental and health effects: Moderately toxic to mammals, toxic to fish, birds, pollinators. Acute oral LD_{50} rats 40-156 mg/kg. LC_{50} fish 0.7-1.0 mg/L. LD_{50} honeybee 0.1 ug/bee. Avian LD_{50} 3.1-137 mg/kg.

[b]Environmental fate: Moderately mobile in soils; mobile in runoff; not expected to contaminate ground water; rapid field dissipation; low bioaccumulation potential. Environmental and health effects: toxic to aquatic organisms and terrestrial non-target insects. Not phytotoxic; relatively non-toxic to birds. Acute oral LD_{50} rats 500-850 mg/kg. LC_{50} fish 1-10 mg/L. Avian LD_{50} 800 - >2000 mg/kg. LC_{50} aquatic invertebrates 1.7-1900 ug/L.

[c]Environmental fate: Persistent in soil for 1-2 months; sorbs to sediment/organics; immobile; fairly high bioaccumulation potential (BCFs 100-2000); fate data limited. Environmental and health effects: Non-phytotoxic. Toxic to aquatic organisms, terrestrial invertebrates, birds, wildlife. Acute oral LD_{50} rat 135-163 mg/kg. LC_{50} aquatic organisms 0.176 ppb-3.2 ppm. Avian LD_{50} 17.7-76.6 mg/kg.

[d]Environmental fate: Hydrolysis important fate mechanism; may sorb to organic matter; relatively non-persistent and immobile; low bioaccumulation potential. Environmental and health effects: Toxic to birds, aquatic organisms, terrestrial invertebrates, livestock. Special Review initiated by EPA in 1986 based on hazard to non-target birds from use on golf courses and sod farms. Acute oral LD_{50} rats 300-850 mg/kg. LC_{50} aquatic organisms 0.8-1700 ug/L. Avian LD_{50} 3.54-4.33 mg/kg.

[e]Environmental fate: Moderately mobile; quick field dissipation; low bioaccumulation potential; sorption to sediments/organic matter. Environmental and health effects: Toxic to aquatic organisms, birds, terrestrial invertebrates, mammals, plants. Acute oral LD_{50} rat 504-800 mg/kg. LC_{50} aquatic organisms 0.003-12 mg/L. Avian LD_{50} 23.6-1662 mg/kg.

[f]Environmental fate: Limited fate data available. Relatively immobile in soils. Field dissipation 14-60 days. Environmental and health effects: limited data available. Restricted use pesticide. Use of protective clothing and equipment required. Human flaggers are prohibited unless in totally enclosed vehicle. Following data for analog cypermethrin: Nonphytotoxic. Toxic to fish and aquatic organisms. Hazard to humans and domestic animals. Acute oral LD_{50} rats 250-310 mg/kg.

[g]Environmental fate: Moderate soil sorption. Rapid biodegradation, field dissipation (10-14 days). Low bioaccumulation potential. Environmental and health effects: Toxic to aquatic organisms and terestrial non-target insects. Acute oral LD_{50} rats 1000-1375 mg/kg. LC_{50} aquatic organisms 3.2-520 ug/L. Avian LD_{50} 167-1485 mg/kg.

[h]Environmental fate: Limited data available. Expected to be persistent, sorb to soils and sediments, and have potential to bioaccumulate on basis of chemical structure. Toxic to aquatic organisms and non-target insects. Acute oral LD_{50} rat 99.2-157.3 mg/kg.

B. Phase 1 (Small Plots)

1. Baseline Data

Small plot study sites (16 ha) will be selected to provide plots which are representative of areas subject to grasshopper infestations. In a typical savanna environment, two habitat types will be identified (open grassland and partly wooded grassland) in addition to cultivated fields. These habitats will be designated as blocks and two replicate plots for each treatment will be randomly assigned to each block. Two control plots will be selected from each habitat block.

The control plots and three treatment plots representing the range of habibat variability in each block will be subjected to baseline surveys. The physical characteristics at each plot will be recorded, i.e., topography, hydrology, soil types, meteorological conditions, and vegetation structure. Dawn and dusk visual surveys for animals (e.g. birds, mammals, reptiles, amphibians) will be performed along 400 m transects within each plot for five consecutive days. On each plot a 120 m mist net line will be run at dusk to census birds. Also on each plot a live-trap line (20 stations with 2 traps each) will be run at dusk.

