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INTRODUCTION

The Technical Development Laboratories were established in Savannah, Georgia, in 1943. Their primary function is to conduct investigations on the control of arthropod- and rodent-borne diseases. Studies involve such disease vectors as mosquitoes, flies, fleas, and triatomids as well as rodents, snails, and cockroaches. The areas of research include: investigations on the habits and tropisms of arthropod vectors, the development of analytical methods for pesticide determination, biochemical studies, investigations on insecticide application equipment and packaging, the development of specifications for insecticides used in vector control, studies on new or improved methods for the chemical control of arthropod vectors and on alternate noninsecticidal techniques, such as biological control, development of new methodology for assessment of arthropod populations, the detection of resistance of insects to pesticides, and the evaluation of molluscicides and rodenticides.

The laboratory is staffed by 26 professional scientists in the biological, chemical, and engineering categories with 63 persons at the supportive level.

Research is conducted along the various professional lines, but the primary characteristic of the Laboratories is the application of the team approach to most of the projects. Experimentation is done at both laboratory and field levels; the latter involves studies in tropical areas in cooperation with national and international agencies.

In addition to their research functions, members of the staff also serve as consultants to local, state, and international health agencies.

CHEMICAL INVESTIGATIONS

A. Examination of DDT Powder Samples Held Under Surveillance for Compliance with the Warranty Clause

During the year, surveillance was kept on 7,334 samples of DDT powder representing 78.9 million pounds of product which had been in storage for 12 months. Of the samples, only 3 failed to meet the shelf-life requirement as required by the warranty clause of Specification PHS/NCDC-I-102. This is by far the lowest rate of failures recorded since initiation of the surveillance program. The two most important factors which affect the shelf life of a water-dispersible powder are increase in particle size and/or decrease in surfactant content. Samples of DDT powder were taken at random from those held under surveillance and analyzed for surfactant content. The average active surfactant content was found to be 1.43%, as compared to 1.08% for those tested the previous year. The improved stability of the FY-1970 samples was probably due to the increase in surfactant in the powders furnished by industry during the past year.

B. Development of Specifications for Pesticide Formulations

After consultation with personnel in the AID Malaria Eradication Program and the contracting officer in GSA who is responsible for purchasing pesticides for the malaria program, the specification for DDT, 75% water-dispersible powder,

was revised and issued on January 15, 1970, as Specification PHS/NCDC-I-102a. With the exception of an increase in the batch size from 10,000 ± 1,000 pounds to 21,000 ± 1,000 pounds, only minor modifications of the specification were made.

C. Study of Foreign DDT Powders

Three samples of DDT, 75% water-dispersible powder, of foreign origin have been tested for compliance with NCDC specifications. Two samples were obtained from Tozai Boeki Kaishaltd (Nippos Soda Company, Ltd., Japan) and one from Pechiney-Pregil, France. The powders were very uniform and finely ground, and all samples passed the suspensibility test as received. One of the Japanese samples was found deficient in suspensibility when tested in hard water after the accelerated storage treatment.

The powders appeared to have a low bulk density so all were compacted to the normal bulk density of DDT powders packaged for shipment. When retested, all failed to meet the suspensibility test after accelerated storage. The fact that the suspensibility of the powders fell sharply after compaction would make their storage stability questionable. These samples are being held for later testing of their performance after shelf-life storage to verify the conclusions drawn from the accelerated storage tests.

D. Study of p,p'-DDT, 90%, Water-Dispersible Powder

Performance tests were made on 90% p,p'-DDT powder purchased for field scale testing on Specification No. PHS/NCDC-I-103 which was issued on May 2, 1969. Of 5 batches of the product prepared by the Montrose Company in July, 1969, only one passed the high suspensibility requirement (2%) after accelerated storage treatment. This batch was accepted and the other 4 batches were held by Montrose for 3-1/2 months and resampled by GSA inspectors. After 3-1/2 months in storage, the suspensibility values for the four batches was 2.25, 2.04, 1.68, and 2.02. The relatively high values obtained after 3-1/2 months storage indicated good probable shelf life, and it was recommended that the batches be accepted. After one year in storage, the batch samples were retested and suspensibility values of 1.41, 1.58, 1.58, and 1.66 were found. The sample which passed the high suspensibility requirement after the accelerated storage test had a suspensibility of 2.21 after one year in storage. If the program should require a 90% p,p'-DDT powder there is little doubt that industry could produce such a product which would have a suspensibility of 2.0% after one year in storage. Lower quality obtained on the first few batches was due to inexperience with the new product.

E. Development of Macro Analytical Methods for Determination of Pesticides

Complications have arisen in the analytical work on malathion and additional work in this area is in progress. One reason for the difficulties presently experienced is the fact that the patent on malathion which was held by the Cyanamid Company has expired and products produced by other manufacturers contain impurities which interfere with previously developed methods. Collaborative analytical work with American Cyanamid Company and the Collaborative International Pesticides Analytical Committee of Europe is in progress.

A titrimetric method for malathion has been developed by TDL employing a potentiometric differential titration of hydrolyzed malathion with silver nitrate. The method is rapid and reproducible but does require the use of a special titrator. Decomposition products do not interfere nor do many of the common impurities.

Simultaneous with the development of the silver titration method for malathion, the American Cyanamid Company has been developing an improved copper-colorimetric method employing a completely anhydrous medium. Their tentative method is currently undergoing evaluation and the results by the two methods are being compared with gas chromatographic results.

The infrared and colorimetric methods in current use yield results that are several percent higher than the true value for malathion content in certain formulations and technical grade products. Typical results obtained on a water-dispersible powder and a foreign made technical product are given in the table below.

Analysis of malathion by different methods

Method	Malathion, percent	
	50% water dispersible (four years old)	Technical (foreign)
Copper-colorimetric (conventional)	-	96.7
Infrared (conventional)	34.0	94.1
Gas chromatography	29.1	85.4
Silver-titration	28.7	84.8
Copper-colorimetric (modified)	24.9	88.8

Although the modified colorimetric method and the titrimetric method have overcome most interferences which affected older methods, neither of the new methods will differentiate between malathion and malathion derivatives which have alkyl groups other than ethyl substituted on the ester group of the malathion molecule. More than 5% of the methyl ethyl ester has been found in some samples of technical material when analyzed by gas chromatography.

The proposed colorimetric method is more time consuming and less reproducible than the titrimetric method. Further work is under way to determine the accuracy of the malathion determination.

A number of analytical problems have arisen in connection with studies on fenitrothion formulations which are being considered by AID and WHO as alternatives to DDT in malaria eradication programs. Two methods were developed which were very useful in studies on fenitrothion powders produced in Japan, Germany, and the United States as well as experimental powders developed at the Technical Development Laboratories. The first was a nitrite titrimetric method adapted from an old procedure used for determination of parathion and the second was a colorimetric procedure which proved to be very useful in determination of the decomposition products of fenitrothion. The latter procedure made it possible to follow the rate of decomposition of the various formulations during storage.

F. Research on Improved Formulations of Organophosphorus Compounds

Work on formulations of organophosphorus pesticides which may be used as alternatives to DDT was accelerated during the past fiscal year. Although most scientists who are intimately acquainted with the problem of anopheline mosquito control are aware of the importance of DDT to malaria programs, pressures from certain conservationists and ecological groups may result in its ultimate demise. Two of the more promising alternatives to DDT are fenitrothion and malathion. During the past year a comprehensive study of fenitrothion water-dispersible powder was completed. Tests were conducted on powders containing

40% to 50% fenitrothion. Short-term accelerated storage and long-term ambient storage tests were conducted on a variety of formulations and some were found to be relatively stable. It was shown that selection of the inert carrier was extremely important to the chemical stability of the compound and accelerated storage tests were developed which would effectively eliminate the undesirable components. Unfortunately, the most sorptive carriers are the most active catalytically and some compromise may have to be made with regard to the concentration of active ingredient in order to obtain long-term storage stability. Powders with 40% active ingredient exhibited little change after one year in storage, while the 45% and 50% powders had reduced suspensibility and chemical content. All samples held up reasonably well in long-term storage. A summary of the data obtained on samples from four manufacturers is given below.

Changes in commercial fenitrothion water-dispersible powders during long-term storage

Company	Nominal content	Decomposition (%)			Suspensibility (%)		
		0 mo.	6 mo.	12 mo.	0 mo.	6 mo.	12 mo.
A	(50%)	2.9	4.3	6.5	67.8	63.4	71.3
B	(40%)	1.6	2.0	2.5	87.2	88.1	91.9
C	(40%)	0.4	0.5	0.8	93.0	90.8	87.9
D	(45%)	0.4	-	3.1	69.5	-	58.6

A = American Cyanamid
B = Bayer
C = Sumitomo
D = Monsanto

Similar studies on malathion formulations are currently in progress. Preliminary studies have shown that malathion is less stable than fenitrothion and, furthermore, the chemistry of malathion is more complex than that of fenitrothion. Commercial formulations of a 50% malathion powder are available; however, it is questionable if such formulations will withstand storage for one year in the field. The chemistry of the decomposition of malathion on inert diluents was undertaken and four decomposition products have been identified and synthesized. It now appears that the cleavage of the molecule is preceded by an isomerization step. This information has been helpful in studying the decomposition rates and in the development of accelerated storage tests. It is now apparent that malathion is less stable than fenitrothion; however, the cost of malathion is considerably less and it would be to our advantage to have a reliable malathion formulation to offer to field programs when an organophosphorus compound is required.

G. Investigation of DDT Pasta Formulations

In some areas in Brazil, acceptance of the conventional DDT powder has been poor especially in communities with good quality housing. The Brazilian program has developed a pasta formulation which contains about 70% DDT and which is acceptable to the above householders. At the present time about 500,000 pounds of the pasta formulation are produced annually in three small plants in Brazil. Unfortunately, some difficulties have arisen in pasta production,

and the Technical Development Laboratories have been asked to assist in the development of an improved formulation. The most frequent problem encountered in the field is poor suspensibility. It has been found that the quality of the pasta can be improved by using a finer grind of DDT starting material or by improving the surfactant system. Some 26 experimental formulations with various surfactants and surfactant mixtures were prepared in order to determine the optimum system for pasta production. Of all systems tested, the products made with a mixture of Triton X-151 and Triton X-172 proved to have the highest suspensibility. Production runs with 50 kg. batches produced in the field confirmed the laboratory results and a significant improvement was observed when the mixed surfactant was substituted for the single surfactant used previously. Some improvement was observed when a superfine grind of DDT was substituted for the 16-mesh product normally furnished; however, the improvement was not as dramatic as that observed with the change in surfactants. Data from the field test are given below.

Effect of surfactant and mesh size
on suspensibility of DDT pastas

Surfactant	Suspensibility, percent	
	16-mesh powder	superfine powder
Triton X-151	21.2	21.2
Octagon 603	19.2	18.4
Triton X-151 + Triton X-172	43.2	48.8
Octagon 603 + Triton X-172	43.2	49.6

H. Instability of Malathion Larval Test Kits

The instability of malathion has caused some difficulty in another area of malaria research. Test kits furnished by WHO for determination of resistance of mosquito larvae have deteriorated in storage. This is of great concern to all who use these kits in the field. Analysis of WHO kits held at ambient temperatures showed approximately 50% decomposition in 6 months. Methods of stabilization of the kits were studied and it was found that addition of 0.01 N benzoic acid reduced the decomposition from 50% to 10% for the 6 months' storage period.

