ENVIRONMENTAL HEALTH PROJECT

ACTIVITY REPORT

No. 58

Entomological Assessment in
Blantyre District, Malawi, in Support of an
Insecticide-Treated Materials Project

January 18 - February 5, 1999

March 1999

by

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and
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Prepared for the USAID Mission to Malawi
under EHP Activity No. 463-CC

Environmental Health Project
Contract No. HRN-5994-C-00-3036-00, Project No. 936-5994
is sponsored by the Bureau for Global Programs, Field Support and Research
Office of Health and Nutrition
U.S. Agency for International Development
Washington, DC 20523

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ACKNOWLEDGMENTS

The authors wish to thank the assessment team members for their special effort in carrying out the entomological collection activities. The ability of the team members to explain our activities to the homeowners led to our being able to search for mosquitoes in numerous homes in the areas selected. Dr. Desmond Chavasse, BITNET Project Director, PSI/Malawi, was instrumental in shaping the final entomological work plan and in providing critical administrative support in the pre-assessment stage. The Environmental Health Project staff (Craig Hafner, Margo Kelley, and Pandu Wijeyaratne) provided essential technical, logistical, and administrative support that significantly contributed to the completion of the entomological activities. USAID staff in Washington and Malawi provided approval and financial support for this activity. The CDC BIMI Coordinator was very supportive of the activities and assisted in establishing official contacts in Blantyre.

ACRONYMS

AIMI	Africa Integrated Malaria Initiative, a USAID regional program
BAC	BIMI Advisory Committee
BIMI	Blantyre Integrated Malaria Initiative, a 5-year effort sponsored by USAID and the Government of Malawi
BITNET	Blantyre Insecticide-Treated Bednet Project, managed by PSI and funded by USAID
CDC	U.S. Centers for Disease Control and Prevention, Atlanta, Georgia
CHAPS	Community Health Partnerships, a project sponsored by USAID/Malawi
CHSU	Community Health Sciences Unit, under MOHP, Malawi
DPD	Division of Parasitic Diseases, a division of CDC
DHO	District Health Officer, employed by the MOHP
EHP	Environmental Health Project
GOM	Government of Malawi
HLC	human landing collection
HSA	Health Surveillance Assistant, employed by MOHP, assigned to local health centers
IMN	impregnated-mosquito net (or bednet)
IRC	indoor resting collection
ITM	insecticide-treated materials (e.g., bednets, curtains)
MOHP	Ministry of Health & Population
PKD	pyrethrum knockdown collection
PSI/M	Population Services International/Malawi, a worldwide, private sector organization specializing in social marketing
RHO	Regional Health Office, MOHP
TDR	Tropical Disease Research/WHO
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

The Blantyre Insecticide-Treated Bednet Project (BITNET) was launched October 31, 1998, and to date (February 1999) has sold some 25,000 bednets and the accompanying cyfluthrin, the insecticide of choice for treatment of the bednets. The primary goal of promoting these insecticide-treated bednets is to reduce malaria morbidity and mortality in children under five and pregnant women. BITNET's early success will certainly lead to widespread use of insecticide-treated materials (ITMs) by the Blantyre District population over the next few years. But in time, widespread and continuous use of cyfluthrin-treated bednets will almost certainly promote vector resistance to the insecticide being used. This resistance could be physiological and/or behavioral. For this reason, baseline information must be collected on the susceptibility of malaria vectors and other pest mosquitoes to cyfluthrin, so that monitoring can be conducted in the future.

To begin this baseline monitoring, Population Services International (PSI), the organization which manages BITNET, and the Environmental Health Project (EHP) developed an assessment work plan in 1998 for obtaining entomological vector mosquito susceptibility data. The primary purpose of the work plan was to carry out cyfluthrin insecticide susceptibility bioassays on adult anopheline vectors and to train local staff to carry out the susceptibility bioassays at selected future intervals. Secondary goals were to obtain limited baseline entomological data on the vectors and to train staff to do the WHO cone bioassays. These bioassays are used to determine the effective residual life of cyfluthrin on the bednets. The assessment was conducted on schedule, January 19 to February 5, 1999. The team that was assembled was composed of four supervisors and eight collectors. Each supervisor had received entomological training in 1991 from the Malaria Branch/CDC with support from USAID and the Ministry of Health and Population's Community Health Sciences Unit. These supervisors then participated in vector assessments in Nsanje, Mangochi, and Dowa in 1991-1992. The eight collectors were from health centers and a local hospital located near the two urban townships (Ndirande and Nkolokoti) and two rural areas (Lirangwe and Lunzu) selected for the collection of vectors for the insecticide-susceptibility testing.

Indoor resting collections (IRCs) to obtain live specimens for the cyfluthrin insecticide WHO adult tube bioassay, the CDC bottle bioassay and the WHO cone bioassay were conducted for 6 to 7 days, depending on the area. Over 1,186 *An. gambiae s.l.* and *An. funestus* were collected by IRC, with all but 8 coming from the two rural areas. It was extremely difficult to find malaria vectors in the urban areas. Pyrethrum knockdown collections (PKDs) were carried out one or two times in each area in selected houses. PKDs are useful in identifying which vectors are present in a house and in what density. But more importantly, PKDs normally produce numerous specimens that can be desiccated and preserved for malaria parasite sporozoite testing, *An. gambiae* sibling species identification, and target-site resistance screening at the Division of Parasitic Diseases/CDC. This information can then be added to the baseline data being collected on the vectors and pest mosquitoes in Blantyre District.

Over 994 *An. gambiae s.l.* and *An. funestus* were collected by PKD in the two rural areas, while 2 *An. funestus* vectors were obtained by PKD in the urban area of Nkolokoti, which illustrates the difficulty of doing assessments in urban situations. Epidemiological data needs to be collected and analyzed to determine the urban locations where malaria is most likely being transmitted before an entomological assessment is undertaken. In addition, anopheline breeding sites should be sought out during both the rainy and dry seasons to pinpoint the best areas to collect the vectors. In general, it is more difficult to obtain permission to enter homes in urban areas then in rural areas, and in general, house density, type of construction, and construction material in urban areas detract from the vectors' resting in homes during the day. In all, some 1,700 anophelines were preserved from the IRC and PKD collections for testing at CDC. In addition, 286 *Culex spp.* and 10 *Aedes aegypti* have been preserved for target-site resistance testing at CDC.

