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central america malaria research station

under

Participating Agency Service Agreement
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with

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
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INTRODUCTION

The Participating Agency Service Agreement (PASA) and associated Project Implementation Order/Technical Services (PIO/T) to implement the establishment of the Central America Malaria Research Station (CAMRS) were signed by the Public Health Service (PHS) and the Agency for International Development (AID) in April 1967. In 1966 during a preliminary survey of possible sites for the Station, the most favorable was offered by the Government of El Salvador, which proposed three buildings originally used by the Institute of Urban Housing (IVU) in Colonia Montserrat, San Salvador. Rent-free use of these buildings was assured in the Project Agreement signed by El Salvador's Ministry of Public Health and Social Welfare and the USAID Mission in San Salvador on June 2, 1967. A contract for renovating and partially equipping the buildings was awarded on June 27, 1967. Valuable assistance in drawing up the specifications for this contract was furnished by the Facilities Planning Branch, National Communicable Disease Center (NCDC).

On July 3, 1967, the contracting firm initiated its renovation job, consisting basically of converting three old but substantial concrete warehouses (which had resisted the worst earthquakes in San Salvador) into modern laboratories. The reconstruction of the old warehouse-type structures included installation of tile floors, false ceilings, fluorescent lighting, a new roof on the main building, refinishing or installing new doors and windows, and installation of security grills on exterior doors and windows. Additionally, special provisions were required to screen all windows and to install laboratory furniture, gas, compressed air, vacuum, electric, and hot and cold water lines in the majority of the laboratories.

Most of the work was completed in late October, but delays in delivery and installation of some electrical items did not permit liquidation of the basic

contract until March 1968. Discovery that existing water lines would not insure adequate water supply necessitated laying special lines in November 1967. Separation of the CAMRS electrical system from that of the IVU, which had kindly extended provisional service, was accomplished in April 1968. Telephones for external use and for intercommunication could not be installed until late June 1968.

Conventional and special equipment for entomology, epidemiology, parasitology, chemistry, health education, engineering, and administrative activities was ordered from the General Services Administration and other U.S. suppliers. These items, as well as vehicles and basic furniture for staff housing, began to arrive in August 1967 and were still being received at the end of the fiscal year. Several seaborne shipments were delayed by port and railway strikes in Guatemala.

Office space and internal administrative and "housekeeping" service were provided the clerk-typist and both epidemiologists of the NCDC Malaria Eradication Program's Regional Evaluation Office, whose operations from San Salvador have included reviews of malaria programs in Haiti, Ecuador, Brazil, Paraguay, and Central American countries.

At a ceremony held on February 16, 1968, the President of El Salvador, Fidel Sanchez Hernandez, formally dedicated the CAMRS in the presence of U.S. Ambassador Raul H. Castro, USAID Mission Director Andre Weissman, and other dignitaries and guests from national government and professional circles, the Organization of Central American States, the Pan American Health Organization (PAHO), the United Nations Children's Fund (UNICEF), the National Malaria Eradication Services of Central America and Panama, and the American community.

EVOLUTION OF THE CAMRS STAFFING PATTERN AND STAFF

The original PASA approved a staff of 13 U.S. and 45 national employees, the latter predominantly field personnel (mosquito collectors and blood survey or drug distribution aides). Because of circumstances described below, only four U.S. staff members and 14 national employees had joined the staff by the end of FY 1968.

The Director of CAMRS reported for duty on July 10, 1967, and was followed to post by the epidemiologist on August 21, 1967; an entomologist, September 2, 1967; and a second entomologist, November 5, 1967.

Concurrence, with a security clearance reservation, was requested and granted in October for a health education specialist. In February, when the nominee became available for assignment, concurrence of the Mission was again sought for a medical technician, a chemist, and a vector control specialist. The Mission concurred, but a further reconfirmation was required because of the "BALPA" reviews of country staffing levels. The reconfirmation could not be obtained. Subsequent discussions involving NCDC, the Department of Health, Education, and Welfare (DHEW), AID, and the Mission indicated approval of 9 U.S. technicians for CAMRS instead of the 13 originally approved. The impasse on moving additional U.S. personnel to post, however, had not been resolved by the end of the fiscal year.

National personnel recruited were initially limited to a clerk-typist, secretary, a janitor, watchmen, and insectary attendants until completed Station facilities and arrival of U.S. personnel could effectively use a larger complement of field aides. In February, when these conditions were almost met, the levels of national personnel were "frozen" by the BALPA review of the country staffing level. Subsequent interagency discussions resulted in mutual acceptance of 35 national employees instead of 45. Complete clarification on employing the additional nationals had not been accomplished, however, by June 30, which found the Station with only 14 nationals.

WORKING RELATIONSHIPS WITH VARIOUS GROUPS

National Organizations

The Ministry of Public Health and Social Welfare actively collaborated in establishing the Station and provided effective liaison with the IVU, the national water authority, and other organizations with whom contacts were essential during the Station's developmental phase. The Director and staff of the national malaria eradication service of El Salvador provided ample reports on current malaria conditions and were helpful in many other ways.

Through the Coordinating Committee for Malaria Investigations in El Salvador, every facility for test areas and assistance has been offered. Library facilities of the Medical School and of the Faculty of Science of the University of El Salvador have been made freely available to Station technical personnel.

Personnel of the national malaria eradication services of Guatemala, Honduras, Nicaragua, Costa Rica, and Panama have been very cooperative. The Station assisted the Nicaragua program in getting an analysis of the insecticide used in their larviciding operations.

International Organizations

Effective working relationships were developed with PAHO and UNICEF officials concerned with malaria problems in the region.

Embassy and USAID Personnel

Ambassador Raul H. Castro has shown much interest in the Station's development and participated personally in dedication activities. Support of the USAID Director, directly or through the Malaria Coordination Officer, helped resolve many problems. The Joint Administrative Services personnel rendered invaluable assistance in many

special and routine activities. Nevertheless, major difficulties in acquiring U.S. technical staff and national employees essential to Station operations have arisen from regulatory conflicts or interpretations not anticipated by signatories of the PASA.