I. Research on Micro Analytical Methods for Determination of Pesticides

During the year, samples of blood of persons exposed to DDT in malaria programs were received for analysis for DDT and its metabolites and proposals were made for further work of this type in the next fiscal year. The interest in DDT levels in exposed people is due to an increase in public awareness and concern over pesticide residues in the environment. The initial samples were analyzed by the simple hexane extraction method of Dale et al., Life Sciences 5: 47-54 (1966), although the method is acknowledged to yield low results. For future work it was decided to investigate methods which would improve the recovery of DDT from the blood and provide more accurate and reliable results.

A variety of solvent systems and extraction techniques were investigated in an effort to improve DDT recoveries from blood samples. The problem was

finally solved by the addition of formic acid to blood serum prior to extraction with hexane. The acid appears to break the bond between DDT and blood protein which allows the DDT to be extracted with hexane. The efficiency of the method was proven by treating rats with C^{14} labeled DDT which enabled analysis of the blood serum by both radioassay and gas chromatography. Quantitative recovery of the DDT from the blood serum by prior treatment with formic acid and subsequent extraction with hexane was shown by parallel radiometric and gas chromatographic analyses. Work on the analytical techniques is now complete and the laboratory is in a position to accept field samples.

J. Biochemical Studies

A large number of compounds, many synthesized at the Technical Development Laboratories, have been formulated in a variety of ways and tested as larvicides, adulticides, and ovicides against two species of mosquitoes, *Anopheles albimanus* and *Aedes aegypti*. A few tests have been run against *Culex pipiens*. The most promising larvicide, presumed to be N-decylputrescine, was synthesized by heating decyl chloride with excess putrescine (1,4-diaminobutane). The following 24-hour LC_{50} values in parts per billion have been obtained with fourth instar larvae of the three species: *Anopheles albimanus* (25), *Aedes aegypti* (2), *Culex pipiens* (1). Polyamines such as putrescine, cadaverine (1,5-diaminopentane), spermine, and spermidine are widely distributed constituents

of biological materials. Since they are often found associated with nucleic acids they are of increasing interest as agents involved in growth regulation. A variety of aliphatic long chain alkyl halides will now be reacted with putrescine and other polyamines, and the products characterized and tested in various insecticidal formulations.

A few compounds have been found to inhibit larval development at a much lower concentration than the LC_{50}. The best example is the commercial surfactant, Barlox 18s, which is recommended for hand and cosmetic cleaners. Stearyl dimethylamine is a fairly good larvicide (LC_{50} of 7 ppm for Aedes aegypti). The oxide of this amine (Barlox 18s) tested against third instar larvae of Anopheles albimanus or Aedes aegypti gave an LC_{50} of about 500 ppm, but inhibited adult formation completely at 7 ppm. The use of such a compound in insect control would be ideal from the standpoint of nontoxicity to higher organisms.

Over a hundred compounds have been tested in adulticide sprays for Anopheles albimanus. A commercial surfactant, Aerosol TR, gave the most promising results. When used in the acid form it was about 1/8 as active as malathion. The salt form of Aerosol TR as well as two syntherized amines, N-hexadecyl-3-amino-1,2-propanediol and N-dodecyl-3-amino-1,2-propanediol, were about 1/2 as active as the acidic Aerosol. The high activities observed with such surfactants, some of which have very little toxicity for

higher organisms, suggests an alteration by such compounds of the permeability of the insect cuticle. Since the eggshell of Aedes aegypti may be considered to be similar in many ways with the adult cuticle, some recently found aedine ovicide formulations are of interest. These aqueous formulations appear to act primarily on the eggshell, killing the larvae by desiccation as evidenced by collapse of the shell. Effective ovicidal formulations have been obtained with use of the following compounds, which are known to break various types of bonds in protein: urea and amines (hydrogen bonds), polar amines (ester bonds), and mercaptoacids (disulfide bonds). Such agents will now be tested in adulticide sprays, with and without the addition of smaller amounts of well-known toxins such as malathion.

Of numerous compounds tested as ovicides for Anopheles albimanus, none have been more potent than the best of those amines reported. Two synthesized amines, N-hexadecyl-2-(2-aminoethoxy)ethanol and N-oleyl-3-amino-1,2-propanediol, each gave an LC_{50} of about 5 ppm. Of the long chain acids tested against anopheline eggs, the best was undecylenic acid which gave an LC_{50} of 20 ppm. Such acids in simple aqueous emulsions have also been found to be effective against aedine eggs, but only when such eggs have been stored in a nearly water-saturated atmosphere. Therefore it appears that fully hydrated aedine eggs have a somewhat similar degree of permeability as anopheline eggs,

which are normally deposited by the insect on water. In the aedine tests, better results were obtained when the long chain acid was supplemented with certain other types of compounds. Such supplements will be used in future testing of acidic ovicides for Anopheles.

ENGINEERING INVESTIGATIONS

A. Hand Sprayers and Nozzle Tips

1. Qualification of suppliers.

As required under the new specifications, the present sprayer and nozzle tip suppliers submitted samples of their products for qualification testing and upon passing were placed on the qualified products list. One other nozzle tip manufacturer submitted samples which failed to qualify. The specifications also require that suppliers furnish samples of each lot of sprayers or tips purchased to TDL for testing. This requirement is now being written into GSA contracts and will insure continuing monitoring of the quality of the products.

2. Sprayer testing.

A cooperative test involving TDL, H. D. Hudson Manufacturing Company, and Pan American Health Organization was undertaken in order to evaluate certain modifications to Hudson sprayers made as a result of suggestions from field personnel in various malaria eradication programs. Fifty sprayers were produced and 44 of these were put into field use in Central America for approximately 6 months.

What does this cost the manufacturer

Detailed histories were kept on the sprayers by field personnel and upon completion of the spraying cycle the sprayers were shipped to TDL for examination. Results of this test, which will be reported elsewhere, probably in the WHO/MAL/VBC series, will be of benefit in the AID-supported malaria programs.

In addition to evaluation of the modifications to the sprayers, very valuable information was obtained on the useful life of certain sprayer components. Action is already being taken to improve some parts in order to increase their service life. These same sprayers are to be returned to the field for further testing following necessary repairs by the manufacturer.

3. Nozzle tip testing.

A field test involving 150 nozzle tips was begun in collaboration with PAHO in Central America. The tips, the same type used by USAID, were first sent to TDL for calibration and then placed in the field. Upon completion of specified periods of time, during which accurate records of quantities of insecticide sprayed were kept, the tips are to be returned to TDL for retesting. The information obtained from this test will reinforce TDL's laboratory evaluations of the new alloy used in these tips and will permit a

when? when will results be available? who will get info?

tip replacement schedule based on actual field experience. Similar field tests are being conducted by MEP-Brazil with TDL's participation in testing the tips, both before and after use. Assistance was also given to MEP-Brazil in furnishing drawings and parts necessary to assemble nozzle testing equipment.

B. Disc Flow Regulator

Extensive laboratory testing of the disc flow regulator has helped to explain the problems encountered in previous field trials of this device. The results of TDL's tests, which have been summarized in a paper submitted for publication, indicate that the DFR is a potentially valuable device which can greatly improve uniformity of application of residual insecticides, reduce nozzle tip replacement costs, and result in less loss of insecticide through "bounce-off" and misting of the spray.

How "potentially"? Give more & copy of report!

C. Ultra Low Volume Insecticide Application

Laboratory and wind-tunnel evaluation of various spray devices designed for ULV use continued. TDL's aircraft has been completely overhauled and new gear installed in preparation for field evaluation of ULV equipment and techniques.

- with what results?

This item was reported last year - what did we do this year?

D. Aircraft Disinsection

1. Baggage containers.

Baggage containers are shelters for insect vectors and they are excellent vehicles for their rapid transportation and dispersion. Tests were made on 747 baggage containers, made available by TWA and Pan American, to determine the efficiency of the dichlorvos vapor aircraft disinsection system. It was observed that containers must be louvered to provide not less than 7% of open area for the vapor passage in order to make the system effective.

2. Boeing 747.

TDL made in-flight tests on the dichlorvos system. The performance was marginal and certification was temporarily withheld.

3. New disinsection cartridge.

TDL has developed a cartridge simpler in design and fabrication, lighter and less bulky, and with an estimated saving of $1.00 per unit over the presently available and recommended cartridge.

4. Approval of disinsection system components.

Four air compressors submitted by vendors were examined and tested. All met the WHO provisional specifications.

BIOLOGICAL INVESTIGATIONS

A. Studies on New and Improved Methods for Control of Mosquitoes[1/]

1. Evaluation of surface-applied residual insecticides.

a. Laboratory evaluation of new compounds as residual deposits.

Procedures:

Approximately 150, 3-day-old adults of susceptible *Anopheles albimanus*, chlorinated hydrocarbon-resistant *Anopheles quadrimaculatus* and *Culex quinquefasciatus* were exposed for 1 hour to treated plywood panels held in a vertical position. In certain tests, *Anopheles stephensi* and *Anopheles sundaicus* also were employed. On removal, the mosquitoes were provided with food and water for 24 hours. Residues were from wettable powders only; dosages were 0.5, 1.0, and 2.0 g/m^2. Each dosage was replicated three times. Panels were stored after each test and the same set of panels was used throughout the test period. Initial evaluations were made

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[1/] The names and chemical formulae of compounds tested are given in Table 1.

Table 1

Compounds Tested, FY-1970

Name	Chemical Composition
Abate	O,O,O',O'-tetramethyl O,O'-thio-di-p-phenylene phosphorothioate
BAY 85032	1,1-dimethyl-4-indanyl-N-methylcarbamate
BAY 93820	O-methyl phosphoramidothioate O-ester with isopropyl salicylate
BAY 99485	confidential
carbaryl	1-naphthyl N-methylcarbamate
Consan 20	Benzyl ammonium chlorides
DDT	dichloro diphenyl trichloroethane
dimethoate	O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate
E.I. 72,016	phosphorothioic acid, 0-5,6-dimethyl-2-pyrazinyl O,O-dimethyl ester
fenitrothion	O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate
fenthion	O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate
Filariol 60	O-(4-bromo-2,5-dichlorophenyl) O,O diethyl phosphorothioate
Flit MLO	petroleum oil

Table 1
(continued)

Name	Chemical Composition
Hercules 9007	M-Isopropylphenyl N-chloroacetyl N-methyl-carbamate
Hercules 14469	confidential
Hexadecane	Hexadecane
methoxychlor	1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane
NC 6897	2,2,dimethyl-1,3-benzodioxol-4-yl-N-methyl carbamate
OMS-1155	confidential
OMS-1169	confidential
OMS-1310	confidential
OMS-1322	confidential
OMS-1336	confidential
OMS-1337	confidential
OMS-1342	confidential
OMS-1343	confidential
OMS-1345	confidential
Ortho 11775	3-(2-butyl) phenyl-N-methyl-N-(Phenylsulfenyl) carbamate

Table 1
(continued)

Name	Chemical Composition
Sulfonated Acid I	Related to methoxychlor - DDT
Sulfonated Ester II	Related to methoxychlor - DDT
T-Det:diesel	Ethoxylated alkylphenol:petroleum oil

2 weeks after treatment and thereafter at 4-week intervals up to 26 weeks. The percent kill for each test was determined 24 hours after exposure with effectiveness based on female mortality only. Evaluation was terminated at 26 weeks or whenever two consecutive tests failed to produce mortalities greater than 70%. Malathion served as the test standard. Untreated check panels were also used.