The results of the WHO tube bioassays and the CDC bottle bioassays indicate that both malaria vectors *An.gambiae s.l.* and *An.funestus*

from the two rural areas were very susceptible to cyfluthrin. In addition, *Culex spp.* from all four areas and the *Aedes aegypti* mosquitoes from a rural area, which are generally considered bothersome pests but in given circumstances can be disease carriers (filariasis and yellow fever), were also found to be very susceptible to cyfluthrin. Although all the bioassays were very favorable, the target-site resistance testing that will be accomplished at CDC will give BITNET a third test to use in establishing a baseline on vector susceptibility to cyfluthrin. The third bioassay was the WHO cone test. The team performed only a few tests to serve as training for the supervisors, in case such tests are used in future monitoring assessments. Although some data are provided in the report, the cone test requires running a large number of assays to overcome the variability often seen. The team was constrained by time and lack of live specimens in running this test beyond a training demonstration. Further, chemical analysis of the bednets to determine the actual amount of cyfluthrin present needs to be assessed at the time of the bioassays as materials differ in their uptake of the insecticide, and the actual treatment of a bednet may vary from person to person.

Overall, the bioassay data gathered in the assessment indicate that the vectors and even pest mosquitoes are susceptible to the cyfluthrin being used to impregnate the bednets sold through BITNET. It is recommended that vector susceptibility monitoring be continued on a two-year basis. The timing will depend, to some degree, on the level of sales of bednets and the consistency of use. At the rate BITNET is going, however, the vectors should be monitored within the next two years. In addition, it is important to follow the behavior of vectors (i.e., biting rates, indoor/outdoor biting, time of biting) to note any changes that exposure to cyfluthrin has caused that could impact the effectiveness of the bednets in reducing malaria morbidity and mortality. Behavioral information could be obtained as part of future insecticide susceptibility assessments.

1 INTRODUCTION

Resistance of the *Plasmodium falciparum* strain of malaria to chloroquine has spread across Africa and has caused renewed interest in utilizing measures aimed at the malaria vector. Recently, the Government of Malawi (GOM) and the United States Agency for International Development (USAID) jointly approved an initiative called the Blantyre Integrated Malaria Initiative (BIMI). One of the principal objectives of BIMI is to strengthen the prospects for long-term control of malaria. And one measure currently receiving widespread attention is the use of insecticide-treated bednets as a means of reducing malaria-related morbidity and mortality in the population, especially in children under five and pregnant women. In 1997, Population Services International (PSI) prepared a proposal through USAID's Africa Integrated Malaria Initiative (AIMI) for a three-year pilot program in the Blantyre District of Malawi for social marketing of insecticide-treated materials (ITMs)—primarily bednets—for the local population to protect themselves from malaria and to strengthen malaria control efforts in Malawi. The PSI proposal was approved in 1998.

With the launching of the Blantyre Insecticide-Treated Bednet Project (BITNET) in October 1998 and the selection of the insecticide cyfluthrin to impregnate the bednets, it became important to establish early in the initiative the susceptibility of the malaria vectors and other pest mosquitoes in Blantyre District to cyfluthrin. Although the primary purpose of the ITMs was to reduce malaria vector biting, the susceptibility of pest mosquitoes was also tested because individuals buying and using the bednets would judge their effectiveness by whether or not they eliminate mosquitoes that prevent sleep at night.

As part of BITNET, the Environmental Health Project (EHP) was selected to design and conduct an entomological assessment of the effectiveness of cyfluthrin in Blantyre District. The primary goal of the assessment was to carry out cyfluthrin susceptibility bioassays of adult anopheline vectors and to train local staff to carry out such bioassays in the future. Secondary goals were to conduct WHO cone bioassays on impregnated bednets to determine the effective residual life of cyfluthrin and to obtain limited baseline vector data. During the actual assessment, which occurred between January 19 and February 5, 1999, several modifications to the work plan became necessary. For example, the schedule called for two all-night human landing collections (HLCs) to learn more about vector habits and to capture live specimens, but this collection method was dropped in favor of additional indoor resting collections (IRCs). IRCs were deemed more important in the long run, as many more live anopheline and culicine mosquitoes are usually collected by this method, and those mosquitoes were needed to complete the insecticide susceptibility bioassays, the primary purpose of the assessment. Other minor changes in the work plan included having two fewer pyrethrum knockdown collections (PKDs) than planned and using one less collector per team, but these changes did not affect the assessment. A major addition was the CDC bottle bioassay which provided an important second bioassay in determining whether vector and pest mosquitoes were susceptible to cyfluthrin.

A list of assessment team members, with their titles, positions, and health facility assignments, are given in Appendix A. The local assessment team leader and the National Malaria Program Manager coordinated the selection of the supervisors, while the team leader selected the entomological collectors from health facilities located near the four areas which were chosen for the activities. One supervisor, the Regional Malaria Officer, had to leave the team after three days to return to his normal duties. The other four supervisors were individuals who had received entomological training from CDC staff in 1991. They had participated in four malaria vector assessments in the districts of Nsanje, Mangochi, and Dowa in 1991-1992. As

mentioned above, the eight collectors work at Blantyre health centers or local hospitals and were trained as health surveillance agents.

Other individuals assisted the team in preparation for the activity (in Washington, D.C., and in Lilongwe) and in local arrangements in Blantyre. Still others in this activity worked on entomological testing in the Division for Parasitic Diseases at the U.S. Centers for Disease Control and Prevention in Atlanta, Georgia. For a list of all these individuals, please see Appendix B.

The work plan for this activity may be found in Appendix C.

2 DESCRIPTION OF BLANTYRE DISTRICT ASSESSMENT AREAS

As part of the pre-assessment planning, the local team leader was assigned the task of selecting two urban areas and two rural areas in Blantyre District for collection of malaria mosquitoes. The areas selected are described below. A map of Blantyre is shown in Figure 1.

2.1 Area A: Lirangwe—a rural area

Lirangwe is a small trading center 40 kilometers from Blantyre City on the highway to Lilongwe. It is in Lunzu township. The villages selected for visits were 1 to 2 kilometers off both sides of the highway and consisted mainly of scattered farm houses situated on slightly hilly land. House construction consisted of mud walls and thatch roofs. The family homes were usually surrounded by cornfields and grassland, a typical rural setting. Anopheline breeding sites would be numerous in this area in road ruts, potholes, and where streams and rivers create puddles when starting to dry up. One dam site is also a source of breeding.