PHS Malaria Personnel in ROCAP, Central American Countries, and NCDC/Atlanta

Exchange of information with the NCDC Malaria Eradication Program's Chief Malaria Advisor, ROCAP, and with Malaria Eradication Program advisors in El Salvador, Honduras, and Nicaragua was accomplished through visits or periodic reports. Contacts with national and PAHO malaria personnel were greatly facilitated by these advisors.

Support from NCDC has been a sine qua non in Station development, beginning with engineering advice from the Facilities Planning Branch and proceeding through all aspects of Station operations. The extensive resources which can be tapped on short notice represent one of the strongest investigational features of the Station.

DEVELOPMENT OF RESEARCH PROJECTS

During renovation and equipping of Station facilities and while awaiting arrival of the full complement of Station personnel, the specialists on duty outlined a number of project proposals based upon published reports of operational difficulties being faced by national malaria eradication services. These were discussed on February 17, 1968, with the directors of all the national malaria eradication services of Central America and Panama and with representatives of UNICEF and PAHO. The directors urged that priority be given to investigations of (1) antimalaria drug combinations with fewer side effects than those already in use; (2) methods for obtaining increased public acceptance of antimalaria drugs; (3) larvicides and larvicide-application techniques for use in special situations; (4) the possible role of secondary vectors in certain areas (*Anopheles punctimacula* and *A. darlingi*); (5) aerial application of insecticides for use in special circumstances; and (6) field application of new residual insecticides against DDT- and dieldrin-resistant anophelines.

To implement research projects within the country, a Coordinating Committee for Malaria Investigations in El Salvador was formed in February 1968, consisting of the director of the national malaria service, the chief of CAMRS, and a representative of PAHO.

Station personnel visited malaria programs in Central America and Panama to observe operational procedures and to discuss problems in specific areas.

Provisions were made in the Station insectary to establish two colonies (which were being abandoned by a PAHO field unit) of DDT-susceptible and DDT-resistant strains of *A. albimanus*, the principal malaria vector in Central America. Establishing colonies from two additional field areas was also being attempted at the end of the fiscal year.

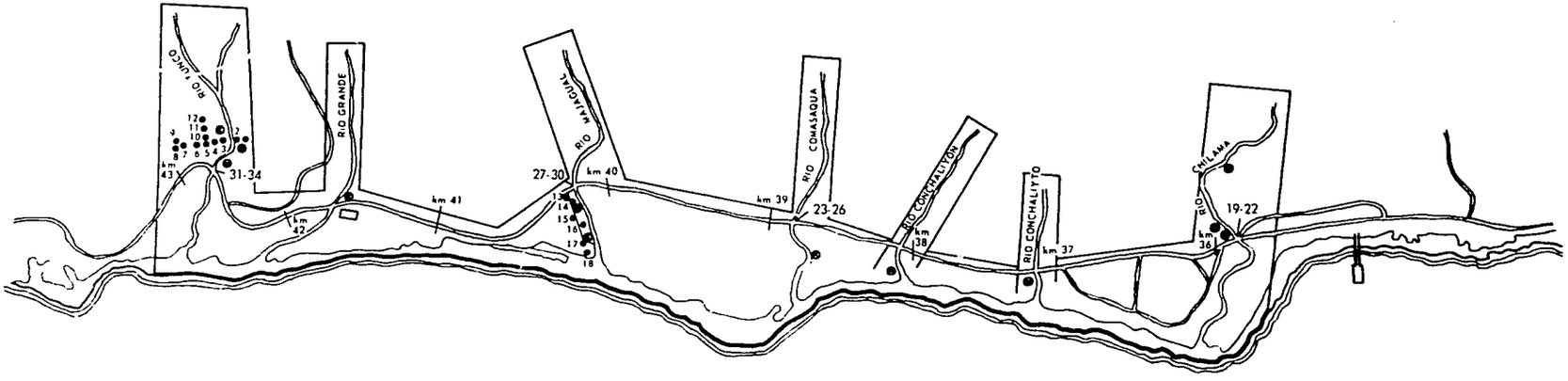
ULTRA LOW VOLUME (ULV) AERIAL APPLICATION OF INSECTICIDES

Following area reconnaissance and preliminary adult and larval mosquito population studies, an area of approximately 2,200 acres along the Pacific coast near La Libertad, El Salvador, was selected as the test site for a limited field evaluation of the ultra low volume (ULV) aerial application of technical malathion. The Marian Air Service of Savannah, Georgia, collaborated in the test, furnishing--without charge--two pilots and an aircraft with calibrated equipment for applying ULV malathion. A representative of the NCDC Aedes aegypti Eradication Program, with previous experimental and operational experience in the use of ULV malathion for mosquito control, assisted in the study.

Several treatments were scheduled to be applied at approximately weekly intervals near the end of the prolonged dry season in this area; effects of the treatments individually and collectively were to be evaluated on adult and larval anopheline populations. Unfortunately, severe weather conditions of fog, smoke, and haze occurred just prior and subsequent to the arrival of the aircraft and prevented the planned sequence. Initial treatment was delayed, and intervals between treatments were prolonged sufficiently to nullify substantially any cumulative effect.

Technical malathion at a dosage rate of three liquid ounces per acre was applied on the mornings of April 17, May 1, and May 9 to the seven rivers and the area between the main highway and ocean (Figure 1). Evaluating the applications was accomplished by using daytime counts of adult anopheline mosquitoes resting under bridges; nighttime counts of adults resting in a stable; larval counts; oil red-dye cards; and, initially, light-trap collections and exposure of A. albimanus larvae and caged adults during treatment.

Figure 1. Map showing ULV Aerial Treatment Boundaries and Light Trap and Dye Card Locations.



⊗ Light trap placement
• Dye card locations

Treatment and weather conditions existing at the time of the first treatment were far from ideal (Table 1).