Results:

The results (Table 2) are based on weeks of 70% kill produced by insecticide treatments. Of the 12 compounds tested, six gave more than 12 weeks of 70% or higher kills of one or more species of test insects and thus met the criteria for further testing. These compounds included methoxychlor[2/], NC 6897, OMS compounds 1310, 1336, 1342, and 1343. NC 6897, OMS-1342 and OMS-1343 were the most effective compounds since each gave extended residual life at the lowest dosage ($0.5\ g/m^2$).

[2/] When tested on mud panels, poor results were obtained.

Table 2

Effectiveness of experimental insecticide residues on plywood panels against *A. quadrimaculatus*, *A. albimanus*, and *C. quinquefasciatus*.

Insecticide Formulation	Dosage g/m^2	Weeks 70% Kill		
		A. quad.	*A. alb.*	*C. quin.*
Abate	0.5	< 2	< 6	< 2
	1.0	< 2	< 6	< 2
	2.0	< 2	< 6	< 2
dimethoate	0.5	< 2	< 6	< 2
	1.0	< 2	< 6	< 2
	2.0	< 2	< 6	< 2
E.I. 72,016	0.5	< 2	< 2	< 2
	1.0	< 2	< 2	< 2
	2.0	2	2	2
methoxychlor	1.0 (mud)	2	2	-
	1.0 (plywood)	6	> 22	-
NC 6897	0.5	> 26	> 26	> 26
	1.0	> 26	> 26	> 26
	2.0	> 26	> 26	> 26

Table 2
(continued)

Insecticide Formulation	Dosage g/m^2	Weeks 70% Kill		
		A. quad.	A. alb.	C. quin.
OMS-1310	0.5	6	14	6
	1.0	14	18	14
	2.0	22	> 26	26
OMS-1336	0.5	< 2	< 6	< 2
	1.0	6	14	2
	2.0	> 26	> 26	> 26
OMS-1337	0.5	< 2	< 6	< 2
	1.0	< 2	< 6	< 2
	2.0	2	< 6	< 2
OMS-1342	0.5	> 26	> 26	> 26
	1.0	> 26	> 26	> 26
	2.0	> 26	> 26	> 26
OMS-1343	0.5	> 22	> 22	> 22
	1.0	> 22	> 22	> 22
	2.0	> 22	> 22	> 22
OMS-1345	0.5	2	< 6	2
	1.0	2	< 6	2
	2.0	2	< 6	2

Table 2
(continued)

Insecticide Formulation	Dosage g/m^2	Weeks 70% Kill		
		A. quad.	A. alb.	C. quin.
VCS-506	0.5	< 2	-	< 2
	1.0	< 2	-	< 2
	2.0	< 2	-	< 2

The acute oral toxicity (LD_{50}) to rats of OMS-1342, however, is 30-40 mg./kg. and that of OMS-1343 is 12-20 mg./kg. which might well present a hazard even at the low dosage. The acute oral LD_{50} for NC 6897 is 80 mg./kg. which would make this compound a much more feasible candidate for field use. The acute oral LD_{50} for rats for the other compounds were: OMS-1310, 250-1,000 mg./kg.; OMS-1336, 110 mg./kg.; and methoxychlor, 5,000-7,000 mg./kg.

In special tests, standard and entrained (described under B. 1.) strains of A. albimanus were exposed for 1 hour to residues of carbaryl (0.1 g/m^2) to determine if any difference in response was detectable. In tests at 2 and 4 weeks the percent mortalities of the standard/entrained strains were 74/91 and 57/81, respectively. At week 6 the mortalities for both strains were 37%. These data suggest that the strain entrained to be active during the daytime hours presumably had a greater pickup of the insecticidal deposits.

b. Simulated field evaluation of promising compounds on different surface materials and in experimental huts.

(1) Outdoor panel studies.

Procedures:

Five different surface materials (clay, whitewashed clay, plywood, thatch, and cement plaster) were treated with BAY 85032, Hercules 9007, Hercules 14469, OMS-1155, fenitrothion, carbaryl, and carbaryl plus RO5-1923. The panels (1' x 4') of the structural surfaces were treated and held under a peaked-roof, outdoor shed (120' long). These panels were treated in position by using a 2-gallon compression sprayer equipped with an 8002 nozzle and charged to 40 psi. The effectiveness of the compounds was evaluated by exposing dieldrin-resistant *A. quadrimaculatus* and/or *A. albimanus* females to the treated surfaces for 60 minutes under plastic cones. Tests also were conducted to determine if adding the by-product remaining after recrystallization of technical DDT to obtain the p,p' isomer would increase the effectiveness of propoxur or malathion. The

dosage was 0.5 g/m^2 to enable the measurement of synergism or potentiation more readily. Virgin female A. albimanus were used in these tests.

Results:

Data against A. quadrimaculatus are given in Table 3. Fenitrothion formulated from a water-wettable powder or emulsifiable concentrate and applied at 2 g/m^2 gave the best results when effectiveness on all surfaces is considered. Methyl Dursban (2 g/m^2) was as effective on clay as on plywood but gave poor results on thatch.

Since adult A. albimanus are much more sensitive to insecticides than are adult A. quadrimaculatus whenever treated panels failed to give 70% kills of A. quadrimaculatus females, tests were made with virgin females of A. albimanus (Table 4). The first tests with BAY 85032 against this species were made 4 weeks after treatment. At 1 g/m^2 the compound failed to give 70% mortality on clay, whitewashed clay, and cement but was highly effective for 14 weeks

Table 3

Number of weeks that surfaces on panel shed treated with listed insecticides as suspensions (S) or emulsions (E) gave mortalities of 90% or more of Anopheles quadrimaculatus females exposed for 1 hour.

Toxicant	Dosage g/m^2	Clay	W. W. Clay 1/	Plywood	Thatch	Cement Plaster
BAY 85032 (S)	1.0	0	0	0	0	0
BAY 85032 (S)	2.0	0	0	0	4	2
Hercules 9007 (S)	2.0	2	2	> 10	> 10	2
Hercules 14469 (S)	2.0	0	2	> 8	> 8	2
methyl Dursban (S)	2.0	8	8	8	2	8
carbaryl (S)	1.0	4	2	> 12	> 12	2
carbaryl plus RO5-1923(S)	1.0+3.0	2	2	> 12	> 12	2
fenitrothion (S)	0.5	4	4	9	6	9
fenitrothion (E)	0.5	6	4	> 9	2	> 9
fenitrothion (S)	1.0	4	6	> 9	> 9	> 9
fenitrothion (E)	1.0	6	9	> 11	6	> 11
fenitrothion (S)	2.0	> 9	> 9	> 9	> 9	> 9
fenitrothion (E)	2.0	> 11	> 11	> 11	> 11	> 11

1/ Whitewashed clay

Table 4

Number of weeks that surfaces on panel shed treated with listed insecticides as suspensions gave mortalities of 90% or more of *Anopheles albimanus* females exposed for 1 hour.

Toxicant	Dosage g/m^2	Clay	W. W. Clay 1/	Plywood	Thatch	Cement
BAY 85032 2/	1	0	0	> 14	> 14	0
BAY 85032 2/	2	14	14	> 16	> 16	14
Hercules 9007 3/	2	0	> 16	> 16	> 16	0
Hercules 14469 3/	2	14	14	16	14	0

1/ Whitewashed clay.

2/ Initial test made when panels were aged 4 weeks.

3/ Initial test made when panels were aged 8 weeks.

on plywood and thatch. BAY 85032 applied at the rate of 2 g/m^2 gave from 97% to 100% mortality for 14 weeks or more on all surfaces. The treatment failed on week 16 on clay, whitewashed clay, and cement. The first test against A. albimanus on surfaces treated with Hercules 14469 or 9007 (2 g/m^2) was made 8 weeks after treatment. Residues of Hercules 14469 were effective on all surfaces except cement through week 14. Residues from Hercules 9007 failed to give satisfactory kills on week 8 on cement and clay but were effective on whitewashed clay, plywood, and thatch for 16 weeks.

Table 5 gives results obtained for the first 6 weeks after treatment when the DDT by-product was combined with propoxur (1:1) as a suspension. The combination did not give any better results than the DDT by-product alone on any surface for the 6-week test period. Mortalities from exposures to residues on clay and whitewashed clay surfaces treated with the DDT by-product alone or in combination with propoxur were 70% or less on weeks

Table 5

Percent mortality of female *Anopheles albimanus* exposed for 1 hour to deposits of several toxicants on five surfaces.

Toxicant	Dosage g/m^2	Percent mortality at weeks after treatment														
		Clay			W.W. Clay[1/]			Plywood			Thatch			Cement		
	wk.→	2	4	6	2	4	6	2	4	6	2	4	6	2	4	6
propoxur (S)	0.5	93	7	7	100	7	0	100	100	100	100	90	100	47	7	-
DDT by-product (S)	0.5	100	63	63	100	63	53	100	100	100	100	87	90	93	93	100
propoxur + DDT by-product (S)	0.5 ea.	100	50	57	100	67	70	100	100	100	100	100	97	100	100	93
malathion (S)	0.5	20	0	-	27	3	-	100	100	100	100	80	60	40	0	-
malathion + DDT by-product (S)	0.5 ea.	100	30	50	100	90	87	100	100	100	100	100	100	100	100	100
DDT, technical (E)	0.5	57	100	80	40	100	20	97	100	100	100	100	73	100	100	97
DDT by-product (E)	0.5	23	30	-	30	47	-	67	97	-	90	77	-	37	100	-
malathion (E)	0.5	13	3	-	10	3	-	100	100	-	100	77	-	37	3	-
DDT, tech. + malathion (E)	0.5 ea.	87	87	90	90	100	80	100	100	100	100	100	100	100	100	100
DDT by-product + malathion (E)	0.5 ea.	3	10	-	0	10	-	100	100	-	-	3	-	83	50	-

[1/] Whitewashed clay

(S) = suspension

(E) = emulsion

4 and 6. On plywood and thatch all formulations gave similar high levels of efficacy. On cement the propoxur alone was inferior to DDT product or to the combination through week 6.

The mortalities from deposits of the DDT by-product plus malathion as a suspension were slightly better than that obtained with the DDT by-product alone on whitewashed clay on week 4. The data for the other surfaces were similar. Subsequent evaluations on weeks 8 and 10 (not shown in Table 5) indicated the malathion or DDT by-product alone gave results similar to the combination.

Later in the season, technical DDT and the DDT by-product were sprayed alone and in combination (1:1) with malathion as an emulsion. Data from tests on surfaces treated with technical DDT were erratic but mortalities were higher than on the surface treated with the DDT by-product. When the technical DDT was combined with malathion, mortalities with some tests were slightly to markedly better than with DDT alone. However

the differences are not considered as sufficient to be of any importance. The combination of DDT by-product with malathion did not show any marked improvement in mortalities over those from the DDT by-product alone.

(2) Experimental hut studies.

Procedures:

The experimental huts were 6 x 6 feet with a 6-foot rear wall and a 7-foot front. The front had a door 2 x 7 feet that opened onto a screened porch (2.5 x 6 feet). A 2-inch opening between the front and back walls and the ceiling provided additional ventilation. The inner walls were lined with clay blocks 2 inches thick; the ceiling and door were plywood.