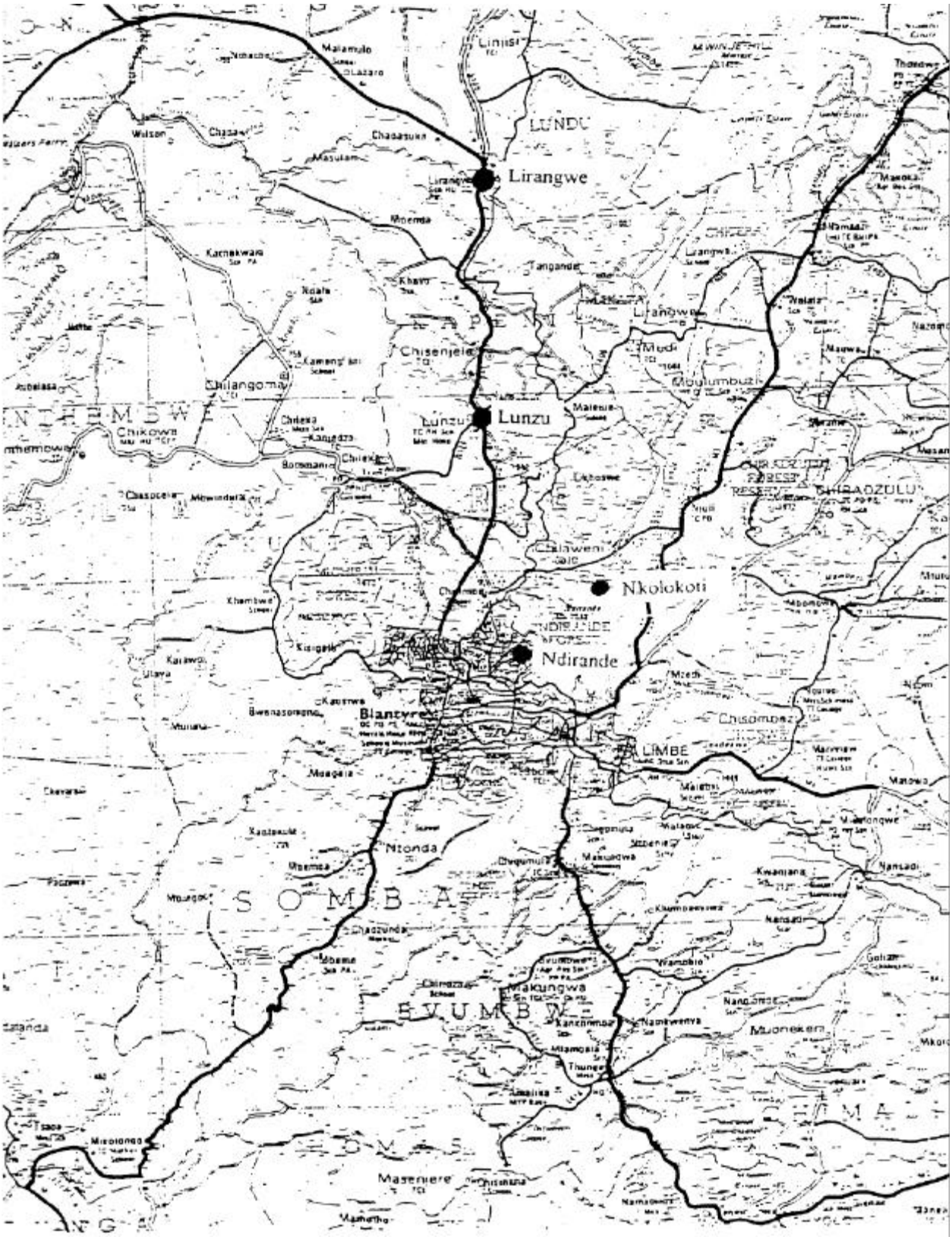
2.2 Area B: Lunzu—a rural area

Lunzu is the name of a township as well as a trading center and market place. It is 20 kilometers from Blantyre City on the highway to Lilongwe. The villages selected in Lunzu were 5 kilometers off the highway on poorly constructed and maintained roads. Housing was typically mud wall and thatch roof. The homes were usually surrounded by cornfields and grassland, another typical rural setting. Like Lirangwe, breeding sites such as road ruts, potholes, and stream and river puddles would be numerous once the heavy rains ceased.

2.3 Area C: Ndirande—an urban area

Ndirande is the name of a township that makes up a portion of the City of Blantyre and is located some 11 kilometers from the city center. It is the most densely populated urban township in the city. It lies to the northeast of the center of the city on the western edge of the Ndirande Mountain. More than 95% of the household structures are of raw and burnt bricks with a corrugated iron sheeted roof. The households are built close together on a planned city road network. The area is serviced with electricity, piped water, and boreholes in some places. The Nasolo River cuts through the township in a southerly direction. The area is well drained for most of the time because of its elevation and position on the edge of the mountain. However, effluent from many households creates surface run-off which results in extensive pollution of the surrounding areas, resulting in fewer anopheline breeding sites. The township is served by a small, busy health center which is situated close to the local market and shops. The Welcome Trust Malaria Project in Blantyre runs its regular clinics and drug resistance studies at this center. It is a difficult setting in which to conduct entomological collections.

Figure 1
Map of Blantyre Assessment Sites



2.4 Area D: Nkolokoti—an urban area

This township lies on the eastern edge of the Ndirande Mountain. It is more of a suburban area, where households are more dispersed than those in Ndirande. The drainage pattern is similar to Ndirande's, with the Lunzu River on the east of the township running northward. The river could provide some mosquito breeding sites at oxbows and on its edges. Nkolokoti is less densely populated than Ndirande, and most of the households are dispersed. A very small section of the township is served by electricity; running water is available in pipes and through boreholes. The area is served by the Limbe health center six kilometers away, but it has its own local market and shops.

3 MOSQUITO COLLECTION METHODS

Daytime collections of adult anophelines and other mosquitoes, as well as larvae, involved three techniques:

3.1 Indoor Resting Collection (IRC)

Collectors and supervisors utilized aspirators and flashlights to search every room in a house for resting mosquitoes. Those found were placed in a pint-sized paper carton, provided with sugar water in cotton, and stored in a cooler under damp paper towelling until being transported to the lab.

3.2 Pyrethrum Knockdown Collection (PKD)

At each home selected for PKD, all family members were requested to stay outside; all water and food were removed from the home, and any fires extinguished. White sheets were then placed on the floor of all rooms. The eave perimeter was then sprayed from both the inside and outside with Doom (which contains propoxur, pyrethroid, and synergist). The person spraying the outside went a little ahead of the inside person to keep the mosquitoes from leaving the house. The inside sprayman then also sprayed the inside ceiling area and under tables and chairs. After that, the sprayman exited the house and closed the door. After l5 minutes, sheets were removed one by one and inspected for adult anophelines and other mosquitoes. The mosquitoes were first placed in petri dishes, and then transferred to microfuge vials which are placed in styrofoam boxes. These styrofoam boxes were then placed in a plastic container containing a desiccant and transported to the lab.