TABLE 1

Treatment and Weather Conditions Existing During 3 ULV Aerial Applications of Malathion Applied in a Coastal Area of El Salavador, April 17-May 9, 1968

	Treatment Number		
	1	2	3
Date of Treatment	April 17	May 1	May 9
Time of Treatment	9:35-10:15 am	7:10-7:50 am	7:15-8:00 am
Temperature	89°F-91°F	78°F-82°F	77.5°F-89°F
Relative Humidity	75%-76%	65%-71%	74%
Wind Speed	1.0-5.0 mph	0- <2 mph	<1 mph
Acres Treated	2,160	2,600	2,600
Dosage (oz/acre)	3	3	3
Aircraft	Beachcraft C-18S	Beachcraft C-18S	Beachcraft C-18S
Speed of Aircraft	150 mph	150 mph	150 mph
Altitude	150 feet	150 feet	150 feet

Treatments were scheduled to begin at approximately 7:00 a.m.; but, because of extreme smoke and haze, the aircraft could not begin treatment until approximately 9:30 a.m. At that time, air temperatures had reached 89° F. Temperature and wind conditions for the second and third treatments were satisfactory except for rapidly rising temperatures during the third treatment.

Malathion droplets were recovered on 4" x 5" oil red-dye cards after each of the three treatments (Tables 2, 3, and 4). The rather adverse weather conditions during the first treatment resulted in an average of only 5.5 droplets per square inch. Although mortality of caged adult female A. albimanus and of third instar A. albimanus larvae was quite high (Table 5), these data are somewhat questionable because of excessive mortalities encountered in untreated control samples.

TABLE 2

Number of Malathion Droplets Recovered on Oil Red-dye Cards after Exposure to ULV Malathion Applications at Rate of 3 Liquid Ounces of Malathion per Acre
Treatment I, April 17, 1968

Site Number	Number of Droplets per Card	Description of Site
1	Lost	-
2	3	In dense jungle--triple canopy.
3	207	Edge of jungle--protected on three sides.
4	32	Edge of grass hut on downwind side.
5	215	On dry river bottom.
6*	56	Open field.
7*	112	" "
8*	34	" "
9*	22	" "
10*	33	Under tree in field.
11*	Lost	-
12*	16	Open field.
13	254	Under heavy canopy at residence.
14	41	Partial canopy at residence.
15	130	Under heavy single canopy.
16	116	Under moderate single canopy.
17	172	Under spreading tree.
18**	14	In hut on beach.
19	179	Under Rio Chilama Bridge.
20	178	" " " "
21	159	" " " "
22	247	" " " "
23	109	Under Rio Comasagua Bridge.
24	214	" " " "
25	177	" " " "
26	140	" " " "
27	163	Under Rio Majagual Bridge.
28	208	" " " "
29	122	" " " "
30	83	" " " "
31*	9	Under Rio Tunco Bridge.
32*	42	" " " "
33*	26	" " " "
34*	29	" " " "
Total	3,541	
Average Droplets per Square Inch	5.5	

* Incomplete treatment because of lack of sufficient insecticide.
** This site too close to first swath--missed with most of treatment.

TABLE 3

Number of Malathion Droplets Recovered on Oil Red-dye Cards after Exposure to ULV Malathion Applications at Rate of 3 Liquid Ounces of Malathion per Acre
Treatment II, May 1, 1968

Site Number	Number of Droplets per Card	Description of Site
1	18	-
2	10	In dense jungle--triple canopy.
3	1	Edge of jungle--protected on three sides.
4	19	Edge of grass hut on downwind side.
5	6	On dry river bottom.
6	62	Open field.
7	147	" "
8	271	" "
9	461	" "
10	104	Under tree in field.
11	54	Open field.
12	58	Open field.
13	305	Under heavy canopy at residence.
14	1,826	Partial canopy at residence.
15	367	Under heavy single canopy.
16	552	Under moderate single canopy.
17	216	Under spreading tree.
18	63	In hut on beach.
19	158	Under Rio Chilama Bridge.
20	171	" " " "
21	315	" " " "
22	416	" " " "
23	Lost	Under Rio Comasagua Bridge.
24	124	" " " "
25	141	" " " "
26	256	" " " "
27	107	Under Rio Majagual Bridge.
28	119	" " " "
29	56	" " " "
30	81	" " " "
31	90	Under Rio Tunco Bridge.
32	203	" " " "
33	361	" " " "
34	354	" " " "
Total	7,492	
Average No. of Droplets per Square Inch	11.3	

TABLE 4

Number of Malathion Droplets Recovered on Oil Red-dye Cards after Exposure to ULV Malathion Applications at Rate of 3 Liquid Ounces of Malathion per Acre Treatment III, May 9, 1968

Site Number	Number of Droplets per Card	Description of Site
1	889	-
2	0	In dense jungle--triple canopy.
3	26	Edge of jungle--protected on three sides.
4	92	Edge of grass hut on downwind side.
5	288	On dry river bottom.
6	198	Open field.
7	627	" "
8	646	" "
9	53	" "
10	Lost	Under tree in field.
11	334	Open field.
12	Lost	" "
13	119	Under heavy canopy at residence.
14	412	Partial canopy at residence.
15	113	Under heavy single canopy.
16	591	Under moderate single canopy.
17	221	Under spreading tree.
18	40	In hut on beach.
19	1,380	Under Rio Chilama Bridge.
20	554	" " " "
21	646	" " " "
22	713	" " " "
23	Lost	Under Rio Comasagua Bridge.
24	Unreadable	" " " "
25	503	" " " "
26	702	" " " "
27	103	Under Rio Majagual Bridge.
28	121	" " " "
29	Unreadable	" " " "
30	457	" " " "
31	2	Under Rio Tunco Bridge.
32	10	" " " "
33	15	" " " "
34	16	" " " "
35*	304	Open field.
36*	380	" "
37*	373	Under single tree--moderate foliage.
38*	265	" " " " "
39*	429	On stump--open field.
40*	133	" " " "
Total	11,755	
Average No. of Droplets per Square Inch	16.8	

*Line of 6 cards between Rio Grande and Establo--total distance approximately 200 yards.