These surveys will provide an inventory of plant and animal species, an identification of dominant species, and estimates of population sizes.

2. Posttreatment Sampling

Aerial spraying will treat 8 small (16 ha) plots per day. On the day of spraying, transect (400 m) surveys will be run on all 8 plots to record animals numbers and observe behavioral effects. On the following day, complete carcass searches will be performed on each plot. Where more than one death is observed for a species all carcasses (up to five) will be preserved for assays of pesticide residues.

On the 8th day posttreatment for each set of 8 plots, a second transect survey will be run. On the 16th day posttreatment, a third transect survey will be run. On control plots and those treatment plots sampled for baseline data, birds will be mist netted and small animals live-trapped on posttreatment days 1 and 14.

C. Phase 2 (Large Plots)

1. Baseline Data

Phase II study sites (256 ha) will be large enough to encompass all habitat types so that all replicates of all treatments can be randomly assigned. Two control plots will be designated. The baseline data from the small plot phase will have identified the general characteristics and principal plant and animal components of these plots. Preliminary sampling in the large plot phase will concentrate on obtaining good estimates of population size for birds, small mammals, and lower vertebrates. Surveys for all animals will be made along 800 m transects for each plot on two different days. Mist nets will be run in a loop at 240 m on each plot to capture and release small birds. Live trap lines (40 traps) will be run on each plot for 3 consecutive days. Mark-recapture (Bailey triple-catch) techniques will provide population estimates and produce marked individuals for posttreatment recapture. Pitfall traps (20 buckets along 20 m of flashing) will be run for 3 days in each plot. Captures will be marked.

2. Posttreatment Sampling

Aerial spraying will treat 2 large (256 hr) plots per day. Carcass searches will be done in each of 4 quadrants within each plot. Surveys along transects of 800 m will be run in each plot on posttreatment days 1, 3, 7, and 14. Bird mist-netting, mammal live-trapping and pitfall-trapping will be performed on each pair

of plots at posttreatment day 7. Each census technique will be applied to the control plots. In addition, carcass searches will be done on controls to determine the level of natural mortality and the efficiency of searching.

VII. RESIDUALS

Based on the characteristics of the chemicals to be tested and the low application rates involved in ULV application, we do not anticipate encountering significant residue problems. Nevertheless, "worst case" assumptions will be followed in the sampling program.

Therefore, the program will involve collecting a full suite of residual samples from: crops, soil, livestock milk, water and affected wildlife. Crop residuals will be sampled just prior to harvesting. The results of these analyses will be combined with information obtained from the literature reviews to provide best estimates of any potential bioaccumulation problems.

Procedures for conducting the residual sampling are set forth in Appendix I. It should be noted that all the crops and substrates listed in Appendix I will not be directly applicable to the tests in the Nara-Dilly area. The Appendix has been prepared to support the overall African program which will involve a wider range of ecological conditions.

VIII. SAFETY PROVISIONS

The following provisions will be made to assure safety of the project personnel and the Malian population in adjacent areas:

A. The pilots and applicator are certified for spray application of pesticides. As certified operators, they will be required to adhere to approved practices in handling and applying the pesticides.

B. All project staff will receive on-site training in safety procedures. The Manager, Field Operations will be responsible for providing the training.

C. During the spray operations, we will work with the representatives of the Plant Protection Service to assure that the population in the area concerned is aware of the activity and takes proper precautions to minimize exposure of humans and domestic animals. The test plots will be selected to avoid spraying in close proximity to villages, and pilots will be instructed to avoid spraying large herds of cattle.

IX. STAFFING

Staffing for the Nara-Dilly operation will be as follows:

A. Management and Coordination

- Manager, Field Operations - J. Atchue
- Logistics Coordinator - A. Stancioff
- Peace Corps Volunteer

B. Efficacy Testing and Beneficials Team

- Leader - G. Schaefers
- Members - D. Kolodny-Hirsh
  R. Campbell

C. Environmental Impact Team

- Leader - M. Southerland
- Members - G. Steiger
  J. Evans

D. Aerial Spraying

- o Pilots (2)
- o Mechanic

E. Participation by Government of Mali

It has been proposed that the Government of Mali participate, if possible, by:

- o Provision of six counterpart personnel (agricultural and environmental scientists) from appropriate ministries.
- o Assignment of a liaison officer to work with the Manager, Field Operations in maintaining communications and expediting clearances.