3.3 Larval Collection

A dipper was used to determine whether anophelines or other mosquitoes were present in a particular breeding site. If they were, the anophelines were then transferred by means of a pipette to a plastic box containing breeding-site water. After all the larvae were collected, they were transferred from the box to a large zip-lock bag and taken to the lab where the larvae were released into another plastic box, fed, and then followed through their development stages. When pupal stage began, the pupae were transferred to a small container of water that had been placed in a large gallon-size paper carton with a netted top. The carton then served as a cage for the adults when they emerged from the pupal stage.

4 SPECIMEN PROCESSING

In the field, live adult anophelines and *Culex spp.* were placed in pint-sized paper cartons with netted tops. Sugar water in cotton was placed on the netting, and the cartons placed in a cooler and covered with damp paper toweling. Upon arrival from the field, the specimens were placed in other coolers or large plastic covered pails, provided with sugar water in cotton, and covered with damp toweling.

Field collected PKD specimens were placed one per vial. These vials were then stored in styrofoam boxes that contain 100 vial slots. The styrofoam bases were then placed in an airtight plastic container with desiccant for 48 hours, and then capped.

Once live mosquitoes had been tested, they are preserved in the same manner as described immediately above and stored for future testing at DPD/CDC.

5 DESCRIPTION OF THE INSECTICIDE SUSCEPTIBILITY BIOASSAY TESTS

Three tests were employed to assess the susceptibility of *Anopheles, Culex*, and *Aedes* to the insecticide, cyfluthrin. The tests are described below.

5.1 WHO Adult Tube Test

This test uses plastic tubes which contain either cyfluthrin-treated paper (0.15%) or a non-treated paper. Replicates of from 10 to 20 mosquitoes are exposed to the cyfluthrin or control papers for 1 hour. They are then removed from the plastic test-tubes and placed in netted pint-sized paper cartons, provided with sugar water in cotton, and placed in a cooler for 24 hours, after which the mortality of the exposed or control mosquitoes is determined and compared.

5.2 CDC Bottle Bioassay

In this test, 250 ml Wheaton bottles are used. Three bottles were treated with 40 micrograms (μg) of cyfluthrin in 1.5 ml of acetone, while three other bottles received only acetone at the DPD/CDC in mid-January. The process involved gently rolling the bottle around to make sure the acetone containing cyfluthrin was spread smoothly throughout the inside of the bottles. During testing, mosquitoes are placed in the bottles, and, after 15, 30, and 45 minutes of cyfluthrin or control exposure, the mosquitoes are checked for knockdown. Based on a test at CDC, it was found that knockdown of a sensitive strain of *An. gambiae* was 100% in 30 minutes, while 50 μg provided no shorter kill time, and 30 μg required 45 minutes to show 100% mortality. Thus, 40 μg was considered the dosage to use to find out if the mosquitoes collected at the four Blantyre District sites were as sensitive as the CDC *An. gambiae* strain.

5.3 WHO Cone Bioassay

This test employs plastic cones that are approximately 4.5 inches in diameter at the base and 3 inches high. In this test, three cones were attached to each of the two bednets that had been treated with cyfluthrin at either 67 or 71 $\mu g/m^2$ of netting. Three other cones were attached to untreated netting, to serve as controls. In the top of each cone there is a hole through which mosquitoes are inserted using an aspirator. A cotton plug is used to cover the hole and contain the mosquitoes. After the mosquitoes are inserted, they are observed at 15 and 30 minutes to determine how many have been knocked down. At the end of 30 minutes, the mosquitoes are removed, placed in netted pint-sized paper cartons, provided with sugar water in cotton, and placed in a cooler under damp paper toweling for 24 hours. After that period, mortality is determined for both the cyfluthrin and control mosquitoes.

6 FINDINGS

6.1 CDC Bottle Bioassay Results

The results of the CDC bottle bioassay are shown in Table 1. All tests produced 100% mortality in *An. gambiae s.l., An. funestus, Culex spp.*, and *Aedes aeqypti* mosquitoes exposed to the cyfluthrin. The only anophelines tested came from the two rural areas, while the *Culex spp.* came from an urban and rural area. The *Aedes* came from a rural area.

Table 1
Results of the CDC Bottle Bioassay Using
40 μg of Cyfluthrin per Exposure Bottle

Total Number Dead and (%) Mortality									
Test*				15 Minutes		30 Minutes		45 Minutes	
Area	Location	No.	Mosquito	Exposed	Control	Exposed	Control	Exposed	Control
A	Lirangwe	1	*An. gambiae*	17 (55)	0 (0)	31 (100)	0 (0)	31 (100)	0 (0)
B	Lunzu	2	*An. gambiae/ funestus*	27 (90)	0 (0)	30 (100)	0 (0)	30 (100)	1 (3)
C	Ndirande	3	*Culex spp.*	50 (100)	0 (0)	50 (100)	0 (0)	50 (100)	0 (0)
B	Lunzu	4	*An. gambiae/ funestus*	24 (73)	0 (0)	33 (100)	0 (0)	33 (100)	0 (0)
A	Lirangwe	5	*An. gambiae/ funestus*	29 (88)	0 (0)	33 (100)	0 (0)	33 (100)	0 (0)
B	Lunzu	6	*Aedes aegypti*	19 (100)	0 (0)	19 (100)	0 (0)	19 (100)	0 (0)
A	Lirangwe	7	*Culex spp.*	30 (100)	0 (0)	30 (100)	0 (0)	30 (100)	0 (0)

Each test consisted of 3 exposure and 3 control bottles with approximately 10 mosquitoes per bottle, except for Test 3 when 16+ *Culex* were used per bottle.

6.2 WHO Adult Tube Bioassay Results

Table 2 gives the results of the WHO adult tube tests that were conducted. All tests indicated that the mosquitoes exposed to papers containing 0.15 % cyfluthrin (the WHO discriminating dose) were all killed (100% mortality).

The anophelines tested came from the two rural areas, and the *Culex* came from both the urban and rural areas.