TABLE 5

Twenty-four-hour Percent Mortality of Caged *Anopheles albimanus* (Adults and Larvae) after Exposure to ULV Aerial Applications of 3 Liquid Ounces of Technical Malathion per Acre, La Libertad, El Salvador, April 17, 1968

Site Number	Adults				Larvae				Number of Malathion Droplets	
	Alive	Dead	Total	Percent Mortality	Alive	Dead	Total	Percent Mortality	Per Card	Per Square Inch
13	0	47	47	100	0	23	23	100	254	12.7
14	14	34	48	71	5	19	24	79	41	2.1
15	1	50	51	98	13	9	22	38	130	6.5
16	1	48	49	98	12	13	25	52	116	5.8
17	0	50	50	100	19	6	25	22	172	8.6
18	1	51	52	98	25	0	25	0	14	0.7
19	2	48	50	96	1	23	24	96	179	9.0
20	0	47	47	100	5	20	25	80	178	8.9
21	1	49	50	98	2	23	25	92	159	8.0
22	0	54	54	100	0	24	24	100	247	12.4
23	1	48	49	98	2	23	25	92	109	5.5
24	0	52	52	100	1	24	25	96	214	10.7
25	0	53	53	100	0	26	26	100	175	8.8
26	0	49	49	100	6	19	25	76	140	7.0
27	1	49	50	98	0	26	26	100	163	8.2
28	0	49	49	100	0	26	26	100	208	10.4
29	0	50	50	100	10	15	25	60	122	6.1
30	1	51	52	98	3	21	24	88	83	4.2
31	19	35	54	65	26	0	26	0	9	0.5
32	2	47	49	96	17	7	24	24	42	2.1
33	2	47	49	96	15	8	23	35	26	1.3
34	1	48	49	98	21	3	24	13	29	1.5
Total	47	1,056	1,103	94.8	183	358	541	66.2	2,810	6.4
Control-1	31	19	50	38	23	2	25	8	-	-
Control-2	21	25	46	54	20	5	25	20	-	-
Control-3	36	14	50	28	21	4	25	16	-	-
Total*	88	58	146	39.7	64	11	75	14.6	-	-

*Controls inadvertently left in back of vehicle and exposed to excessive heat and travel over rough terrain.

Counts of adult mosquitoes resting under bridges were made in the early to mid-afternoon period. (Tables 6, 7, and 8). Mosquito counts under Rio Majagual Bridge were quite low before the first treatment with a total of only 10 mosquitoes captured during 4 pre-treatment periods. Although only 3 mosquitoes were captured in 6 post-treatment periods, populations under this bridge were considered too small to warrant drawing specific conclusions. The maximum effect from ULV malathion application on adult Anopheles mosquitoes under Rio Comasagua Bridge was noted after the second treatment on May 1 (Table 7). On April 30, 125 adult mosquitoes were captured under this bridge; however, on May 2 (24 hours after the second treatment), only 2 adult mosquitoes were captured. Reductions in adult mosquitoes under Rio Grande Bridge after the first and second treatment approached 90 percent (Table 8).

Nocturnal counts were made of adult mosquitoes resting in Establo San Jose (dairy barn) (Table 9). The last pre-treatment count made on April 8 showed a capture rate of 51.0 mosquitoes per man hour. The first post-treatment count on April 19 (48 hours after treatment) showed a capture rate of only 6.0 mosquitoes per man hour reflecting a reduction of approximately 90 percent when compared with the pre-treatment count on April 8. Although a considerable period elapsed between the last pre-treatment and first post-treatment evaluation, the rapid increase in mosquito count on April 22 (48.5 mosquitoes/man hour) and on April 30 (90.5 mosquitoes/man hour) tends to substantiate the apparent reduction of adult mosquitoes after the first treatment. The second and third treatments failed to reflect significant reduction. This establo was very near one of the seven rivers, and newly emerged adults could have been replenishing the population.

Evaluation of the effects of the ULV treatments on aquatic stages of the Anopheles population (Tables 10-14) revealed that, although some reductions in larvae were noted in specific rivers, overall reduction was insignificant. Highly significant reduction in larval populations was noted in the Estero Majagual; however, the area was flooded with salt water, which probably played an important part in reducing the larvae.

TABLE 6

Diurnal Counts of Resting Adult Mosquitoes under Rio Majagual Bridge, La Libertad ULV Studies, 1968

Date	A. albimanus			A. pseudopunctipennis			Both Species		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
March 26	0	2	2	0	0	0	0	2	2
April 1	1	5	6	0	0	0	1	5	6
April 3	1	0	1	0	0	0	1	0	1
April 8	0	1	1	0	0	0	0	1	1
April 17	TREATMENT I								
April 19	0	2	2	0	0	0	0	2	2
April 22	0	1	1	0	0	0	0	1	1
April 30	0	0	0	0	0	0	0	0	0
May 1	TREATMENT II								
May 2	0	0	0	0	0	0	0	0	0
May 6	0	0	0	0	0	0	0	0	0
May 9	TREATMENT III								
May 14	0	0	0	0	0	0	0	0	0

TABLE 7

Diurnal Counts of Resting Adult Mosquitoes under Rio Camasagua Bridge, La Libertad ULV Studies, 1968

Date	A. albimanus			A. pseudopunctipennis			Both Species		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
March 26	0	0	0	5	5	10	5	5	10
April 1	0	0	0	9	15	24	9	15	24
April 3	0	0	0	2	3	5	2	3	5
April 8	0	0	0	10	10	20	10	10	20
April 17	0	TREATMENT I							
April 18	0	0	0	9	2	11	9	2	11
April 19	0	0	0	25	30	55	25	30	55
April 22	0	0	0	32	19	51	22	19	51
April 30	0		0	91	34	125	91	34	[illegible]
May 1	TREATMENT II								
May 2	0	0	0	1	1	2	1	1	2
May 6	0	0	0	2	2	4	2	2	4
May 9	TREATMENT III								
May 10	0	1	1	3	5	8	3	6	9
May 14	0	0	0	3	15	18	3	15	18