Treatment to the hut interior was made with the same equipment used to treat the outdoor panel shed. After the spray application, the floors were lined with clean paper. The screened porch was not sprayed. Treatments were evaluated by (1) releasing free-flying

mosquitoes in the hut and (2) suspending caged mosquitoes near the center of the hut. The free-flying and caged mosquitoes were exposed for 2 hours and then held for 24-hour mortality determinations. All tests were conducted during the day with the door open between the screened porch and the interior of the hut. Approximately 60 female mosquitoes were released in the hut and 25 were confined in the suspended cage. The two types of tests were duplicated in an untreated hut. Records were also made of the location of free-flying mosquitoes at the end of the tests. Unless otherwise noted, the mortalities given represent specimens found both inside the hut and on the porch. The compounds tested were BAY 85032, Hercules 9007, propoxur, malathion, and the DDT by-product.

Results:

BAY 85032 at 1 and 2 g/m^2 was ineffective against *A. quadrimaculatus* (Table 6) but gave kills ranging from 88% to 100% for 14 weeks when *A. albimanus* were used.

Table 6

Mortalities (inside and porch) obtained after 2-hour exposure of free-flying female *Anopheles quadrimaculatus* in treated clay-lined plywood huts. 1969.

Toxicant[1/]	Dosage g/m^2	Percent Mortality											
		Residue age, weeks											
		2	4	5	6	7	8	9	10	11	12	13	14
BAY 85032	2.0	73	33	-	-	-	-	-	-	-	-	-	-
BAY 85032	1.0	21	33	-	-	-	-	-	-	-	-	-	-
Hercules 9007	2.0	100	100	-	100	-	100	-	96	-	100	-	100
propoxur	0.5	100	-	100	-	100	-	95	-	36	-	88	-
DDT by-product	0.5	38	-	38	-	21	-	55	-	18	-	21	-
propoxur + DDT by-product	0.5 ea.	-	-	97	-	100	-	100	-	89	-	100	-
malathion	0.5	-	-	36	-	20	-	59	-	28	-	23	-
malathion + DDT by-product	0.5 ea.	-	-	64	-	54	-	30	-	49	-	6	-

1/ Formulated from water-wettable powder.

Hercules 9007 at 2 g/m^2 gave kills of 96% to 100% of A. quadrimaculatus for 14 weeks.

A. quadrimaculatus and A. albimanus also were used in the hut tests to determine if combining the DDT by-product with propoxur or malathion would increase the effectiveness of either compound as compared to the separate components (Tables 6 and 7). The dosage selected was 0.5 g/m^2 so as to provide a rapid assessment of the potential of the combination formulations. With the exception of the test on week 11, propoxur was highly effective against A. quadrimaculatus, giving mortalities ranging from 88% to 100%. The DDT by-product was ineffective against this species, giving no more than 55% mortality. When the two compounds were combined, mortalities ranged from 89% to 100% through week 13. The results with the combination were only slightly better than those with propoxur alone. Neither malathion alone nor in combination with the DDT by-product was effective, 64% being the highest mortality obtained.

Table 7

Mortalities (inside and porch) obtained after 2-hour exposure of free-flying female A. albimanus in treated clay-lined plywood huts. 1969.

Toxicant[1/]	Dosage g/m^2	Percent Mortality					
		Residue age, weeks					
		2	4	6	8	10	14
propoxur	0.5	100	100	98	100	100	97
DDT by-product	0.5	59	59	73	57	5	-
propoxur + DDT by-product	0.5 ea.	100	93	100	100	100	98
malathion	0.5	100	100	96	47	77	20
malathion + DDT by-product	0.5 ea.	100	95	78	59	84	24
Control		19-4	2	0	4	5	0

1/ Formulated from water-wettable powder.

When A. albimanus were used as the test specimens, propoxur alone gave kills of 97% to 100%, and similar results were obtained with propoxur plus the DDT by-product. The DDT by-product gave erratic and ineffective results. Malathion alone gave kills of 96% to 100% for 6 weeks but only 47% on week 8. When combined with the DDT by-product, the mortalities obtained were not improved over those with malathion alone.

Only propoxur and Hercules 9007 gave greater than 70% kills of mosquitoes in the suspended cages. Propoxur gave 92% kill of A. quadrimaculatus 2 weeks after treatment and 72% kills of A. albimanus 4 weeks after treatment. Hercules 9007 gave complete kill of A. quadrimaculatus 2 weeks after treatment and complete kill of A. albimanus 4 weeks after treatment. In earlier studies with propoxur at 2 g/m^2, the fumigant action against A. quadrimaculatus persisted for more than 20 weeks, that of Hercules 9007 at the same dosage for 13 weeks.

2. Evaluation of space applications.

a. Laboratory evaluation of new compounds as space applications.

Procedures:

Adult 3-day-old female susceptible *A. albimanus* were exposed for 30 seconds to a mist spray discharged within a modified Hoskins-Caldwell chamber. Each toxicant was formulated at concentrations of from 0.002% to 0.01% in a glycerol (15 parts):95% ethanol (85 parts) carrier. Two milliliters of each formulation were atomized through the exposure carton, each carton containing approximately 50 female specimens. After exposure, the insects were removed to clean holding containers and provided with food and water for 24 hours at which time the percentage kill was determined. Three replicates of each dosage were used.

The criterion for further testing is a 95% mortality of the test insects at a concentration of $< 0.025\%$. Check specimens were exposed to solvents alone. Malathion served as the standard toxicant.

Results:

The data are shown in Table 8. OMS-1102 was as toxic to A. albimanus as the standard, but OMS-1310 was inferior.

A sample of DDT emulsifiable concentrate was submitted by the National Malaria Eradication Program, Philippine Islands, for chemical and biological assessment. The formulation was tested against four species of Anopheles: quadrimaculatus, albimanus, stephensi, and sundaicus. Results are given in Table 9. Against A. albimanus at 0.01% the DDT treatment was essentially as effective as malathion (Table 8). The difference in response of the four species is apparent from the data, the lowest mortalities being achieved against A. quadrimaculatus and A. stephensi.

3. Evaluation of new compounds applied as larvicides.

a. Laboratory evaluation of new compounds as larvicides.

Procedures:

The LC-95 level in parts per million was determined for A. quadrimaculatus, A. albimanus, and Culex quinquefasciatus

Table 8

Percent mortality of *A. albimanus* exposed to mist applications at three concentration levels.

Toxicant	Percent Concentration		
	0.01	0.005	0.002
malathion	98	83	5
OMS-1102	99	78	11
OMS-1310	76	14	3

Table 9

Percent mortality obtained with most applications of 25% emulsifiable DDT against four species of *Anopheles*.

Species	Percent Concentration of Toxicant			
	0.025	0.01	0.005	0.0025
A. quadrimaculatus (dieldrin-resistant)	46	7	1	0
A. albimanus (dieldrin-resistant)	100	92	59	7
A. stephensi (susceptible)	68	8	< 1	1
A. sundaicus (susceptible)	95	55	17	25

using the standard WHO test method which involves a 24-hour exposure of 25 third instar larvae in 250 ml. of water with the toxicant introduced in serial dilutions in an ethanol solution. Each dosage was replicated three times. Fenthion served as a standard. Ethanol-water and water checks were employed.

Surface treatments with oil larvicides were evaluated in the same manner except that they were not diluted with ethanol but added directly to the test container. The surface of the container measured 8.25 sq. in. Volumes of 0.01 to 0.08 ml. were used which are roughly equivalent to from 2 to 20 gal./acre.

Results:

Twenty-one compounds (Table 10) were evaluated; of the 18 compounds tested as nonsurface treatments, 10 met the criterion for further testing by giving LC-95's of 1 ppm or less against one or more of the test species. OMS compounds 1169, 1342, and 1343 were the materials most toxic to the three species of larvae. Of the data for these 10 insecticides, the results for Abate, fenthion, and methoxychlor

Table 10

Concentration in parts per million (ppm) of insecticide required for the LC-95 of three mosquito species.

Toxicant	A. quad. (ppm)	A. alb. (ppm)	C. quin. (ppm)
Abate	0.004	0.02	0.004
BAY 93820	0.5	-	0.5
BAY 99485	0.5	-	0.5
Consan 20	> 10.0	> 10.0	> 10.0
E.I. 72,016	0.1	0.5	0.1
fenthion	0.02	0.02	0.005
Filariol 60	0.1	0.1	0.02
methoxychlor	0.5	0.5	0.5
NC 6897	2.5	2.5	0.5
OMS-1169	0.02	0.02	0.02
OMS-1322	0.1	0.1	0.1
OMS-1328	> 2.5	2.5	0.1
OMS-1336	> 2.5	-	2.5
OMS-1337	> 2.5	> 2.5	> 2.5
OMS-1345	0.02	0.02	0.02
Ortho 11775	0.1	0.02	0.02
Sulfonated Acid I	> 10.0	-	> 10.0
Sulfonated Ester II	> 10.0	-	> 10.0
T-Det:diesel oil	0.02 (ml.)	-	0.04 (ml.)
Flit MLO	0.01 (ml.)	-	0.02 (ml.)
Hexadecane	0.04 (ml.)	-	0.04 (ml.)

represent current evaluation to up date data obtained in earlier tests. The results with Flit MLO, Hexadecane, and T-Det:diesel oil indicate that Flit MLO is somewhat better against C. quinquefasciatus and A. quadrimaculatus than either of the other two oil larvicides. A. albimanus was added to the species tested in FY-1970 because tests with Abate indicated that as a larva this species was more difficult to kill than the larvae of A. quadrimaculatus. This response was surprising in view of the fact that as an adult A. albimanus is much more susceptible to insecticides than A. quadrimaculatus.

4. Evaluation of improved malathion solutions for use in larval susceptibility tests.

The modified larval test solutions of malathion containing benzoic acid (0.01 N) as reported on page 11 were evaluated against the larvae of A. quadrimaculatus during March 1970. The data indicated that the benzoic acid had no adverse effect upon the larvae and that a malathion-benzoic acid solution prepared in July 1969 was as toxic to the larvae as was a malathion solution prepared in March 1970.

B. Studies on the Responses and Habits of Mosquitoes and on Methods for Measuring Mosquito Densities

1. Studies on the responses of mosquitoes to ultraviolet light.

In order to demonstrate the susceptibility of the normal behavioral cycles of A. albimanus and A. stephensi mosquitoes to photoperiod manipulation and confirm the feasibility of conducting light trap evaluations during the day when the mosquitoes normally show little response to such stimuli, experimental work continued on photoperiod entrainment. An artificial photoperiod technique previously used to produce A. albimanus adults with a high level of flight activity around noon when normally they are inactive has now been applied successfully to A. stephensi. Adult mosquitoes subjected from the pupal stage onward to timer-controlled photoperiods were tested at 5-6 days of age in 6 cu. ft. chambers in a light-proof room. Peak activity and responsiveness to an ultraviolet light trap have been obtained at three predetermined times during the day for A. albimanus and at two times for A. stephensi. A summary comparing the combined daytime catches of all artificially conditioned mosquitoes of both species with those of specimens exposed only to the natural photoperiod is given in Table. 11.