F. Other

We anticipate some additional on-site personnel, such as observors from some chemical companies and FAO.

X. Reporting

All field results will be fully documented. A report on the field tests will be prepared for USAID and presented to FAO in December 1987. Copies will be provided in French for the Government of Mali. The findings will be incorporated into a comprehensive final report at the completion of the overall African program.

APPENDIX I

APPENDIX I

Contract AFR-0517-C-00-7035-00

FIELD RESIDUE SAMPLING DIRECTIONS AND PROCEDURES

I. INTRODUCTION

The critical nature of conducting a specific program EXACTLY as requested to ensure proper samples for residue analysis cannot be over-emphasized. A sampling program is necessary because of residue data needs and is not a biological efficacy trial. If a particular study is unrealistic or confusing the questions should be brought to our attention IMMEDIATELY.

If you have any questions, please ask them. It is much easier to correct an error on paper than to correct it once the study is underway.

This set of directions contains guidelines on how to obtain representative residue samples. It is directed toward presenting only general principles for field sampling. It is NOT INTENDED, however, to issue an INFLEXIBLE set of step-by-step directions, due to the very diverse nature of problems that will be encountered in the field.

II. COORDINATION OF RESIDUE PROGRAM

All coordination and communication regarding the residue program should be directed through a Dynamac representative. If possible, any changes in the residue sampling program should be discussed and approved by Dynamac prior to the initiation of the change. Please contact:

_____

_____

_____

Tel. _____

Field Personnel collecting samples should receive the following:

1. Field Residue Sampling Directions and Procedures.
2. Specific sampling instructions for each type and sample to be taken.
3. Dynamac field trial number and a Dynamac experiment number.
4. Pesticide Residue Sample Record form.
5. Sample bags or other containers and sampling supplies.
6. Shipping boxes.
7. Shipping directions.

III. GENERAL SAMPLING GUIDE

RESIDUE DATA ARE NO BETTER THAN THE SAMPLES THEY REPRESENT. IT IS IMPORTANT THAT THE SAMPLES ACCURATELY REFLECT THE RESIDUES WHICH CAN BE EXPECTED FROM THE USE PATTERN BEING EXAMINED. AS A RESULT, SEVERAL CRITERIA CAN INFLUENCE THE QUALITY OF A RESIDUE SAMPLE:

A. Contamination

To prevent cross-contamination of samples, always use separate bags for each sample collected. Wash tools and knives between samples. Also, TAKE A CHECK SAMPLE (see below) FIRST and then sample in order of increasing treatment rates to minimize sample cross-contamination. For all root crops, rinse or brush the soil off the raw agricultural commodity (RAC) prior to placement into the sample bags. After rinsing, air dry the root sample. Chopping and other processing should be done on a clean surface using newspapers between treatments.

B. Degradation/Freezing

To prevent residue degradation, freeze all samples as soon as possible. This aspect of residue sampling is especially important because significant metabolism/degradation can continue even after taking the samples.

Ship all plant, biota, and soil samples frozen.

C. Collection of Untreated Check Samples

A CHECK (CONTROL) IS A SAMPLE NOT CONTAMINATED WITH PESTICIDES OF THE SAME CLASS (CARMBAMATE, PYRETHROID, ETC.) AS THE PESTICIDE BEING STUDIED.

Since check samples must be free from contamination with the pesticide under study, special care must be taken during the collection. Unless otherwise specified, always collect the check sample with the treated sample series.

One check of each type is sufficient for all samples of the same type collected from the same plot at the same time interval. As mentioned above, collection of the check first will minimize the chances of contamination.

Check samples representing all sample types collected from the treated plot must be sent. If an untreated sample is unavailable, a sample of the plot treated with another pesticide (determine that the specific chemical used will not interfere with the existing analytical method) is preferable to none; of course, indicate the treatment received. These check samples should obviously be representative of the treated samples. RESIDUE CHECK PLOTS SHOULD NOT BE TAKEN FROM AREAS TREATED WITH CURRENT TEST CHEMICAL IN THE PREVIOUS YEARS. This is especially true for soil samples.