TABLE 8

Diurnal Counts of Resting Adult Mosquitoes under Rio Grande Bridge, La Libertad ULV Studies, 1968

Date	A. albimanus			A. pseudopunctipennis			Both Species		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
March 20	21	10	31	25	24	49	46	34	80
March 26	25	30	55	24	45	69	49	75	124
April 1	6	15	21	7	15	22	13	30	43
April 3	5	5	10	17	25	42	22	30	52
April 8	2	10	12	16	19	35	18	29	47
April 16	9	13	22	5	19	24	14	32	46
April 17				TREATMENT		I			
April 18	0	3	3	1	1	2	1	4	5
April 19	4	4	8	3	3	6	7	7	14
April 22	1	2	3	3	5	8	4	7	11
April 30	0	6	6	14	33	47	14	39	53
May 1				TREATMENT		II			
May 2	0	1	1	3	2	5	3	3	6
May 6	0	0	0	2	2	4	2	2	4
May 9				TREATMENT		III			
May 10	0	2	2	4	12	16	4	14	18
May 14	0	0	0	5	10	15	5	10	15

TABLE 9

Nocturnal Counts of Resting Adult Mosquitoes* from Establo San José, La Libertad ULV Studies, 1968

Date	Time Interval	Total Mosquitoes	Man Hours	Mosquitoes/Man Hours
April 1	8:00-8:30 pm.	82	1.5	54.7
April 3	8:00-9:00 pm.	72	2.0	36.0
April 8	7:30-8:00 pm.	102	2.0	51.0
April 17		TREATMENT	I	
April 19	7:30-8:10 pm.	12	2.0	6.0
April 22	7:45-8:15 pm.	97	2.0	48.5
April 30	7:30-8:00 pm.	181	2.0	90.5
May 1		TREATMENT	II	
May 2	7:30-8:00 pm.	139	2.0	69.5
May 6	7:30-8:00 pm.	84	2.0	42.0
May 9		TREATMENT	III	
May 10	7:30-8:00 pm.	132	2.0	66.0
May 14	7:20-8:00 pm.	120	2.0	60.0

*All mosquitoes counted and captured were A. albimanus except on April 1 and 3 when the count was not recorded by species. Most specimens in these establo captures are blooded females.

TABLE 10

Aquatic Stage Mosquito Survey
Rio Tunco, La Libertad ULV Studies, 1968

Date	Dips			Larval Instars				Total Larval	No. of Pupal	No. of Larval Per Dip	Sample Identification (Alb/PP)
	No.	Pos.	Percent	1	2	3	4				
Mar. 27	100	84	84.0	85	176	75	22	358	0	3.58	5/20
Apr. 2	200	39	19.5	27	30	19	31	107	20	0.53	No sample
Apr. 16	200	42	21.0	20	34	24	26	104	9	0.52	3/15
Apr. 17	TREATMENT I										
Apr. 18	200	35	17.5	107	58	8	11	184	4	0.92	0/8
Apr. 22	200	65	32.5	97	84	63	51	295	20	1.48	No sample
Apr. 30	200	40	20.0	82	52	31	6	171	9	0.85	0/7
May 1	TREATMENT II										
May 2	200	37	18.5	43	39	26	17	125	5	0.62	1/14
May 6	100	16	16.0	5	13	21	14	53	11	0.53	0/21
May 9	TREATMENT III										
May 14	100	12	12.0	36	12	6	0	54	0	0.54	-

TABLE 11

Aquatic Stage Mosquito Survey
Rio Grande, La Libertad ULV Studies, 1968

Date	Dips			Larval Instars				Total Larval	No. of Pupal	No. of Larval Per Dip	Sample Identification (Alb/PP)
	No.	Pos.	Percent	1	2	3	4				
Mar. 27	95	47	49.5	14	30	39	37	120	5	1.26	10/14
Apr. 2	200	135	67.5	72	157	201	49	479	0	2.39	18/21
Apr. 9	200	66	33.0	60	51	55	40	206	51	1.03	-
Apr. 16	200	90	45.0	115	64	33	57	269	0	1.34	-
Apr. 17	TREATMENT I										
Apr. 18	200	89	44.5	164	87	38	32	321	8	1.60	5/9
Apr. 22	200	89	44.5	40	36	43	75	194	11	0.97	-
Apr. 30	200	101	50.5	201	90	48	59	398	10	1.99	8/14
May 1	TREATMENT II										
May 2	200	45	22.5	62	30	14	15	121	2	0.65	-
May 6	100	61	61.0	169	100	69	31	369	7	3.69	8/34
May 9	TREATMENT III										
May 10	100	14	14.0	30	14	10	7	61	2	0.61	3/12

TABLE 12

Aquatic Stage Mosquito Survey
Rio Comasagua, La Libertad ULV Studies, 1968

Date	Dips			Larval Instars				Total Larval	No. of Pupal	No. of Larval Per Dip	Sample Identification (Alb/PP)
	No.	Pos.	Percent	1	2	3	4				
Mar. 27	40	6	15.0	15	11	10	4	40	0	1.0	0/5
Apr. 17	TREATMENT I										
Apr. 30	50	14	38.0	10	14	18	22	64	20	1.28	0/10
May 1	TREATMENT II										
May 2	50	12	24.0	24	4	4	10	42	2	0.84	0/14
May 6	50	35	70.0	20	48	76	4	148	0	2.96	0/32
May 9	TREATMENT III										
May 10	50	0	0	0	0	0	0	0	0	0	-