Table 2
Response of Adult *An. gambiae s.l.*, *An. funestus*, and *Culex spp.* Exposed to Cyfluthrin (0.l5%) Treated Papers in the WHO Adult Tube Test

							Exposed		Control	
Test	Area	Location	Mosquito	Number Exposed	Number Control	Number of Replicates	Dead	%	Dead	%
3	A	Lirangwe	*An. gambiae/ funestus*	15	15	2	30	100	0	0
5	B	Lunzu	*An. gambiae/ funestus*	15	15	2	30	100	0	0
6	A	Lirangwe	*An. gambiae/ funestus*	10	10	2	20	100	0	0
9	B	Lunzu	*An. gambiae/ funestus*	17	18	2	34	100	1	3
10	A	Lirangwe	*An. gambiae/ funestus*	13	12	2	25	100	0	0
11	A	Lirangwe	*An. gambiae*	12	13	2	24	100	0	0
12	A	Lirangwe	*An. gambiae/ funestus*	15	15	2	30	100	0	0
1	A	Lirangwe	*Culex spp.*	10	10	2	20	100	0	0
2	A	Lirangwe	*Culex spp.*	20	20	2	40	100	0	0
4	B	Lunzu	*Culex spp.*	10	10	2	19	100	0	0
7	C	Ndirande	*Culex spp.*	15	16	2	31	100	0	0
8	D	Nkolokoti	*Culex spp.*	15	15	2	30	100	0	0
13	C	Ndirande	*Culex spp.*	9	9	2	18	100	0	0
14	C	Ndirande	*Culex spp.*	16	13	2	32	100	0	0

Note: *An. gambiae* and *An. funestus* were tested together due to limited number of each species collected during a given period.

6.3 WHO Cone Bioassay Training Experience

As outlined in the work plan, training for the supervisors in conducting the WHO cone bioassay occurred during the last two days of the assessment. The cone bioassays were performed on two different types of bednets promoted in the PSI project. The bednets were treated with the appropriate cyfluthrin dosage by the local team leader. The data obtained were somewhat variable. When a mixed number of *An. gambiae s.l.* and *An. funestus* were exposed to a cyfluthrin treated *blue* bednet for 30 minutes, 100% mortality occurred, but when the mosquitoes were exposed to the cyfluthrin treated *green* net for the same time, the mortality rate was 80%. Yet, an assay using *Culex spp.* mosquitoes produced 100% knockdown after only 15 minutes. In other tests, the supervisors had trouble with control mortality. Such variability is not uncommon when doing tests with bednets, as it is difficult to apply the cyfluthrin evenly on the fabric. The data obtained in these exercises have no real meaning regarding susceptibility of the vectors to cyfluthrin on bednets because this was only a training session and was not a controlled test situation.

6.4 Pyrethrum Knockdown Collection Results

The results of the pyrethrum knockdown collections are listed in Table 3. As the table indicates, this method is a good one for collecting large numbers of mosquitoes, if live specimens are not needed.

Table 3
Pyrethrum Knockdown Collections of *An. gambiae s.l., An. funestus,* and *Culex spp.* Using Doom Insecticide Spray

			Adult Mosquitoes Collected (average number per house)		
Area	**Location**	**No. of Houses Sprayed**	***An. gambiae***	***An. funestus***	***Culex spp.***
A	Lirangwe	9	148 (16)	328 (36)	171 (19)
B	Lunzu	6	105 (18)	455 (76)	164 (27)
C	Ndirande	6	0 (0)	0 (0)	491 (82)
D	Nkolokoti	3	0 (0)	2 (0.7)	546 (182)

6.5 Indoor Resting Collection Results

Table 4 summarizes the number of vectors collected through indoor resting collections. The results clearly indicate that the vectors are quite abundant in the rural areas, but extremely rare in the urban houses in which the team attempted collection.

6.6 Samples Preserved for Testing at DPD/CDC

Over 1,700 anopheline malaria vector mosquitoes and 296 culicine pest mosquitoes were preserved for testing at the Division of Parasitic Diseases/CDC in the United States. Anopheline and culicine mosquitoes will be tested to determine if point mutations can be detected that cause target-site resistance to pyrethroids. In addition, the anophelines will be tested for the presence of malaria sporozoites (and the parasite species), and the *An. gambiae s.l.* will be sampled to determine whether they are *An. gambiae s.s.* or *An. arabiensis.*

6.7 Evaluation of Supervisors and Collectors

The field supervisors readily recalled their 1991 field training and were able to teach the collectors how to do indoor resting collections and pyrethrum spray sheet knockdown collections. The collectors learned quickly and carried out their activities in a very satisfactory manner.

6.8 Larval Collection Results

An. gambiae s.l. and *Culex spp.* larvae were collected in Lirangwe (Area A) and reared to adult stage in the laboratory. The anophelines were used in Test 1 of Table 1 and Test 11 of Table 2, while the culicines were used in Test 7 of Table 1. *Aedes aegypti* larvae were collected in Lunze (Area B) and reared to adult stage in the laboratory. They were used in Test 6 of Table 1. Both the anophelines and culicines were completely susceptible to cyfluthrin in the bioassays used.

Table 4
Total Anopheline Adults from Indoor Resting Collections, Rural and Urban Sites

			Adult Mosquitoes Collected (average number per house)*	
Site	**Location**	**No. of Houses Searched**	***An. gambiael An. funestus***	***Culex spp.***
A	Lirangwe	53	405 (7.6)	285 (5.4)
B	Lunzu	72	773 (10.7)	187 (2.6)
C	Ndirande	38	0 (0)	221 (3.8)
D	Nkolokoti	20	8 (0.4)	250 (12.5)

* Vectors not separated in the field

7 CONCLUSIONS

A. The WHO adult tube bioassay results indicated that when *An. gambiae s.l., An. funestus,* and *Culex spp.* were exposed to cyfluthrin (0.15%) impregnated papers for 1 hour and then held for an additional 23 hours, their mortality was 100%, while the mortality in the controls was 0%. At the discriminating dosage of 0.15% selected by WHO for cyfluthrin, it is clear that the malaria vectors from rural Areas A and B and pest mosquitoes from all four areas were very susceptible to the insecticide.

B. The CDC bottle assay results showed that when *An. gambiae s.l., An, funestus, Culex spp.,* and *Aedes aegypti* were exposed to 40 mg cyfluthrin/bottle for 15 minutes, 55% to 100% were knocked down. By the end of 30 minutes' exposure, 100% mortality occurred. These results indicate that the vectors and pest mosquitoes tested in Malawi are as susceptible to cyfluthrin as a sensitive strain of *An. gambiae s.s.* which is maintained at DPD/CDC.

C. The cone bioassay results were somewhat variable. Cone bioassays on materials such as bednets often vary since it is difficult to apply the insecticide solution evenly over the whole net. Thus, it is often necessary to run a large number of assays to even out the data. In addition, chemical analysis of the net at the locations where the cones are attached provide additional information on the actual amount of cyfluthrin present on that portion of a net. Since the WHO cone bioassay was a training exercise for the supervisors, the results obtained were not presented in table form in the results section, but were given only as information for PSI to consider when planning future work. PSI and the BIMI Coordinator should work with DPD/CDC and have that unit perform a chemical analysis of PSI's cyfluthrin-impregnated bednets on a periodic basis to determine the quantity of cyfluthrin present as well as to run the cone bioassays under laboratory conditions. If that were done, the bioassay results could be compared to the cyfluthrin dosage present on a given location bioassayed.