D. Collection of Treated Samples/Plot Design

In general, the idea is to produce a sample of modest size that reasonably represents the entire treated area at the time of sampling. This requires the collection of units from the treated area in some regular and random fashion. No less than eight (8) randomly selected units (individual plant or parts of plants, biota, soil cores, etc.) should be considered adequate to represent a treatment under any circumstances. If eight units together exceed the bulk or weight requested, then some means of taking an aliquot of the total sample must be devised. For example, 1) chop, mix, withdraw a double handful, mix again and repeat withdrawal until the desired sample size is obtained; 2) quarter or eighth on the vertical axis and discard one or more quarters to obtain desired size sample that consists of an equal number of pieces from each unit sampled. Do not sample peripheral areas of the treatment plot and as far as possible, collect the individual units of sample from random areas throughout the plot. Avoid the edges of a plot and the ends of the rows. Do not take all large or small plants, rather take those plants that reflect average plant development at the particular sample interval of concern.

Replicate samples are to be taken if possible. Dividing one sample into three sub-samples is not a valid procedure and is a waste of the sampler's time. Replicate samples are individually collected, i.e., three sets of eight (8) units each.

See enclosed Specific Sampling Instructions.

E. Sample Containers and Their Identification

A MISLABELED SAMPLE IS A LIABILITY AND IT MAY INVALIDATE A WHOLE SERIES OF SAMPLES: AN UNLABELED SAMPLE IS WORTHLESS; USE EXPERIMENT NUMBER ON ALL SAMPLES.

Use Dynamac-provided sampling supplies. Do not try to put a 10 pound sample in a bag designed for 3 pounds. Send water samples in plastic jars inside foil-lined bags All sample containers shall be furnished by Dynamac.

F. Marking Plots for Long Term Studies

Residue trials should be staked and mapped showing measurements to permanent landmarks. These plots may have to be sampled during subsequent years and considerable time and money can be saved if the trials can be accurately located.

G. Identification of Sample Types

To avoid confusion, always use the following terms for residue samples:

Fodder	Crop parts of value as cattle feed, which remain after harvest of the desirable part, i.e., corn stems and leaves after removal of harvest ears.
Forage	Green, whole plants (above-ground parts) at any stage prior to crop maturity (harvest); corn, bean, pea, alfalfa, peanut, ryegrass and wheat samples prior to harvest.
Grain	The seed of a cereal crop such as wheat, oats, barley, rice and corn.
Hay	Usually whole and mature plants, e.g., alfalfa, which have been cut and dried until storable (less than 25% moisture). Refer to peanut, green and lima bean, pea and soybean plant parts (dried) after harvest of the desirable food parts as hay, also.
Processing Fractions	Items of food or feed produced by the further processing of raw agricultural commodity, e.g., corn grain - crude oil, meal; rice grain - hulls, bran, white rice; peanuts - crude oil, meal, refined and bleached oil, soapstock.
Straw	The "fodder" of a cereal grain crop which is used as bedding and not fed directly, i.e., dry rice and wheat parts left after harvest of the grain as straw. Field corn by convention will be called fodder, however.
Soil	Surface soil from plot areas.
Sediment	Soil collected form a body of water, i.e., river or lake sediment or hydrosoil.
Biota	Animal life collected from plot areas. Describe by type and species, if known.
Vegetation	Natural vegetation of plot areas, i.e., non-agricultural plants. Describe by type and species, if known.
Water	Identify by type, surface water, potable water, etc.
Body Fluids	Samples of blood, urine, etc. collected from humans or animals. Identify fluid type and species collected from.
Miscellaneous	Samples of unspecified type collected as necessary.

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IV. RESIDUE TRIAL INFORMATION

It is extremely that the documentation of residue sampling be filled out accurately and completely as soon as the residue sample is collected. Without this information, the residue samples are useless, and will be discarded.

A. Residue Sample Identification

The sample bag must be identified with the contents of the sample. The information must be consistent with that given on the residue form.

Since the sample bag is the only means of identification, it should be filled out completely after each sample has been taken. If it is incompletely filled out, the samples collected may be worthless.

The tag is composed of three parts. Upon completion the white copy is sealed inside a 3" X 5" plastic bag and then placed insided the sample bag. The yellow copy should be retained for personal records. If a bag does not have a tag attached to it, the cardboard tag can be stapled to the bag. If there is a tab sewn on the bag, it may be filled out as above, or the cardboard tag may be stapled to this existing tag. When the sample is collected not later, accurate notes should be written on the sample tag prior to transfer of this information to the Pesticide Residue Sample History Sheets. Accurate identification of the sample is critical.