Table 11

Captures of artificially and naturally entrained mosquitoes

in 2-hour daytime ultraviolet light trap tests.

Anopheles	Entrainment	Males		Females	
		No. tested	% trapped	No. tested	% trapped
albimanus 1/	Artificial	4447	91	4364	89
	Natural	4334	31	4111	22
stephensi 2/	Artificial	977	95	733	87
	Natural	936	3	728	3

1/ Trapping period 1/2 to 2-1/2 hours after artificial or natural sunset.

2/ Trapping period 4 to 6 hours after artificial or natural sunset.

As an illustration of the variability in behavior which may occur within the genus Anopheles, it may be pointed out that the results shown for A. albimanus were obtained with trapping periods beginning one-half hour after subjective sunset. A. stephensi behaved differently. Table 12 shows that male response to the trap was comparable to that of A. albimanus males shortly after subjective sunset (Table 11), but that females were only moderately attracted at that time. Only when the trapping periods began 4 hours after subjective sunset did the female stephensi catch attain the level shown by female albimanus at the earlier time. A manuscript [3/] detailing the procedure for photoperiod entrainment and the results outlined above, has been prepared for publication.

2. Response of adult anophelines to physical attractants.

For possible application to anopheline surveillance, the behavior of adult A. stephensi in response to light of different wavelengths between 254 mμ in the ultraviolet and 790 mμ in the infrared is being tested by means of a monochromator and

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[3/] Wilton, D. P. and R. W. Fay. Photoperiod entrainment of two Anopheles mosquitoes. In manuscript.

Table 12

Daytime captures by ultraviolet light trap of artificially entrained *Anopheles stephensi* at three intervals after subjective sunset.

Subjective sunset	Test period	Males		Females	
		No. released	% trapped	No. released	% trapped
11:00 a.m.	11:30 a.m.[a] - 1:30 p.m.	346	94	231	30
11:00 a.m.	2:00 - 4:00 p.m.[a]	513	94	290	71
8:30 a.m.	12:30 - 2:30 p.m.[b]	977	95	733	87

a = Three replicates.

b = Six replicates.

a short flight tunnel (see Figure 1). Following introduction of test specimens into the tunnel at its center, the mosquitoes are exposed simultaneously to a test wavelength and to an incandescent light standard of equal brightness positioned at opposite ends of the tunnel. At the end of the exposure period the tunnel is divided into equal parts by a central sliding gate and the numbers of mosquitoes in the two halves are determined. A minimum of four replicates have been run at each of 23 test wavelengths using an average of 136 males and 128 females per replicate.

When subjected to Chi-square analysis, the counts showed statistically significant preferences for three wavelength regions over incandescent light. These regions are 254-315 mμ, 365-415 mμ, and 640-765 mμ. As indicated in Figure 2, the most pronounced responses were to 290 mμ and 740 mμ by females and to 265 mμ and 765 mμ by males. At nearly all wavelengths, male response was the stronger.

3. Field studies in El Salvador.

An exploratory field trial of ultraviolet light as an attractant for *A. albimanus* was made during June 1969 on the seacoast

FIGURE 1

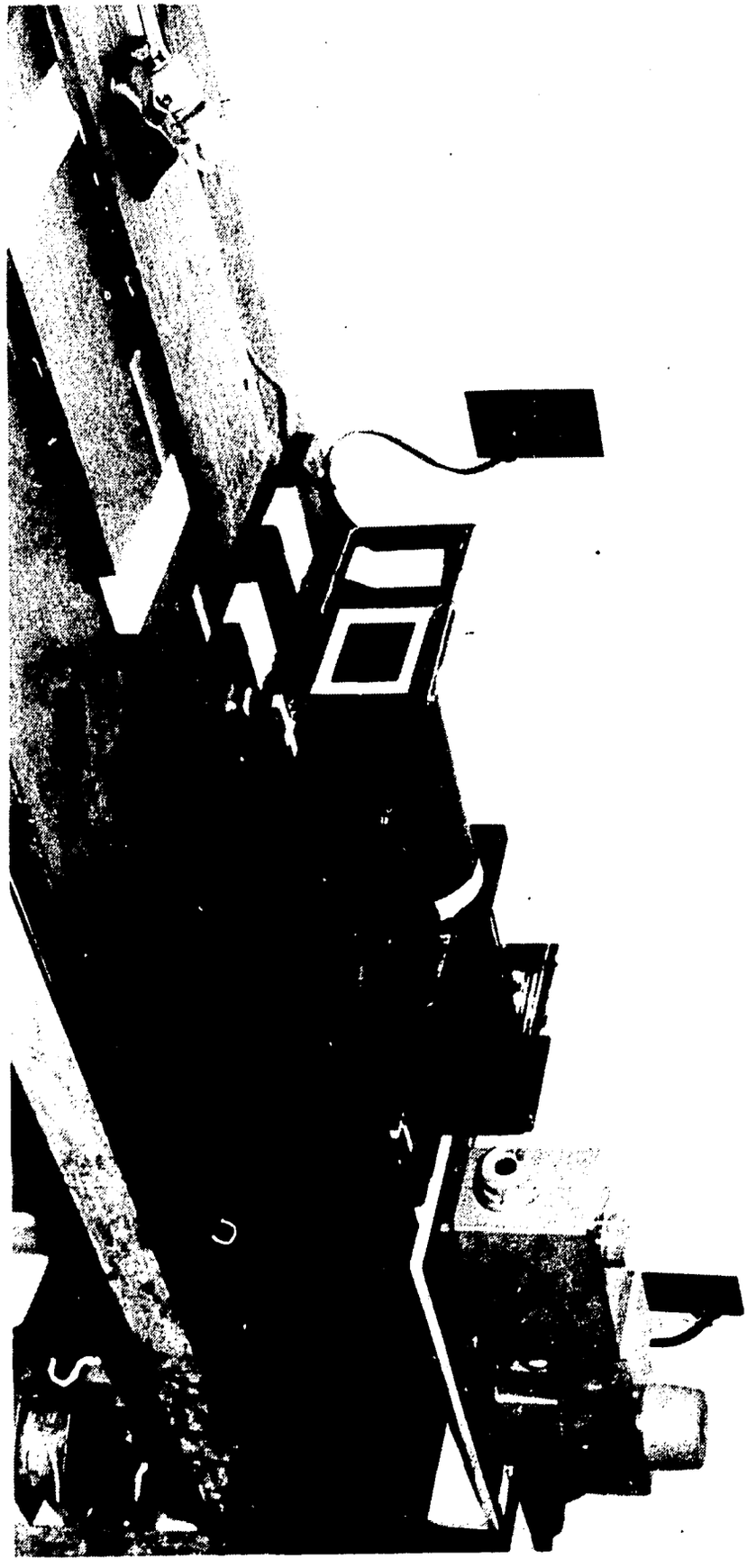
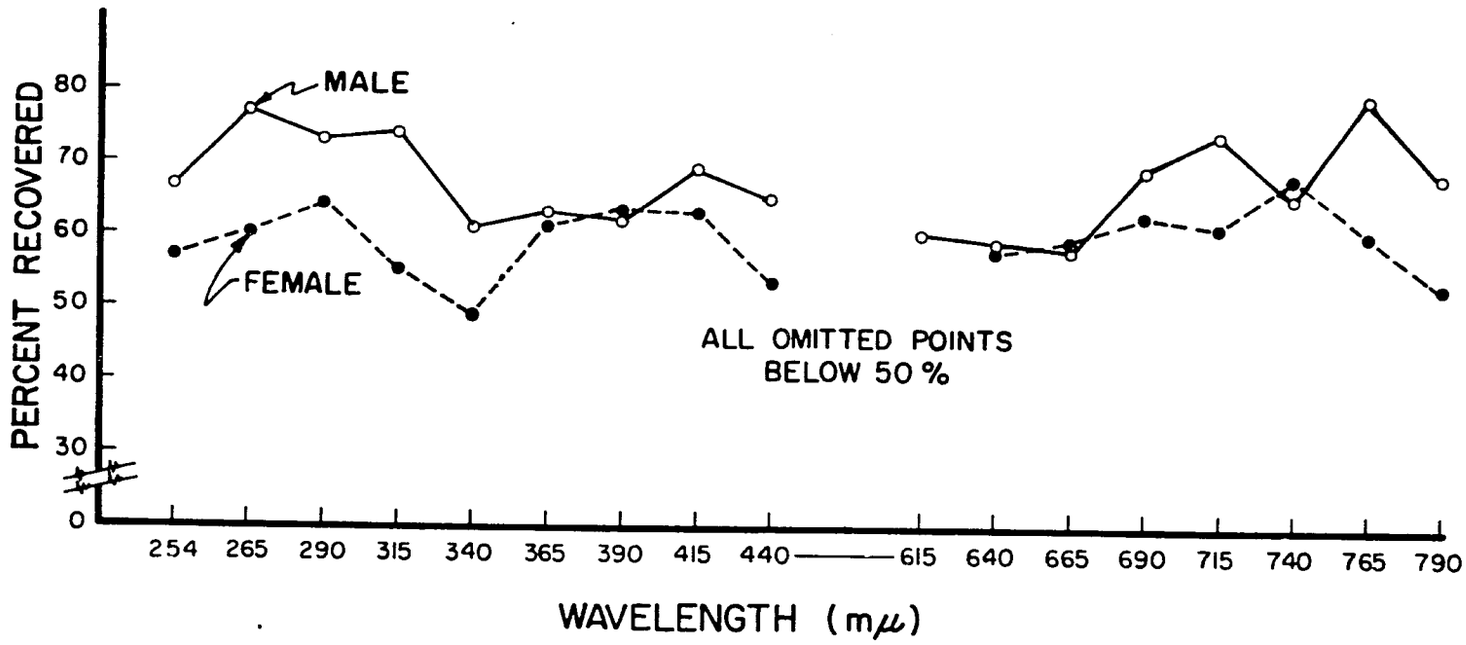


FIGURE 2


PERCENT RECOVERED
80
70
60
50
40
30
0
MALE
FEMALE
ALL OMITTED POINTS
BELOW 50 %
254
265
290
315
340
365
390
415
440
615
640
665
690
715
740
765
790
WAVELENGTH (mμ)

near La Libertad, El Salvador, a department which had a recent history of malaria transmission and high albimanus populations. A 115-volt commercially produced trap which contains a horizontally mounted 8-watt BLB fluorescent tube was employed. The trap was operated on three successive evenings between 6:30 and 9:00 p.m. at three localities. Results were inconclusive, however, because the albimanus population had already undergone its seasonal decline and because the AC current requirement of the trap severely limited its placement.