D. The supervisors were able leaders and were valuable resources in this assessment. They were very professional in their dealings with the homeowners and, in almost all cases, were able to convince the homeowners to permit the teams to search their homes for mosquitoes. The collectors are professional health personnel and, as such, were quick to learn their responsibilities and carried out their activities correctly. These supervisors and collectors will be very helpful in any future entomological assessment undertaken by BIMI. The supervisors should also be considered valuable resources that the MOH can call upon in future entomological activities within Malawi.

E. The results of the indoor resting and pyrethrum knockdown collections clearly show that the malaria vectors could easily be collected in the two rural areas, but not in an urban setting. This is not an unusual finding as it requires a concentrated and continuous effort to locate the urban breeding sites and the adult vectors. Before additional entomological activities are planned for Blantyre City, the PSI Director and the BIMI Coordinator should study any and all malaria epidemiological data available for the townships of Blantyre City to see if any areas present moderate to high malaria transmission. If there are such areas, future entomological monitoring could concentrate on them.

8 RECOMMENDATIONS/FUTURE PLANNING

8.1 Future Monitoring of Vectors for Insecticide Susceptibility/Resistance

At least every two years, insecticide susceptibility monitoring should be conducted on the malaria vectors and pest mosquitoes in Blantyre District. Repeated vector monitoring is important as the BITNET Project is likely to expand the sale of cyfluthrin-treated bednets as well as the sale of cyfluthrin for retreatment of bednets throughout the district. As this occurs, more vectors and pest mosquitoes will be exposed to cyfluthrin, which increases the potential that the mosquitoes will develop cyfluthrin resistance. Vector monitoring will help protect the investment the public has made by buying bednets and then treating them with cyfluthrin. The public needs to know that when cyfluthrin is used, it is going to be effective. If that is not the case, then they need to know which insecticide to use. In addition to susceptibility monitoring, it is important to gather information on the effect the treated bednets are having on the behavioral patterns of the vectors (i.e., time of biting, resting habits, indoor/outdoor biting). Behavioral changes could impact the effectiveness of the bednets on malaria morbidity and mortality, especially in children and pregnant women.

As for the future, both the BITNET Project Director and CDC BIMI Coordinator agree that a two-year monitoring interval is reasonable for testing the susceptibility of anopheline and culicine adult mosquitoes to cyfluthrin. The best locations to collect mosquitoes will be where the project is having the most success in marketing the bednets and where retreatment of the bednets is high. Thus, future testing will probably include the rural areas, but not Blantyre City. Not including the city in this monitoring should not be a problem as DPD/CDC and the BIMI Coordinator appear to the authors to be very interested in evaluating the malaria situation in Blantyre City within the next several years. Preliminary plans call for DPD staff to visit Malawi in March or April 1999 to discuss/review BIMI's plans. These plans apparently include looking at the epidemiology of urban malaria in Blantyre and/or evaluating the use of a rapid assessment technology for obtaining information. Under both situations (i.e., rural and urban), entomological activities such as identification of vector breeding sites, vector collections, and sporozoite determination would be important aspects of the work. Insecticide susceptibility testing could also be included. If CDC's plans for Blantyre City continue as presently outlined, then the goals of this recommendation (for monitoring insecticide susceptibility) will be met within the timeframe suggested. In conducting the future urban malaria assessment, the BIMI Coordinator should request the services of the local leader and assessement team members for the entomological activities. The team members are capable, experienced, and locally available.

8.2 Testing the Bednets

The BITNET Project Director and the BIMI Coordinator should request that DPD/CDC evaluate a number of the cyfluthrin-treated bednets to determine the quantity of cyfluthrin present on the nets as well as to determine how evenly the insecticide is spread on the nets. At the time of such testing, CDC could use the cone bioassay to evaluate, under laboratory conditions, whether the cyfluthrin present on the bednets is sufficient to kill sensitive malaria vectors.

The first step in implementing this recommendation has been taken. At the request of PSI and the BIMI Coordinator, bednets of four different styles used by BITNET and the cyfluthrin necessary to treat them were delivered to DPD/CDC in Atlanta, Georgia. Staff of the DPD Entomology Branch will treat and evaluate the bednets. The chemical and biological analysis of these cyfluthrin-treated bednets under laboratory conditions will provide important baseline information to the BITNET Project. Additional and equally important information will be gained from bednets purchased from BITNET and then treated and used by individuals living in Malawi. The information obtained in Malawi will help determine how well the bednets were treated

by the buyers and how effective the insecticide is over time in protecting individuals during actual use. The CDC BIMI Coordinator will need to coordinate this additional information-gathering with the appropriate DPD/CDC staff and the BITNET Project Director.

8.3 Vector Testing at CDC

The BITNET Project Director and CDC BIMI Coordinator need to follow up on the vector and pest mosquito specimens that were carried back to DPD/CDC for pyrethroid resistance, sporozoite, and sibling species testing. The results will provide important additional baseline information on the malaria vectors and pest mosquitoes in Blantyre District. The specimens are now with Dr. William Brogdon, DPD/CDC, for testing. Dr. Brogdon believes the testing will take up to six months to complete, but as of February 1999, the process has started. When additional epidemiological/entomological assessments are conducted in Blantyre City and District, plans should include preservation of mosquito specimens for testing at CDC in order to detect any changes that may be occurring in the vector/pest mosquito population over time.

APPENDIX A: LIST OF ASSESSMENT TEAM MEMBERS

NAME	TITLE	PROJECT ROLE	DUTY STATION AND LOCATION
1. Fredson Kamchira	Health Assistant	Supervisor	Thyolo Hospital
2. John Zoya	Sr. Health Asst.	Supervisor	RHO (Mzuzu)
3. Prince Kabambe	Health Assistant	Supervisor	Ntcheu Dist. Hospital
4. Violet Chiphaso	Matron	Supervisor	Zomba Nursing School
5. Ben Kalonga	Regional Malaria Officer	Supervisor	RHO (Blantyre)
6. Felix Kumalonje	Health Surv. Asst.	Collector	Ndirande Health Center
7. Vanessa Menyele	Health Surv. Asst.	Collector	Ndirande Health Center
8. Patricia Lipenga	Health Surv. Asst.	Collector	Nkolokoti Health Center
9. Martin Chisiano	Health Surv. Asst.	Collector	Nkolokoti Health Center
10. Rose Botso	Health Surv. Asst.	Collector	Mlambe Hospital/Lunzu
11. Alfred Phiri	Health Surv. Asst.	Collector	Mlambe Hospital/Lunzu
12. Olive Bitoni	Health Surv. Asst.	Collector	Lirangwe Health Center
13. Blessings Namtchukwa	Health Surv. Asst	Collector	Lirangwe Health Center
14. Phillimon Tambala	Principal Parasitologist	Local-EHP Coordinator	RHO (Blantyre)
15. John Sexton	EHP Consultant		Atlanta, Georgia