Please press down firmly when filling out residue tags.

The Sample Number of the trial must correspond to that on the PESTICIDE RESIDUE SAMPLE HISTORY SHEET

The sample number is assigned by the sampler for each sample taken from an experiment. Samples are numbered sequentially within each experiment regardless of sampling time so that no test will have duplicate numbers.

The sample number is of the form xx-xx-xxxxx. The first two digits are the country and location code, the next two digits are the pesticide formulation code and the cost four digits are the sequential sample number. All codes will be assigned in the field by the Task leaders.

B. Pesticide Residue Sample History Sheets.

A form for collecting and transmitting residue trial information will be used. A History Sheet is a 4-page carbonless form. This form should be filled out as completely as possible. The WHITE & YELLOW copies should be forwarded to Dynamac. The PINK copy should be retained by the sender. The GOLDENROD copy is sealed in a plastic bag and enclosed in the first box of the shipment i.e., Box 1 of X. A copy of the form is attached.

RESIDUE SAMPLING PROTOCOL FOR AERIAL APPLICATION

Collection: Locate nine (9) sampling stations within the test plot area in a 'x' pattern. At each station, place a 20 x 20 cm square of what? 3mm paper in such a fashion that it is held horizontally approximately 2 feet above the ground. The paper should be located such that it can receive the direct impact of the aerial application.

Following application and drying of the test material, the paper squares are collected, individually wrapped in foil. Then combined in a single residue bag and properly labeled.

Be sure that each paper square is labeled by pencil with the test article, ? rate, time and date, sampling station, and plot number

Preservation: Store in cool area, refrigerate of possible. Freeze as soon as possible.

V. SHIPPING

A. Packaging

Each box should be packed to accommodate about 40 pounds of dry ice per box.

If multiple boxes are used, designate the number of boxes in bold ink on each box (1 of 10, 2 of 10, etc.).

Ensure that the copy of the Residue Sample Record is sealed securely in a plastic bag and is included in the first box (i.e., box 1 of 10 of the shipment). Make sure to reinforce the box(es) with appropriate tape.

B. Shipment

The residue samples must be shipped via air freight to the appropriate airport.

Each carrier may have its own form of "Shippers Certification for Restricted Articles" which must be completed when sending CO_2 is "ORM-A" and the Identification Number is 1845.

TO AVOID UNNECESSARY COMPLICATIONS, SAMPLES SHOULD BE SHIPPED NO LATER IN THE WEEK THAN WEDNESDAY.

Also indicate that the materials are PERISHABLE GOODS.

Notify the laboratory the same day as shipment. The samples are your responsibility until they are at the storage location. Follow-up to ensure that the samples arrive.

1. Carrier - airline, flight number
2. Airbill number
3. Number of boxes
4. Appropriate weight
5. Arrival date and time
6. Whether or not samples are packed in dry ice.

RESIDUE SAMPLING PROTOCAL

FOR

CITRUS FRUIT GROUP

Members: Grapefruit, kumquats, lemon, lime, mandarin, orange (sweet and sour).

Sample Size: 8-10 whole fruit, minimum 3 pounds (for larger fruit) per replicate.

12-20 whole fruit, minimum 3 pounds (for smaller fruit) per replicate.

Sampling Procedure:

1. Pick 8-10 whole harvestable fruit representing the entire treated area by random selection. Do not sample fallen fruit.

2. Bag fruit.

RESIDUE SAMPLING PROTOCOL

FOR

LEAFY VEGETABLES GROUP

Lettuce Sub-Group:	Celery, celtuce, endive, garden cress, lettuce, parsley, spinach, swiss chard.
Cabbage Sub-Group:	Broccoli, broccoli raab, brussel sprouts, cabbage, cauliflower, collard, kale, kohlrabi, mustard greens.
Sample Size:	8-10 heads or bunches, minimum 3 pounds per replicate.
Sampling Procedure:	1. Harvest one head or bunch from 8-10 random locations in the treated area. Field trim only browned or decaying outer leaves; include healthy wrapper leaves. 2. Bag heads or bunches. Reduce volume halving or quartering each unit, if necessary.