TABLE 13

Aquatic Stage Mosquito Survey
Rio Chilama, La Libertad ULV Studies, 1968

Date	Dips			Larval Instars				Total Larval	No. of Pupal	No. of Larval Per Dip	Sample Identification (Alb/PP)
	No.	Pos.	Percent	1	2	3	4				
Apr. 2	205	100	49.3	106	86	53	71	316	9	1.54	7/38
Apr. 9	200	49	24.5	42	25	45	35	147	18	0.73	-
Apr. 16	200	62	31.0	143	61	11	16	231	6	1.15	7/9
Apr. 17	TREATMENT I										
Apr. 18	200	26	13.0	58	39	18	9	124	1	0.62	0/9
Apr. 22	200	62	31.0	30	68	47	49	194	17	0.97	-
Apr. 30	200	78	39.0	275	130	67	58	530	43	2.65	0/30
May 1	TREATMENT II										
May 2	200	104	52.0	97	73	93	108	371	82	1.85	-
May 6	100	68	68.0	115	109	85	11	320	5	3.20	2/21
May 9	TREATMENT III										
May 10	100	45	45.0	47	36	17	18	118	18	1.18	12/16

TABLE 14

Aquatic Stage Mosquito Survey
Estero Majagual, La Libertad ULV Studies, 1968

Date	Dips			Larval Instars				Total Larval	No. of Pupal	No. of Larval Per Dip	Sample Identification (Alb/PP)
	No.	Pos.	Percent	1	2	3	4				
Apr. 2	210	68	32.4	54	40	13	20	127	13	0.60	10/0
Apr. 9	200	13	6.5	0	0	9	7	16	0	0.08	3/0
Apr. 16	200	11	5.5	34	11	5	0	50	0	0.25	No Sample
Apr. 17	TREATMENT I										
Apr. 18	200	5	2.5	6	4	1	0	11	0	0.05	No Sample
Apr. 22	100	1	1.0	1	0	0	0	1	0	0.01	No Sample
Apr. 30	100	8	8.0	5	1	1	1	8	9	0.04	No Sample
May 1	TREATMENT II										
May 2	100	0	0	0	0	0	0	0	0	0	-
May 6	100	0	0	0	0	0	0	0	0	0	-
May 9	TREATMENT III										
May 14	100	0	0	0	0	0	0	0	0	0	-

Some NCDC minature light traps were operated before the first treatment and for four nights after the treatment (Table 15). Since returns from the traps were only two to four mosquitoes per night, operations were discontinued.

The evaluation tools used should show that, in some situations, as much as 90 percent reduction in adult Anopheles populations could be obtained. Although treatments were scheduled for 7-day intervals, smoke and haze prevented any logical sequence of treatments. As a result, adults were allowed to oviposit in the seven rivers under study; thus populations of adults were present throughout the study. Also, applying insecticide on a "spot treatment" basis instead of to the entire area may have caused some decrease in overall effectiveness of treatments.

The three trials in La Libertad area did not produce results comparable to those previously observed with this type of treatment in other situations against other applications. Despite this fact, the study did demonstrate that the technique offers

some promise in malaria eradication; therefore, consideration is being given to designing a larger scale study at an appropriate future time.

TABLE 15

Results of Mosquito Light Trap Collections
Before and After the First ULV Malathion Treatment

Date Traps Were Operational	Total Number of Mosquitoes Taken*
April 16-17	23
Treatment April 17	
April 17-18	10
April 18-19	5
April 22-23	49
April 23-24	33

*Data obtained from 9 to 12 light traps operated throughout the area under treatment.

Studies of Natural Daytime Resting Places of Anophelines

In anticipation of using portions of La Libertad area for larviciding and ULV studies, measurements of anopheline fauna in this area began in February 1968. Collections became more specific as these projects developed. Since the completion of ULV trials in April and May, CAMRS has recognized the need for developing techniques to measure anophelism not previously used in Central America. Apart from indoor measurements, there is a notable lack of information about anophelism, particularly relating to outdoor daytime resting habits. After several casual collections of anophelines from such places as hollow tree trunks and beneath stones, a more concerted effort was made to discover natural daytime resting places. Since February 27, 1968, when the first resting adults were taken, a number of successful searches have been made. Excepting one large collection at Lake Jocotal, near San Miguel, all collections were from the coastal plain near La Libertad (Table 16).

TABLE 16

Summary of Captures of Diurnally Resting Adult Anophelines from Natural Situations
February-June 1968

Type of Site	No. of Collections	*A. albimanus*			*A. pseudo-punctipennis*			Both Species			Comments
		Male	Female	Total	Male	Female	Total	Male	Female	Total	
Beneath or among small rocks, shaded margin of stream beds	3	0	12	12	3	0	3	3	12	15	Offers scattered, isolated resting sites; requires diligent searching, low-yield results for time expended; however, probably an important niche, especially during the dry season.
Rock or stone walls near breeding sites, partially shaded	1	-	-	400	0	0	0	-	-	400	Mosquitoes in a high density situation, Lake Jocotal, near San Miguel. Mosquitoes rest on or near surface, possibly a good index situation.
Beneath and between large, angled or flat-surfaced rocks, in shaded woodland areas or woodland-field ecotones	9 2	12 -	13 -	25 7	133 -	127 -	260 140	145 -	140 -	285 147	Mosquitoes in concentrated numbers--*albimanus* in more exposed sites, *pseudo-punctipennis* in darker and more protected sites.
Small horizontal cavity in cliff-side, behind water-fall, very dark interior	1	0	0	0	16	14	30	16	14	30	Exclusively *pseudo-punctipennis*, a very dark and well-protected situation.

Type of Site	No. of Collections	*A. albimanus* Male	*A. albimanus* Female	*A. albimanus* Total	*A. pseudopunctipennis* Male	*A. pseudopunctipennis* Female	*A. pseudopunctipennis* Total	Both Species Male	Both Species Female	Both Species Total	Comments
Large cliffside with jagged, small rock outcroppings, range from heavy to light shading, various degrees of protection	2 2	9 -	9 -	18 90	8 -	3 -	11 106	17 -	12 -	24 196	*A. albimanus* in the more exposed situations; *A. pseudopunctipennis* in the more shaded and protected sites.
Hollows of large trees, shaded bases of trees, or between large roots of trees	5	4	8	12	22	6	28	26	14	40	*A. albimanus* in more exposed sites; *A. pseudopunctipennis* in darker recesses.
Rotted stump, open field	1	3	1	4	0	0	0	3	1	4	Exclusively *A. albimanus*, may be too exposed for *A. pseudopunctipennis*.
Shallow, small cave, very dark	1	0	0	0	7	0	7	7	0	7	Exclusively *A. pseudopunctipennis*, may be too dark for *A. albimanus*.
Miscellaneous rocks, stones, tree trunks, etc.	1	13	3	16	54	58	112	67	61	128	One man-hour near Pantano Ticuiziapa.
Total	28	41 -	46 -	87 497 584	243 -	208 -	451 246 697	284 -	254 -	538 743 1,281	