APPENDIX B: LIST OF PERSONS CONTACTED

Dr. Pascal Mkanda, Officer-in-Charge, CHSU/MOH&P/Lilongwe

Mr. Alan Macheso, Malaria Program Manager, CHSU, MOH/Lilongwe

Dr. Charles Ziba, Deputy Malaria Program Manager, CHSU, MOH/Lilongwe

Dr. Lawrence Marum, Blantyre Integrated Malaria Initiative Coordinator, Blantyre

Dr. Kelita Kamoto, Acting Regional Health Officer & District Health Officer, Blantyre

Dr. Desmond Chavasse, BITNET Project Director, PSI/Malawi

Dr. Terri Taylor, Queen Elizabeth Hospital, Blantyre

Ms. Joan LaRosa, HPN Officer, USAID, Lilongwe

Mr. Mexon Nyirongo, CHAPS Team Leader, USAID, Lilongwe

Mr. Craig Hafner, Deputy Director, EHP, Washington, DC

Dr. Pandu Wijeyaratne, Program Director, Tropical Disease Prevention, EHP, Washington, DC

Dr. Dennis Carroll, Project Manager, AIMI, USAID, Washington, DC

Dr. William G. Brogdon, Research Entomologist, DPD/CDC

Dr. Janet McAllister, Entomologist, DPD/CDC

Mr. Dwight Mount, Chemist, DPD/CDC

APPENDIX C: WORK SCHEDULE/PLAN FOR MALAWI *INSECTICIDE SUSCEPTIBILITY TESTING AND ENTOMOLOGICAL ASSESSMENT 1999*

Section I: The primary purpose of the entomological activity scheduled for Malawi is to 1) carry out pyrethroid insecticide susceptibility bioassays of adult anopheline vectors and 2) train local staff to carry out the insecticide susceptibility bioassays at selected future intervals. Secondary goals will be 1) training and completion of a limited baseline entomological assessment and 2) training in carrying out cone bioassays to determine the effective residual life of the insecticide on the bed nets. A work schedule/plan is provided in Section III for conducting the primary and secondary activities and is based on the following:

1. Availability of maps for the selection of 2 rural and 2 urban areas of Blantyre District for the collection of live anopheline vector specimens and for entomologic sampling. In the work plan these areas are identified as areas A, B, C, and D. The PSI medical entomologist could speed up the process by identifying these areas.

2. That active anopheline breeding sites can be located for the collection of immature stages which then will be held until maturity for use in the susceptibility bioassays. These sites will serve to back up live adult collections for the bioassays.

3. There will be WHO Adult Tube Test Kits with appropriate insecticide treated papers available.

4. Selection of at least 6 houses in each area in which to do indoor resting, pyrethrum knockdown, and all-night collections. If the PSI medical entomologist could identify these houses then the assessment would move forward more quickly..

5. There will be four teams, each with a supervisor and 3 collectors. Delays in lining up supervisors and hiring collectors could affect the work accomplished. Retraining of the supervisors is expected to be minimal and collectors will receive mostly on-the-job training. The supervisors will also do collections.

6. There will be adequate transport. Minimum of two vehicles and drivers necessary as there will be a minimum of 6 people going to the field with equipment and supplies.

7. Appropriate equipment and supplies are available to collect immature anopheline stages, rear these stages to adulthood, and then to conduct the insecticide susceptibility testing. In addition, the 4 teams will need to be equipped to do the resting, PKD, all-night landing collections.

8. That the collection techniques listed are satisfactory. If additional collection methods or a different combination is desired then the schedule would need to be adjusted. Since live vector specimens are the first priority, it may be necessary to spend most of the efforts on collecting the specimens and less on assessment measurements. This will depend on the number of anophelines being captured by resting collections and those reared from larval collections. It also may be necessary to add bed net trap collections.

9. The dates and time available are acceptable. If the rainy season is delayed then the trip may need to be delayed. A few days leeway also should be built into the number of days available as collection of live specimens for susceptibility bioassays may require more time.

10. There will be no immediate identification of An. gambiae species, sporozoite rates, or blood meals. If these measurements are important, every attempt will be made to preserve anophelines for future DNA analysis, sporozoite determinations, and source of blood meal. To do this, adequate supplies and freezer storage must be available. There may be resources available in Malawi to do the sporozoite and blood meal work (Mr.Tambala may be able to help identify the resources and equipment). Arrangements with CDC or a University would probably be necessary to do the DNA work for An. Gambiae species identification, but the anopheline legs could be frozen until the work is accomplished.

11. A small room or at least space in a room will be needed to serve as a primitive lab for the bioassay testing and holding of live specimens. This space must be free of insecticides or insecticide treated materials.

Section II: The purpose of the insecticide susceptibility bioassays are to insure that anopheline vectors are susceptible to the insecticide applied to the bed nets. The entomological collection techniques will provide limited data on:

1. Vector resting behavior and density indoors.
2. Vector species composition: indoors and outside near houses.
3. Vector biting behavior; indoor/outdoor; by time of day. (Limited information only).
4. Vector species identification from the resting, pyrethrum knockdown, and human landing collections.
5. Sporozoite rate determinations by parasite species and anopheline vector (if arrangements for testing can be made).
6. Biting preference through blood meal identification (if arrangements for testing can be made).
7. An. Gambiae species identification (if arrangements for DNA testing can be made).
8. It may be possible have biochemical insecticide susceptibility testing done on selected specimens back in the U.S., if a lab, such as at CDC/DPD would become a collaborator for research information they may be able to obtain for publication. EHP could follow-up on this.

Section III: Malawi Work Plan - January 15, 1999-February 6, 1999

DATE	**DAY**	**TIME**	**SUBJECT**
1/15/99	Friday	Evening	• Leave Atlanta, Ga.
1/17/99	Sunday	Morning	• Arrive Lilongwe, Malawi. • Reservations for Lilongwe Hotel. Transport arranged by PSI?
1/18/99	Monday	0800 - 1200	• Meetings with USAID, PSI, and other interested parties. Arrangements made either by PSI and/or EHP.
		1200 - 1800	• Drive to Blantyre. Vehicle and driver arrangements made by PSI and/or EHP. • Meet with Phillimon Tambala and 3 team leaders. • Advanced Hotel Reservations made by EHP/PSI.