Since it was clear that proper evaluation of ultraviolet light would require a battery-powered trap, tested at a time of higher albimanus population density, further field work was scheduled in April 1970. During this series of tests the attractiveness to anophelines of the CDC, 4-volt miniature light trap, equipped with a 0.2-watt tungsten-filament bulb[4/], was compared with that of an experimental 12-volt ultraviolet trap which had been developed at Technical Development

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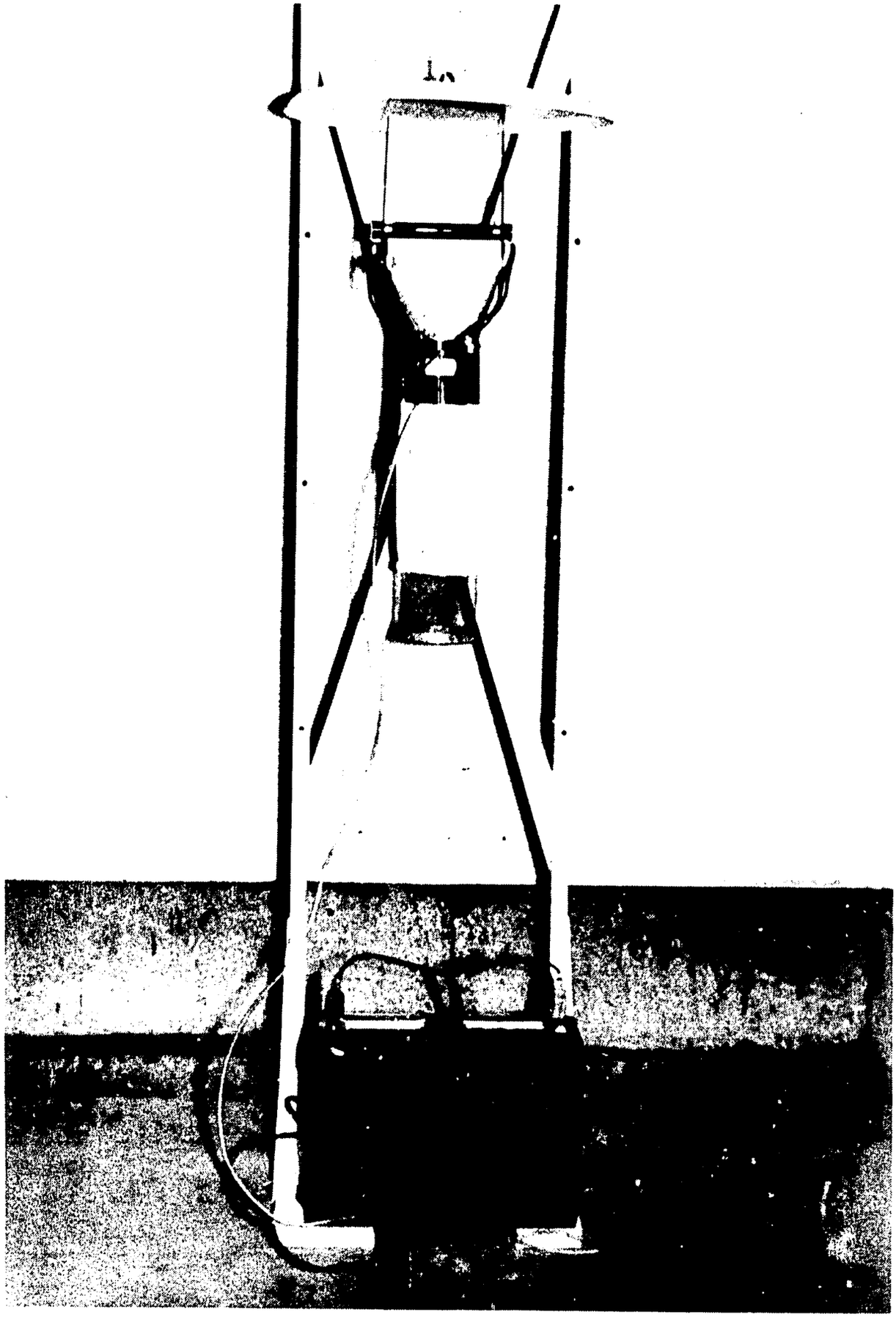
4/ Sudia, W. D. and R. W. Chamberlain. 1962. Battery-operated light trap, an improved model. Mosquito News 22:126-129.

Laboratories (TDL) for these tests. The CDC trap was selected for comparison because of its routine use as a standard tool for mosquito surveillance in arbovirus field work and also because information on its effectiveness in capturing Central American anophelines would be of specific interest to malaria workers in this area.

The TDL trap (Figure 3) employs a 4-watt BLB fluorescent lamp and a 2-blade suction fan, both powered by a 12-volt auto battery. The use of a converter ballast permitted operation of this lamp, which normally requires 115-volt AC. Attempts by industry to develop converters which permit operation on a 6-volt DC power supply have not been successful. The CDC light traps employed in these trials operated with 4-volt rechargeable dry cells.

A village near a small lake with a high anopheline population was chosen as a test site. Dwellings in the village were largely single room buildings with tile or thatch roofs. Generally their cane pole construction was so open as to present no barriers to insect movement in or out of the dwellings. Chickens, dogs, cats, and pigs shared the interiors with the human inhabitants.

FIGURE 3



Traps were situated both inside and outside occupied huts. The inside traps were suspended at eye level from a rafter in the center of the room. Outside traps were similarly hung from the roof overhang or other nearby support. A series of seven 6-8 p.m. trap runs were employed at selected locations using each type of trap and both trap placements. Resulting captures, shown in Table 13, may be summarized as follows:

a. Both types of trap captured more mosquitoes inside dwellings than immediately outside. Total inside catch with the CDC trap was 58 mosquitoes (17 Anopheles) in 14 operating hours. Outside tallies for the same hours were 18 mosquitoes (5 Anopheles). Comparable figures for the TDL trap were: inside, 247 (103 Anopheles) in 14 hours; outside, 60 (16 Anopheles) in 10 hours. The TDL trap caught approximately four times as many total mosquitoes and six times as many Anopheles per trap hour as the CDC trap.

Table 13

Mosquito captures by CDC miniature and TDL ultraviolet traps operated from 6-8 p.m. inside and outside occupied huts in El Salvador, April 13-21, 1970.

Trap	A. albimanus		A. pesudo-punctipennis		Mansonia titillans		Ae. taenior-hynchus		Culex spp.		Totals		Comb. Total
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
CDC miniature:													
Inside (14)[a]	0	17	0	0	1	29	0	0	0	11	1	57	58
Catch/trap hr.	0	1.2			<0.1	2.1				0.8	<0.1	4.1	—
Outside (14)	1	4	0	0	3	2	0	1	0	7	4	14	18
Catch/trap hr.	<0.1	0.3			0.2	0.1		<0.1		0.5	0.3	1.0	—
TDL ultraviolet:													
Inside (14)	5	75	3	20	26	60	0	0	18	40	52	195	247
Catch/trap hr.	0.4	5.4	0.2	1.4	1.9	4.3			1.3	2.9	3.7	13.9	—
Outside (10)	5	10	0	1	13	21	0	2	1	7	19	41	60
Catch/trap hr.	0.5	1.0		0.1	1.3	2.1		0.2	0.1	0.7	1.9	4.1	—

[a] ( ) indicates total trap hours.

b. Small numbers of A. pseudopunctipennis were recovered from the TDL trap. The CDC trap captured none of this species.

The best TDL trap catches averaged 12% of concurrent human bait catches (Table 14). Although limited, the data on this point indicate that the ratio of TDL inside captures to human bait captures made at the entrance to similar huts was reasonably constant.

4. Factors involved in food selection by anopheline larvae. Looking toward development of improved control methods for anopheline larvae, a technique for the quantitative determination of their feeding rate is under development using A. albimanus as the test organism. Equal age larvae of similar size are transferred in lots of 50 to fine mesh wire baskets partly submerged in clean tap water in 600 ml. beakers. Ten such lots usually constitute a trial. Food material of known particle size dyed with insoluble Sudan black B is added to the baskets and the larvae are left to feed for predetermined periods. At intervals the baskets are withdrawn from the beakers, the larvae are washed to remove clinging food particles, and then crushed

Table 14

Anopheles albimanus captures by TDL ultraviolet trap inside occupied huts in El Salvador and by human baits near similar dwellings.

Numbers captured		Ratio
Human bait	TDL trap	HB/TDL
124	12	10.3
189	9	21.0
203	20	10.2
78	8	9.8
50	6	8.3
644	55	11.7

between two layers of filter paper. After the papers have thoroughly dried, the dye is extracted with acetone. The optical densities of the extracts are then read in a spectrophotometer for comparison with the optical densities of standards made from similar extracts of known weights of the same dyed material. In this way, food ingestion can be expressed as μg./larva/unit time.

Initial work on this technique revealed unsuspected variables to be dealt with; for example, both the nutritional state of the larvae and the amount of food on the water surface affect their feeding rate. When starved overnight prior to testing, 3rd instar larvae ingested 18 μ diameter food (ragweed pollen) at an average rate of 1.8 μg./larva/hour. Without prior starvation, the rate was 3.2 μg./larva/hour. The reasons for this apparent anomaly are not presently known.

Fifteen mg. of pollen resulted in a lower ingestion rate than did 5 mg. on the same surface area. The greater density of particles produced by the 15 mg. application tended to clog the larval mouth parts, reducing considerably the efficiency of food intake.

Ragweed pollen served well to indicate the feasibility of the technique but for quantitative assessment of the influence of particle size a material which is available in a graded series of diameters is needed. In addition to larval acceptance, criteria which such a material must satisfy include floatation and dispersal characteristics because anopheline larvae feed almost entirely in the water surface film. The following materials were tested but found unsuitable for the reasons indicated:

Material	Reasons for rejection
Charcoal	Particles too large
Corn pollen	Difficult to dye
Powdered cellulose	Too fibrous, clumped
Norit A (decolorizing carbon)	Particles too small, coated larvae
Chromasorb 101 (styrene polymer)	Particles floated too high, did not wet
Diatomaceous earths	"
Chromasorb G	"
Chromasorb Q	"
Chromasorb W	"
Diataport	Particles sank
Ground cornmeal	Particles failed to disperse

The search for a material with the required characteristics is continuing.