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RESIDUE SAMPLING PROTOCOL

FOR

GRAPES

Sample Size: 1-2 pounds grapes per replicate.

Sampling Procedure:

1. Select fresh, harvestable bunches (at least one dozen) at random. If necessary to reduce sample size, cut bunches in two with a knife, discarding one-half of each bunch, randomly. Freeze.

2. Bag grapes removed from the stem.

Process Fraction Samples:

Raisins:

1. Sample 30-50 pounds of grapes representing all three replicates and process into raisins. Withdraw a 1-2 pound subsample and freeze. Make raisins by laying grapes on large sheets of paper for approximately three weeks in the sun (simulates commercial practice).

2. Collect all dry material (raisins, stems and all waste) for shipment. This should yield 3-4 pounds raisins; 1/2-1 pound raisin waste.

RESIDUE SAMPLING PROTOCOL

FOR

SUNFLOWER SEEDS

Sample Size: 8-10 seed heads subsampled to yield 2 pounds of seed per replicate.

Sampling Procedure:

1. Harvest one head from 8-10 random locations in the treated area. Avoid taking samples from within three (3) feet of the ends of the row.

2. Remove seeds by shaking; combine seeds in one bag.

3. Bag seeds.

4. Ship approximately 2 pounds of seeds sampled randomly from #2 above.

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RESIDUE SAMPLING PROTOCOL

FOR

POME FRUIT

Members: Apple, crabapple, loquat, pear, oriental pear, quince.

Sample Size: 10-12 fruit (more if needed to make a 2-3 pound sample) per replicate.

Sampling Procedure:

1. Following typical practice for the area as to time of picking, pick at least 10-12 fruit per replicate. Randomly hand pick from top, middle, bottom and inside the tree. Describe picking process, if other than above, on residue forms.

2. Bag fruit.

RESIDUE SAMPLING PROTOCOL

FOR

TREE NUT GROUP

Members: Almonds, beechnut, brazil nut, butternut, cashew, chestnut, chinquapin, filbert, hickory nut, macadamia nut, pecan, black walnut, english walnut.

Sample size: Sufficient sample to generate 1/2-1 pounds of nut meat per replicate.

Sampling Procedure:

1. Following typical practice for area as to time and manner of picking, collect sufficient sample to provide amount of nut meat requested. If hand picking, randomly pick from the top, bottom, middle and inside of tree.

2. If harvesting practice involves contact of nuts with ground as a result of shaking or natural drop, sample nuts at random from the treated area. In either case, note the sampling technique on the residue form.

3. Provide a 1/2-1 pound nut sample for each replicate. For other than almonds, discard shell or hull.

4. Bag sample.

RESIDUE SAMPLING PROTOCOL

FOR

CEREAL GRAINS

Members: Barley, buckwheat, proso millet, oats, pearl millet, rice, rye, sorghum, wheat, wild rice.

Sample Size: 1-2 pounds of grain, 1/2-1 pound straw per replicate.

Sampling Procedure:

1. For forage residue samples; randomly cut plants 1-2 inches above soil line.

2. Harvest straw is also taken randomly but must have seed-head removed.

3. For hand harvested grains, randomly sample at harvest and remove seed heads. Strip seed head contents into a sample bag.

4. If mechanically harvested, withdraw sized random sample (1-2 pounds of grain) from the harvester.

RESIDUE SAMPLING PROTOCOL

FOR

COTTON

Sample Size: 1-2 pounds of ginned cotton seed.

Sampling Procedure:

1. When bolls are developed and at maturity, randomly pick sufficient cotton to produce a 1-2 pound sample of cotton seed after ginning.

2. Samples are to be ginned before shipping. Only cotton seed with linters are to be shipped.

3. Bag.

RESIDUE SAMPLING PROTOCOL

FOR

PEANUTS

Sample Size: 1/2-1 pounds shell; 1-2 pounds nut meat; 2 pounds vine per replicate.

Sampling Procedure:

1. At harvest dig peanuts in customary manner and field dry as customary for thrashing.
2. Collect a sufficiently large random sample for thrashing.
3. After thrashing collect sufficiently large trash including peanut stems (label peanut hay).
4. Shell the peanut. Sufficient nut plus hull sample must be collected to obtain 1/2-1 pounds of shell sample.
5. Bag separately nut, shell and vine samples.