Summary of Comments:
Some 28 collections from 8 types of sites yielded 1,281 resting adults. Of these, 584 (45.6%) were *A. albimanus* and 697 (54.4%) were *A. pseudopunctipennis*. The sex ratio of *A. albimanus* was 45.6 percent males and 54.4 percent females; for *A. pseudopunctipennis*, 53.9 percent males and 46.1 percent females; and for both species, 52.8 percent males and 47.2 percent females. *A. pseudopunctipennis* showed a decided preference for darker and more protected places than did *A. albimanus*. Protection offered by rock or stone cover, crevices, and tree hollows dominated the natural resting places discovered.

Studies of Artificial Daytime Resting Places of Anophelines

The ULV and larviciding studies indicated that such man-made structures as bridges and culverts were used freely by both A. albimanus and A. pseudopunctipennis as diurnal resting shelters. Since this is true of some common North American anophelines which also use such places for shelter, it seemed reasonable to presume that A. albimanus and A. pseudopunctipennis might also respond to portable artificial devices, such as red boxes. Although these devices were used by CAMRS late in the reporting year, the relatively few studies done so far indicate the validity of the assumptions. In a high density situation, as many as 200 A. albimanus have been caught in a single red box (Lake Jocotal, near San Miguel); in a low density situation (Pantano Ticuiziapa, near La Libertad), three red boxes used for a three-day period performed very well (Table 17). A sustained study to evaluate the use of these and other artificial devices as indices of Anopheles density is being developed.

TABLE 17

Summary of Anopheline Collections from Three Red Boxes, Pantano Ticuiziapa, La Libertad, El Salvador, June 18-20, 1968

Box		A. albimanus			A.pseudopunctipennis			Both Species		
Number *	Date	Male	Female	Total	Male	Female	Total	Male	Female	Total
1	June 18	0	2	2	15	8	23	15	10	25
	June 19	0	0	0	16	8	24	16	8	24
	June 20	0	0	0	23	17	40	23	17	40
Total		0	2	2	54	33	87	54	35	89
2	June 18	2	1	3	0	0	0	2	1	3
	June 19	0	0	0	0	0	0	0	0	0
	June 20	0	0	0	5	0	5	5	0	5
Total		2	1	3	5	0	5	7	1	8
3	June 18	1	1	2	3	0	3	4	1	5
	June 19	0	0	0	16	6	22	16	6	22
	June 20	0	0	0	7	3	10	7	3	10
Total		1	1	2	26	9	35	27	10	37
Grand Total, 3 boxes 3 days		3	4	7	85	42	127	88	46	134

*Box No. 1 Situated in a hillside north side of pantano, beneath a large root and rock outcropping under rather dense vegetation along a fence row, box at ground level with opening facing south.

Box No. 2 Situated in a woodland on north side of pantano, box at ground level under dense foliage, facing west.

Box No. 3 Situated under a large slanting rock in small woodland on north side of pantano, heavily shaded, box opening facing southeast.

Comment: Probably all of these sites were too shaded for A. albimanus.

LARVICIDING STUDIES

Studies on anopheline larval breeding conditions in El Salvador have indicated that applying larvicides by hand equipment would be most feasible during the dry season when larval sources are most restricted. On this basis, studies were initiated by CAMRS in an area east of La Libertad to determine the effectiveness of applications with manual equipment on anopheline larval sources located primarily along river courses.

During the dry season, these sources are characterized as pools of quiet water along the river margins, frequently with algal mats, emergent vegetation, or both. Penetrating the algal mats with a forceful delivery of spray is required to obtain good distribution of insecticide and good control. For this reason, Tee-jet 8002 fan-type nozzle were used on the spray wand of the 2-gallon hand sprayers used in the larviciding tests.

The insecticide studied was Abate(R) 4 lb/gal emulsion concentrate. This material was applied where anopheline larval breeding was occurring in an area heavily used by local residents as sources of water for domestic use, bathing, and washing clothes. Its high toxicity to anopheline larvae and its extremely low mammalian toxicity offer great potential for using this material in malaria eradication programs. The emulsion concentrate was diluted with water to form the finished spray. Various dosages were applied at the rate of 8 gallons of finished spray per acre, a volume that allowed for good spray distribution in algal mats despite such obstacles in the river bed as large rocks and deep holes that could not be waded.

Pressure gauges were not available for the spray cans; therefore, air pressure

was estimated by pumping the plunger 45 times to give an estimated pressure of 30 psi. Determining the exact pressure exerted by 45 strokes will be made upon arrival of the pressure gauges.

Several dosage rates were applied to determine the minimum dosage that could be used routinely to give complete larval control. The dry season ended before the series of tests contemplated was completed. Replication of some dosages was possible; in other cases, only single tests were made.

Plots were laid out along the river courses by first estimating width of the river to determine the average width for a given section. Then linear measurements were made to form an area with the required square footage for a 1/8 acre plot. The beginning and end of the linear measurement were marked, and the finished spray was applied to the entire water surface area within the bounds. The usual average width when the rivers were low was 10 feet, which required a linear measurement of 544 ft.; one gallon of finished spray was applied to each 1/8 acre plot.

Before applying spray, 20 random dips were taken within the plot and the total number of larvae counted were recorded. Approximately 24 hours later, post-treatment larval counts were made by taking another 20 dips at random.

The total numbers of surviving larvae were recorded, and the difference between pre-treatment and post-treatment counts was used to determine percent mortality. All four stages of larvae, sometimes with both species present, were used in determining the totals in pre-treatment and post-treatment counts. In most cases when surviving larvae were found, they were third and fourth instar (Table 18). A limited number of tests were accomplished before onset of the rainy season.