C. Studies on Potential Biological Control of Anopheline Species

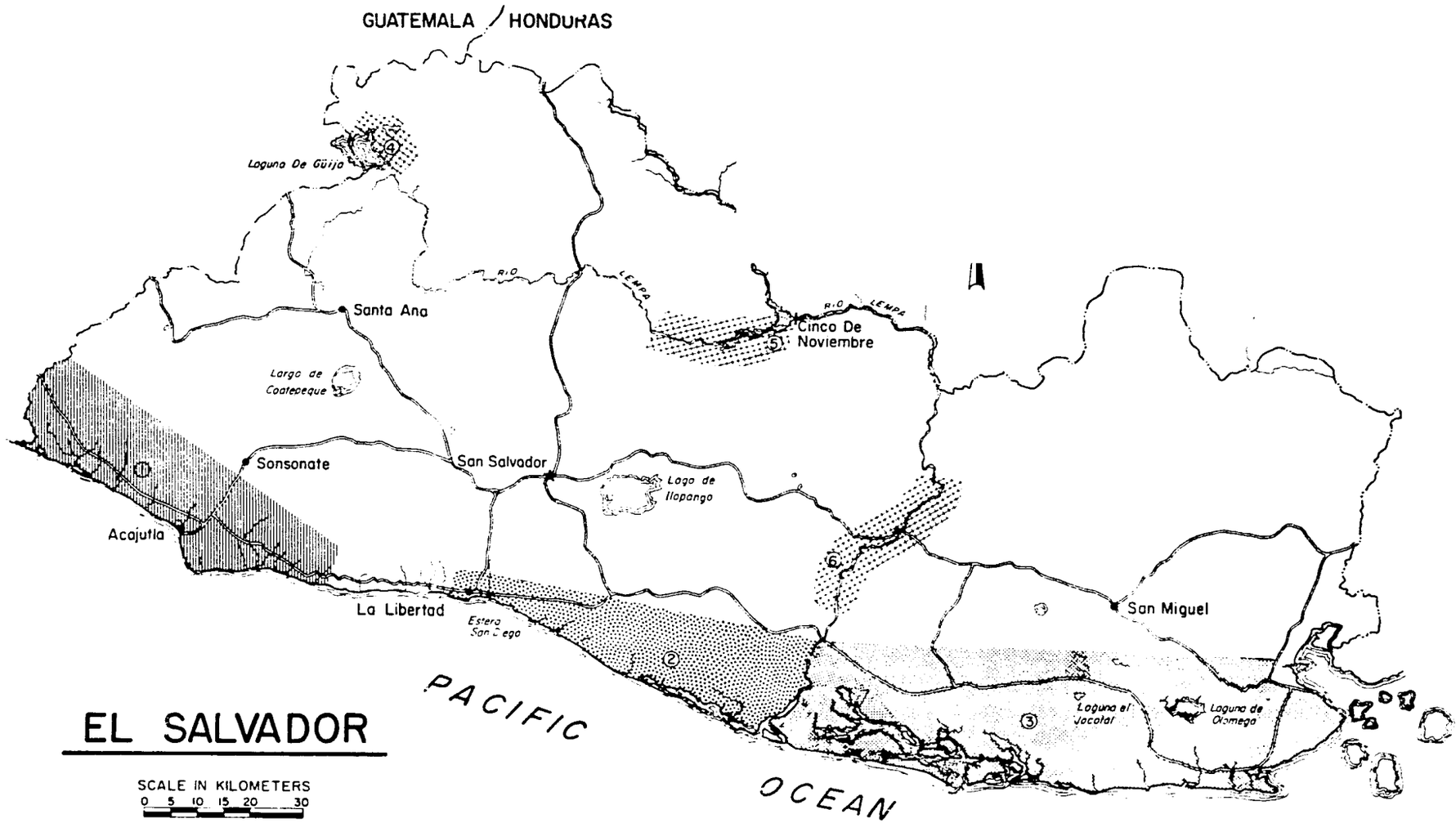
1. Field biology of *A. albimanus*.

In 1969 and in 1970, field studies were conducted in El Salvador, Central America, to obtain information on those ecological factors that might have direct bearing upon the success or failure of current and future investigations on biological control. Such information as temperature, topography, rainfall pattern, nature of larval breeding sites, larval and adult behavior, etc., were necessary to visualize and plan possible use of and research on experimental organisms.

a. Topography.

In the initial visit, six areas were selected for study (Figure 4). The country is almost entirely composed of mountains and volcanoes except for a narrow coastal plain extending along the Pacific Coast from the east to the west borders. Except for a short portion west of La Libertad which consists of precipitous mountains coming right down into the sea, this coastal plain is flat rich soil, with many small streams, lakes and ponds, and is extensively utilized for raising cotton and beef cattle. This coastal

FIGURE 4


GUATEMALA
HONDURAS
Laguna De Güija
Santa Ana
RIO
LEMPA
Cinco De Noviembre
Largo de Coatepeque
Sonsonate
San Salvador
Lago de Ilopango
Acajutla
La Libertad
Estero San Diego
San Miguel
Laguna el Jocotal
Laguna de Olomega
PACIFIC
OCEAN
EL SALVADOR
SCALE IN KILOMETERS
0 5 10 15 20 30

plain, the major anopheline breeding area was separated into a western, central and eastern search area (1, 2 and 3, respectively). Other areas (4, 5 and 6) were along the basin of the Rio Lampa. Area No. 4, in the vicinity of the new hydroelectric installation at Laguna de Guija (altitude 2,000 feet) on the Guatemalan border, was reported to have persistent malarial cases, but because of its inaccessibility was to be studied only if time permitted. Area No. 5, primarily the reservoir backed up by the hydroelectric dam Cinco de Noviembre near the Honduran border, was an extremely large, slow moving body of water that was difficult to reach by car. Search area No. 6 was a section of the Rio Lampa below the hydroelectric dam and was accessible only where the Carretera del Litoral crosses it near the coast and where the Carretera Panamericana crosses about 35 kilometers further upstream.

b. Rainfall.

Two seasons, a wet summer and a dry winter, occur with the dry season having occasional scattered showers. During

the dry season these intermittent showers do not produce enough water flow in the streams to keep the estuaries open into the sea. The action of the surf builds up the sand so that the streams are totally blocked for several months. This blockage results in the formation (several hundred yards from the ocean) of shallow elongated fresh water lakes, known as esteros which are usually associated with an extensive mangrove swamp. The rains normally begin in May and build up in frequency and intensity until June. For about 3 weeks in June, the temporales occur. These intense, torrential downpours scour all of the streams. At this time the esteros are opened to the sea using hand labor or machinery. This produces a salt water inlet that is flushed daily by the 4- to 6-foot tides. The frequency and intensity of the rains then stabilize until the fall when the rainy season is terminated by another brief period of temporales.

The timing of the two field studies were such that A. albimanus were observed during the early rainy season (June 1969) and the last of the dry season (April 1970).

c. Larval breeding sites.

(1) June 1969.

The opening of the esteros and the almost daily flushing of the streams result in a transition period for anopheline breeding which lasts about 4-6 weeks. What happens to the anophelines at this time is unknown. On the initial survey, the first few days revealed no larvae despite the fact the search was conducted in areas known to be major breeding sites 1 week earlier. It quickly became apparent that the transition period had begun. In subsequent reconnaissance, it was found that breeding had shifted to small previously dry depressions in the farm lands and pastures away from the streams. Here, seeps were holding water constantly because of the daily rains. Newly hatched and first-instar larvae were found in little holes and depressions made by the hooves of the cattle and in small isolated pools 2-3 inches deep. Occasionally, depressions similar to borrow pits were found and these usually were breeding *A. albimanus*.

Two to 3 weeks after the beginning of the temporales 3rd- and 4th-instar larvae were found in the seeps and depressions. The staff of the Central America Malaria Research Station stated that throughout the rainy season Anopheles breed extensively in any depression or seep holding water. The esteros, which during the dry season are major breeding areas, no longer produced anophelines, presumably the result of the daily tidal flushing with sea water.

In the area above the Cinco de Noviembre dam (Area No. 5) the water moved very slowly, and there were many sloughs with an abundance of floating vegetation. Access to the river was limited and difficult, and the matted vegetation extended 20-50 feet out from the shore. This environment is such that mosquito breeding could occur year-round.

In area No. 3 there were several lagunas that appeared to be excellent breeding sites throughout

the year. Specifically, these included the Lagunas Negritos, Maquique, Olomega, San Juan, Aramuaca, El Jocotal and perhaps El Pacayal. El Jocotal, the only one searched during the June study, had a large bog and extensive semi-floating vegetation extending around the shore and was supplied with water from numerous springs. The bog was filled with small pot holes and depressions produced by the hooves of cattle and practically every little hole checked contained larvae of all instars in relatively large numbers.

(2) April 1970.

The second study was made at the end of the dry season, a time when the localities for larval breeding were sharply delineated. For several miles east and west of La Libertad (Area No. 2), the numerous rocky streams on the slopes of the mountains and volcanoes consisted primarily of small isolated pools, most of which contained large numbers of *Anopheles*.

Counts in many such pools ranged from 50 to 75 larvae and pupae per dip. These pools characteristically contained masses of filamentous green algae, numerous small fish and tadpoles. Occasionally, some red algae were seen in some of the pools. Microscopic examination of the gut contents of larvae collected from these pools did not reveal any green algae but did indicate that the larvae were ingesting some of the red algae, though it did not appear to be a major portion of their diet. The filamentous green algae was an important part of the habitat, however, for it was used as protective cover. Since these rocky pools contained almost no growing vegetation other than the algal mats, the algae were the only protection from predation by the large numbers of small fish. The larvae were so adapted to the algae that dipping in the clear areas of the pools caught no larvae, but dips into the algal masses were almost always productive. When the larvae were disturbed, they would dive

immediately into the mass of algae and attach themselves to the filaments so firmly that it was very difficult to dislodge them with a pipette. Pools free of algae generally contained few or no Anopheles larvae.

In the same vicinity at this time of year, the esteros [described under 1.c.(1)] constitute a second major breeding area. In 1970, however, the sand beach had not built up high enough to prevent the breakers from washing over into the estero at each high tide and the salinity level, as monitored by the staff of CAMRS, was much higher than normal and large numbers of Anopheles were not present. Searches of pot holes in the mangrove swamps were unproductive, though some Anopheles larvae were reported in open water of the upper reaches of the esteros. Whether the increased salinity of the estero was responsible for the absence of Anopheles is conjectured only.

A third clearly defined area of Anopheles breeding was at the western end of the country (Area No. 1) near the city of Acajutla. The lowlands here remain wet throughout the year. On the hacienda Atalaya were very many freely flowing springs. The waters were impounded in numerous ponds and small lakes and irrigation channels were widespread. While the numbers of larvae per dip were small, the total acreage of water available for breeding was such that the area was a major production site for Anopheles. The large cattle herds on this hacienda provided an ample blood supply for the female Anopheles and apparently acted as an attractant, for many adult Anopheles were observed resting during the day on the walls and thatched roofs of the cattle sheds. The impounded waters in this area were clear and had few algal masses, but there was ample vegetative growth of other aquatic plants within the ponds and all around the water edge. Microscopic examination of the larval gut

contents revealed neither filamentous green algae nor the red algae as their diet.

The fourth Anopheles breeding area studied was at the east end of El Salvador (Area No. 3) at the Laguna El Jocotal, a lake constantly fed by clear flowing springs and about 1-1/2 square miles. The central portion of the lake is clear of vegetation and the fish population is sufficient to support several small fishing villages. The shoreline of the lake was heavily matted with water hyacinth, pickerel weed, duck weed and other emergent vegetation. Here the Anopheles larvae were found in the vegetation. Though few larvae per dip were found, the adult activity on the shore at the peak biting time in the evening was extremely high. During one 2-hour period, 300+ female Anopheles were aspirated by a single human bait. Such high adult populations indicate that though larval density per square foot of water surface was low, the total available breeding area in this one lake was of major proportions.

d. Microscopic examination of larvae for pathogens.

As the larvae were collected at the field sites, they were grossly examined for signs of apparent infection or illness. Visible signs specifically looked for were color pigmentation, physical deformities, abnormal movement, and abnormal response to light and probing. Such larvae as well as samples of healthy larvae were retained for microscopic examination. The specimens were examined for *Coelomomyces*, other fungi, protozoans and nematodes. No *Coelomomyces* were observed in any of the larvae. Although the water collected with the larvae abounded in nematodes and protozoa, none were observed as internal parasites of the larvae. Numerous ciliated protozoa as ectoparasites were observed, but none which appeared to be pathogenic. The numerous green larvae collected usually were partially coated with a unicellular green algae. Such larvae are routinely found in the field and no pathogenicity is associated with this condition. Larvae were occasionally found with the bristles missing from the abdominal segments.

These specimens were usually from the small pools with high larval population densities. No parasites could be found on or in these larvae that potentially could have been responsible for this loss of bristles. Since some Anopheles larvae chew on the bristles of others when in crowded conditions, this act may be the explanation.

e. General.