RESIDUE SAMPLING PROTOCOL

FOR

SOYBEANS

Sample Size: 1-2 pounds of seed, 1-2 pounds of hay.

Sampling Procedure:

1. For forage samples randomly cut 8-10 plants two inches above soil level.
2. Separate the pods from the hay.
3. Chop the soybean hay for shipping.
4. Separate seeds from pods.
5. Bag separately seeds from hay samples.

RESIDUE SAMPLING PROTOCOL

FOR

CORN

Sample Size:

Field Corn:
- Forage - 1-2 lbs
- Silage - 1-2 lbs
- Fodder - 1-2 lbs
- Grain - 1-2 lbs (from at least 8 ears)
- Cob - 1-2 lbs (from at least 8 ears)

Sweet Corn:
- Forage - 1-2 lbs
- Kernel + Cob - 1-2 lbs (at least 8 ears)

Sampling Procedure:

Field Corn:

1. For pre-harvest samples in which the ears are immature cut eight or more plants 2 inches above the soil level. Chop into 6-8 inch sections and mix well in a plastic bag or on paper. Withdraw random handfuls (mixing again between handfuls) to produce a 1-2 pound subsample. Make sure the immature ears are chopped and represented in this subsample.

2. At harvest cut eight or more plants as above, remove the ear. Remove the husk from the ear, set the ear aside and combine the husk with the fodder. Chop the fodder into 6-8 sections; subsample as above.

3. Remove the grain from the cob. Ship the grain and cob separately.

RESIDUE SAMPLING PROTOCOL

FOR

WATER

Collection:

Tap Sampling

Flush the spigot for 3 to 5 minutes at moderate flow. For wells equipped with hand or mechanical pumps, run the water to waste for 3 to 5 minutes before the sample is collected. Fill a 500 ml polyethylene bottle with sample, cap, and label.

Grab Sampling

Collect a grab sample directly into a polyethylene sample bottle. Remove bottle cap and protect from contamination. Grasp bottle securely at the base with one hand and plunge it, mouth down, into the water, avoiding surface scum. The sampling depth should be 6 to 8 inches below the surface of water. Tip the bottle slightly upwards to allow air to exit and the bottle to fill. Remove bottle, cap, and label.

Preservation:

Store sample in cool location and freeze as soon as possible.

RESIDUE SAMPLING PROTOCOL

FOR

SEDIMENT

Collection:	Collect stream sediment or runoff sediment with a suitable device such as a polyethyline scoop or shovel. Select sampling points with a high probability for pesticide residues such as obvious siltation from runoff. Emphasize sampling of surface sediments. Place sample in 500 ml polyethyline bottle, cap, and label. Use sample water to wash container.
Preservation:	Sample should be cooled when collected and frozen as soon as possible.

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RESIDUE SAMPLING PROTOCOL

FOR

FISH

Collection: In the case of small fish, a sufficient number should be combined by sampling site location and species to obtain a 250 gram weight.

In the case of large fish, take a subsample of edible muscle and viscera (especially liver) from a number of individuals.

Preservation: The sample should be wrapped in aluminum foil, labeled, and placed in a sample bag. The samples should be frozen as soon as possible.

RESIDUE SAMPLING PROTOCOL

FOR

SOIL

Collection:	Collect surface soil samples from at least nine locations on a X pattern within the test plots. Use a clean polyethyline scoop to collect about 50 grams of soil that was directly impacted by the application. Soil should be sampled to a depth of 1 inch. Combine the 9 samples into a 500 ml polyethylene bottle. Shake to mix, cap, and label.
Preservation:	Samples should be coded when collected and frozen as soon as possible.

RESIDUE SAMPLING PROTOCOL

FOR

BIOTA/WILDLIFE

Collection:

Vegetation

Samples of test plot vegetation should be collected by hand. The sampled vegetation should be frim areas directly exposed to the aerial application. Collect 1/2 to 1 pound of vegetation which received direct impact of the applied material.

Wildlife

Samples of the adversely affected test plot wild life (small size birds, mice, etc.) should be collected. Individually wrap in aluminum foil, group by species, and bag. If organism size pesticides collection, try to obtain samples of liver and blood.

Insects

Grouping species, and bag.

Preservation:

Freeze sample as soon as possible.