TABLE 18

Results of Limited Dry Season Larviciding Tests with Abate(R) on *A. albimanus* and *A. pseudopunctipennis* in River Systems of El Salvador, 1968

Number of Tests	Dosage lb/acre	Pre-treatment Count	Post-treatment Count	Average 24-hour Mortality (Percent)	Range of Mortality (Percent)
3	0.002	392	86	78.0	76-80
2	0.005	274	41	85.0	74-96
1	0.01	25	1	96.0	--
2	0.02	289	6	98.0	98-98.6
1	0.04	423	2	99.5	--
2	0.05	618	0	100.0	--

THERMAL FOGGING TESTS

Studies were initiated to determine the feasibility of using thermal fog applications of insecticide as an adjunct in malaria eradication programs. This technique may be useful in causing a rapid decline in adult mosquito density in areas where there is an urgent need for quick reduction of vectors--for example, during an explosive epidemic.

The area selected for these studies was a small village consisting of 34 thatched-roof houses in a row along the margin of a large lake. Steep hills rise abruptly behind the houses. Continuous emergence of adults from the swampy lake margin provided an abundant supply of A. albimanus for experimental purposes. Population of the village consisted of 89 adults and 60 children (younger than 16 years of age).

An index of adult anophelines around the dwellings before treatment was determined by placing eight artificial resting shelters consisting of one-foot square boxes painted red at strategic points outside houses throughout the village. Additionally, as an index of insecticidal efficacy, caged adult mosquitoes were placed in selected houses. These mosquitoes were obtained by capturing A. albimanus females in the evening at a stable near the village. Twenty blooded females were aspirated from stable surfaces into cylindrical, 16-mesh screen cages, 3 inches in diameter and 6 inches in length. These mosquitoes were held in a cool place and provided with a 5 percent white Karo syrup-water solution on a cotton pad until used the following evening. This involved holding the mosquitoes for a total of 20 to 24 hours before exposure to insecticide applications and for 24 hours afterward; with proper handling, however, the controls exhibited very little or no mortality, seldom exceeding 10 to 15 percent.

Before applying thermal fog, red boxes were placed in selected stations at 5 or 6 p.m. The following morning, adult mosquitoes resting in each box were counted. This morning index preceding evening treatment was compared with the post-treatment index made the morning after fogging to determine the percentage reduction (Table 19). The caged adults were taken from the houses approximately 30 minutes after fogging and were held for observation to verify insecticidal effectiveness. Correlation between box counts and cage counts was evident in some tests, but lacking in others.

Technical malathion was formulated as an oil solution by adding the indicated fluid ounces of insecticide to a volume of diesel oil to make one full gallon. A small amount of tenac additive (2 ml/gal) was added to provide good mixing of oil and insecticide.

Fenthion 8 lb/gal emulsion concentrate was prepared as an oil solution by adding the indicated number of fluid ounces to a volume of diesel oil to make one gallon of finished solution. Two milliliters of tenac were added, although a fairly uniform mixture appeared to result even without the additive.

Thermal fog was applied between 7 and 8 p.m. to all 34 houses, inside and outside, and required an average of 1½ gallons of oil solution. This time was selected since it appeared to offer maximum protection from biting adult mosquitoes. Although this time was indicated as a peak activity period, unaffected adults from outlying areas undoubtedly moved into the area that had been fogged during later hours of the nights. The fog was applied with a portable Dyna-Fog 150, with the dial setting for particle size at No. 6 for malathion and No. 4 for fenthion, which, according to the manufacturer, produces a particle size of approximately 5 to 10 microns. Impactor tests were not done to verify that this size was actually being obtained.

TABLE 19

Results of Initial Thermal Fogging Tests on Adult *Anopheles albimanus* at Jocotal Village, El Salvador, Central America, 1968

Date of Test	Insecticide and Concentration		Red Box Counts* Pre-Treatment	Red Box Counts* Post-Treatment	Percent Reduction in Red Boxes	Percent Kill of Caged Adults in Houses
6-11-68	Malathion	5 oz/gal	269	55	80	40
6-25-68	Malathion	6 oz/gal	133	38	79	75
7-15-68	Fenthion E.C.	3 oz/gal	38	19	50	100

*Figures shown are totals for all eight boxes.

ANTIMALARIAL DRUG STUDIES

Arrangements were made with the El Salvador malaria program for a test area with a population of approximately 5,000 people to evaluate the effectiveness of a combination of pyrimethamine and primaquine as an antimalarial drug regimen for possible use in mass drug treatment programs in Central America. Tablets with this drug combination being formulated by a pharmaceutical firm were expected to arrive by late summer. The Station epidemiologist responsible for this project visited the Sambu River area in Panama where this combination has given promising results during a two-year period.

Plans were also being made with El Salvador program personnel for a field study in population groups of approximately 5,000 people to evaluate the relative acceptability of tablets containing a new formulation of amodiaquine-OH + primaquine, as compared to presently available tablets containing amodiaquine-Cl + primaquine, with respect to gastrointestinal upsets. This study was scheduled to be initiated simultaneously with the evaluation of the pyrimethamine-primaquine combination.

POSSIBLE SYNERGISTIC ACTION OF PYRIMETHAMINE AND PRIMAQUINE

Arrangements were completed for a mouse colony and a strain of *Plasmodium berghei* for animal trials of possible synergistic action of these two drugs.

CHARACTERIZATION OF THE LOCAL STRAIN OF PLASMODIUM VIVAX

The Director of the Sonsonate Hospital has cooperated in making available untreated malaria patients for anopheline blood meals. Excessive mortality among the initial lot of fed *A. albimanus* precluded the transmittal of infected specimens to the National Institutes of Health malaria study group at the Federal Penitentiary, Atlanta, Georgia, which is collaborating in this study. This effort will be continued until sufficient successful feedings have been obtained on the desired number of patients to provide an adequate sample of *P. vivax* from the region to characterize it.