1/19/99	Tuesday	0800 - 1200	• Meet with PSI staff to go over organizational details. • Review PSI Medical Entomologist selection of: a) 2 urban and 2 rural areas for entomological assessment sampling; b) 6 - 10 houses in each area that could be used for permanent sampling; c) anopheline breeding sites, and d) location for rearing anophelines and storing supplies and equipment (this can be a small room or even a part of a small room).
		1300 - 1800	• If the 4 areas and houses and breeding sites have been pre-selected then drive through the areas and look over the breeding sites. Set up appointments to discuss activities with village or community leaders for next day. Indicate the need to select three to four local people to assist in the entomological assessment. It may be that the supervisors could make prior arrangements for meeting with such leaders and take names of potential collectors this would speed up the operation. If no pre-selection has been done then the schedule will be backed up at least one to two days to visit local leaders; select areas, houses, and breeding sites, and hire local collectors.
		1900 - 2000	• Inventory the supplies and equipment. All items should have been transported to Blantyre prior to consultant arrival with PSI medical entomologist and 4 supervisors. Determine if additional materials needed and where they can be obtained.
1/20/99	Wednesday	0800 - 1200	• Hire at least 3 collectors for each of the 4 areas. PSI medical entomologist to go over administrative details with collectors. Depending on supervisors, up to 4 teams organized.
		1300-1700	• Begin training of the collectors in the use of the supplies and equipment for larval collections, resting collections, pyrethrum knockdown spray sheet collections, and human landing collections.
1/21/99	Thursday	0700 - 1200	• Train teams to do indoor adult anopheline resting collections. All teams will work together for training purposes until supervisors satisfied their teams can independently do the work.
		1300- 1700	• 3 teams will continue to do indoor resting collections. Styrofoam coolers needed to keep specimens alive. • 1 team will be trained to do larval collections.
		1800- 2000	• Supervisors will place the anopheline adults from the resting collections in gallon cartons and provide sugar water for 24 hours. The cartons can be stored in styrofoam

			coolers with damp paper towels or even cardboard boxes. Coolers or boxes must be protected from ants. • Supervisors will place anopheline larva in pans and feed, while pupa will be placed in small cups containing water and covered with netting. At maturity these anophelines will be bioassayed.
1/22/99	Friday	0700 - 1500	• Indoor resting collections will be conducted in as many houses as possible by 3 teams. The purpose is to collect as many adult anopheline as possible for susceptibility bioassays. Depending on need, and availability, 1 team will do larval collections and then will sort the anophelines larva at the rearing room. All pupa will be kept until maturity.
		1500 - 1700	• WHO adult tube test bioassays started. If sufficient adult female *An. gambiae* and/or *An. funestus* are available from the resting collections of the first day, they will be bioassayed for insecticide susceptibility. • The day's resting collections will be placed in gallon containers and fed. • Any pupae found in the larval pans will be picked and placed in adult holding containers.
1/23/99	Saturday	0700 - 1700	• Repeat of Friday's schedule. Do bioassays on any resting collected anopheline adults that have been held 24 hours. When larva mature then the 3 day-old adults will be bioassayed—if sufficient numbers are available. • Record results of previous day's bioassay. • At any point sufficient anopheline adults available, train supervisors to do the cone tests on impregnated bed net material.
1/24/99	Sunday	1000- 1700	• Depending on whether supervisors (possibly collectors) are paid to work on Sunday, larval collections will be done; taken to the lab and placed in pans. • Record results of previous day's bioassay. • Begin new bioassays if sufficient *An. gambiae* and/or *funestus* adults available.
1/25/99	Monday	0700 - 1700	• Continue resting collections; larval collections; bioassays through as on previous days. Record results.
1/26/99	Tuesday	0700 - 1700	• Same activities as Monday.
1/27/99	Wednesday		• Same activities as Tuesday.
1/28/99	Thursday	0700 - 1400	• Begin Pyrethrum knockdown collections in areas A & B. All teams will work together for training purposes until supervisors satisfied their teams can work

			independently. The objective will be to PKD at least 6 houses in each of 2 areas each time PKD collections are conducted. This will depend on number of workers, supplies and equipment available.
		1500 - 1700	• Sort, identify, and store PKD anopheline specimens. • Do bioassays of lab reared material if sufficient mosquitoes available. • Record results of previous day's bioassay and cone test.
1/29/99	Friday	0700 - 1400	• PKD in Areas C & D using 2 teams. • Resting collections in Areas C & D using 2 teams.
		1500 - 1700	• Sort and identify anophelines. Store dead specimens and place live specimens in cartons and feed. • Begin bioassays. • Record results of previous day's bioassays and cone test.
1/30/99	Saturday	0800 - 1500	• PKD in Areas A & B using 2 teams. • Resting collections in Areas A & B using 2 teams.
		1500 - 1700	• Sort and identify anophelines. Store dead specimens and place live specimens in cartons and feed. • Begin bioassays. • Record results of previous day's bioassays and cone test.
1/31/99	Sunday	1600 - 1930	• Begin bioassays and cone tests if specimens available. • Record results of previous day's bioassay and cone test • Prepare for all-night human landing collection.
		2000- 0500	• Two man teams will be trained to do these collections inside and outside houses (within 3 feet). All staff will work in one area.
2/1/99	Monday	0600 - 1400	• Team sleeps. • Supervisors place live specimens in cartons and feed.
		1500 - 1700	• Sort, identify, and store dead anophelines from human landing collection. • Begin bioassays and cone tests if specimens available. • Record results of bioassays and cone test from previous day.
2/2/99	Tuesday	0700 - 1100	• Resting collections in 6 houses of each area.
		1100 - 1200	• Live specimens put in cartons and fed.
		1200 - 1800	• Rest and sleep before all-night collections • Record results of bioassays from previous day.
		1930 - 0500	• All-night human landing collections in/outside of 2 houses in each of the 4 areas. All specimens stored.
2/3/99	Wednesday	0500 - 1400	• Team Sleeps.
		1600 - 1800	• Sort and identify the HLC anophelines. • Teams given instructions on future activities, paid, and thanked for their efforts.
2/4/99	Thursday	0700 - 1700	• Data prepared in a preliminary report. • Plans finalized for any follow-up on entomological activities. • Meet with PSI staff and other interested parties. • Drive to Lilongwe. Reservations at Lilongwe hotel.

2/5/99	Friday	0800 - 2400	• Meet with USAID, PSI, and other interested parties. • Work on preliminary report. • Leave Lilongwe for London
2/6/99	Saturday	0600 (?) 1300 (?) 1730 (?)	• Arrive London • Leave London • Arrive Atlanta.

This first draft prepared by Dr. John D. Sexton, June 15, 1998