Although no pathogens were found in the samples of larvae taken during this visit, it is inconceivable that a place exists where Anopheles do not have natural disease organisms and parasites. The dry season, when the larvae are restricted to specific breeding areas, would appear to be the most likely time to find any disease organisms that are present, but the finding of any given organism at any given time is largely a matter of chance. Like human diseases, there is always a low level of incidence in the total population that only becomes readily apparent at the time of an epidemic. The search for pathogens in the Anopheles of El Salvador should therefore

be of a continuing nature, for a pool that appears negative for diseased larvae one week might be the site of a heavy epizootic the following week. If possible, larval collections should be periodically screened for the presence of parasites and pathogens and when any organisms appear, immediate collections of larvae and water would be made. Such material would be sent to the Technical Development Laboratories in Savannah for further studies, by the fastest means possible.

Subsequent to the above studies, A. albimanus larvae that appeared to have external parasitic growth were forwarded to TDL by personnel of the CAMRS. Although several ectoparasites were found on some of the preserved larvae, no potentially pathogenic forms were detected. However, some larvae contained structures that warrant further study with living larvae and water collected from the field site.

2. Anopheles crucians - Coelomomyces sp. studies.

Early attempts to produce a laboratory infection using field collected Anopheles larvae parasitized with Coelomomyces as the infective material were unsuccessful. The infected larvae had been forwarded to TDL from the USDA laboratory in Lake Charles, Louisiana. The Coelomomyces sporangia present in the larval carcasses appeared to be C. punctatus, a parasite of A. quadrimaculatus. Although some sporangial germination did occur after the material was in the laboratory, no infections occurred in the A. quadrimaculatus larvae that were exposed to the motile zoospores. Subsequent information indicated that A. crucians was the principal species found infected in the single field collection site, and that A. quadrimaculatus were only infrequently affected. Additional A. crucians infected larvae were forwarded to TDL and again no infection resulted when A. quadrimaculatus larvae were exposed. Since there appeared to be a preferred host, an attempt was made at TDL to establish a laboratory colony of A. crucians from local field collected specimens. Since colonization of Anopheles from wild specimens frequently is

a difficult and not always successful task, one staff member was assigned to it for an entire season. However, all attempts to produce a self-sustaining colony failed.

In another phase of this study, eggs from one of the TDL colonies of A. quadrimaculatus were shipped to the Louisiana collaborator and placed in the pool from which the parasitized larvae were being collected. Larvae of A. crucians collected from the pool the week prior to, during, and the week after the A. quadrimaculatus eggs and larvae were present were positive for Coelomomyces. None of the introduced A. quadrimaculatus larvae became parasitized, thereby verifying the suspicion that a host species immunity did exist.

Since the Coelomomyces from Lake Charles apparently would not produce an infection in our A. quadrimaculatus and a colony of A. crucians was not established, this line of investigation was terminated.

3. Coelomomyces punctatus in Anopheles quadrimaculatus.

Two shipments of dead A. quadrimaculatus larvae parasitized with C. punctatus were received from Dr. John Couch of the University of North Carolina (UNC). With the initial shipment, tests were started with A. quadrimaculatus in three different environments. Clear plastic containers were used in the tests to hold 1 liter of (1) tap water or (2) a mixture of tap water and algae from Chapel Hill. Three containers of each type were held at 80° F. and 80% relative humidity in normal daylight, so that the first group received filtered sunlight in the morning, the second group received filtered sunlight in the afternoon, and the third group, placed in a green house with temperatures ranging from 70° F. to 100° F., received sunlight throughout the day. Eggs of A. quadrimaculatus and sporangia of C. punctatus were placed in each container every third day. In this procedure, the hatching of the eggs and subsequent development of the larvae correlated with the germination of the fungal spores and the release of zoospores resulted in each stage of larval development being exposed to motile zoospores. No

infections were found in any test following microscopic examination of the larvae.

The second shipment was used in a series of tests at 80° F. in which sporangia and newly hatched larvae of A. quadrimaculatus were combined in 150 ml. of water at 2-day intervals for a total of three inoculations in each container. The larvae were then reared until they died or pupated. All dead larvae and all pupae were examined for infection but none was detected.

Since a possible cause of the failure to secure infection was the suitability of the local tap water for the fungus, a sample was sent to Dr. Couch who showed it to be satisfactory. Because of difficulties in maintaining the Coelomomyces at UNC, no further material has been supplied. Until more is known about the mode of infection and a suitable method devised for propagating the fungus, no further work on C. punctatus at TDL is contemplated. Twice weekly shipments of 10,000 eggs of each of two strains of A. quadrimaculatus to UNC will be temporarily continued, however, to facilitate work on the basic biology of the fungus towards developing an improved technique for propagation.

4. Laboratory studies on the parasitism of mosquito larvae with an aquatic fungus *Lagenidium*.

An undescribed fungus, tentatively considered to be of the genus *Lagenidium*, was received from Dr. Clyde Umphlett who isolated it from an unidentified immature *Culex* sp. at the University of North Carolina (UNC). *Culex pipiens quinquefasciatus* was used at TDL as the initial host in experiments to obtain a laboratory infection and to establish a culture "in vivo." The initial infection, from material on artificial media, was successful though mortality was not extensive. However, serial passages through successive inoculations of *C. p. quinquefasciatus* established a thriving laboratory culture. Observations on the biology of the fungus indicate that approximately 24 hours following death of an infected larva, the fungus, which inhabits the coelomic cavity of the larva, shifts into a reproductive phase. Reproductive structures penetrate the cuticle and motile zoospores are released into the water. These zoospores infect new host larvae but the mode of entry is unknown.

Tests to determine species resistance or susceptibility were initiated with Ae. aegypti, Ae. taeniorhynchus, Ae. triseriatus, Culex pipiens fatigans from India, A. quadrimaculatus, A. albimanus, and A. stephensi. In each test, four containers of 100 ml. of unchlorinated tap water with 25 third-instar larvae per container were used for each species. Four fungus infected carcasses of C. p. quinquefasciatus larvae that had been dead for 24 hours were added to each beaker. An equal number of controls were used, the larvae were fed standard diets, and mortalities were recorded daily. Except for the Ae. taeniorhynchus and all of the Anopheles, the larvae became infected and usually died within 48-72 hours after being placed with the fungus. It was noted that the Ae. taeniorhynchus ate the carcasses within a few hours after they were placed in the container. The procedure was then modified so that the carcasses were in the container for 24 hours before the Ae. taeniorhynchus larvae were added. They then became infected and died 48-72 hours later. Apparently, in the earlier test the larvae had eaten the

carcasses before the vegetative stage of the fungus had an opportunity to produce reproductive structures and release the zoospores.

Cross resistance among the several species that were susceptible was then tested by using infective material from each species against every other species. The tests were conducted in the same manner. The data showed that the zoospores produced from all host species were equally infective and pathogenic for all other species.

Since the propagation of the culture and execution of the tests were dictated by the timing of the release of the zoospores from any given carcass, studies were initiated to determine a method of temporarily halting the development of the fungus to facilitate the scheduling of tests. Larvae that had just died were stored in water at temperatures of 40, 50, 60, 70, 80, 90, and 100° F. for varying periods of time. Though these tests are still in progress, data to date show that storing of infected larvae immediately after death at 60° F. for periods up to 1 week does not reduce the infectivity or pathogenicity of the fungus.

To date, the majority of the studies on this fungus have been done with culicines to devise culture methods and obtain data on the life cycle and bionomics of the fungus. The fungus is being maintained at this laboratory by successive inoculations of culicine larvae and maintained at UNC with artificial media. Data tentatively suggest that material from the artificial media is either low in infectivity or pathogenicity, whereas material from the line maintained with parasitized larvae is highly active. In the various tests thus far completed, all culicine larvae tested routinely die within 48 hours after initial exposure to the fungus.

Now that the fungus is in production and part of its biology known, tests are being conducted to determine its potential for Anopheles spp., particularly methods for obtaining infection. If infection can be produced in anophelines by any method, even artificial inoculation, then serial passages through several anopheline hosts may result in a satisfactory pathogenic strain. All efforts, at present, are directed toward obtaining infection in anophelines.

D. Colonization of Malathion-resistant *A. albimanus*

Resistance to organophosphorus insecticides has rarely been encountered among anopheline mosquitoes so a report of a population of *A. albimanus* resistant to malathion is of considerable interest. Approximately 300 presumably resistant females of this species captured in the Department of La Libertad, El Salvador, plus an estimated 800 eggs obtained from them, were hand-carried to Savannah in December 1969 for colonization at Technical Development Laboratories.

The females, released in a standard 2 x 2 x 2 ft. mosquito cage, did not respond to a rabbit offered to them between 4:30 and 8:00 p.m., but eagerly fed and quickly engorged on a chicken at 3:00 p.m. the following day and subsequently laid eggs. These eggs, and those received on wet mud in petri dishes, hatched readily when flooded with tap water. The larvae were reared on the standard formulation used for anophelines in the TDL insectary. The adult females thus obtained also took chicken blood readily.

Although the second generation adults appeared normal and fed readily, the females in this and in two subsequent trials with additional reared material from El Salvador failed to produce viable eggs.

An investigation of the reasons for the failure of this strain of *A. albimanus* to reproduce under insectary conditions is planned, as are attempts to establish and maintain a colony by forced matings if necessary.

E. Summary of Activities on Biological Studies

The laboratory evaluation of new chemicals as residues against adult *Anopheles* indicated that five compounds had marked biological activity against *A. quadrimaculatus* and *A. albimanus*. However, two of the compounds which remained lethal to the mosquitoes for the longest periods (OMS-1342, 1343) possess mammalian toxicity values that render them unlikely candidates for field usage. Two of the remaining three (OMS-1336 and NC 69[illegible]) warrant further testing at dosages of 0.5 and 1.0 g/m^2; the [illegible] (OMS-1310) at 2.0 g/m^2. NC 6987 gave > 26 weeks of effectiveness against all species at 0.5 g/m^2.

Because of its biodegradability, methoxychlor was evaluated at 1.0 g/m^2 as a possible substitute for DDT. It showed promise against *A. albimanus*, giving effective kills for more than 22 weeks. BAY 85032, which was highly effective against *A. quadrimaculatus* in last year's laboratory tests, gave poor results against this species in

outdoor panel studies. However, against A. albimanus it was highly effective for 14 weeks on clay, whitewahsed clay, and other materials. Hercules 9007 and 14469 also gave poor results against A. quadrimaculatus but kills of A. albimanus were excellent. Fenitrothion emulsion or suspensions showed similar periods of effectiveness on the different surfaces; each formulation persisted > 9 weeks on clay and whitewashed clay at 2 g/m^2. Methyl Dursban, a compound of much lower mammalian toxicity than ethyl Dursban, showed much promise against A. quadrimaculatus, providing 8 weeks of 90% kills on clay, whitewashed clay, and cement. This compound generally is less effective than ethyl Dursban against anopheline mosquitoes but its acute oral LD-50 for rats is 1,000 mg./kg. versus 135 mg./kg. for ethyl Dursban. Thus, it offers greater potential for ultimate field use. In experimental hut studies, BAY 85032 was far less effective against free-flying A. quadrimaculatus than against A. albimanus. Fourteen weeks of kills between 88% to 100% were obtained with A. albimanus.

Although 10 of the 21 compounds tested as larvicides met the criterion for further study, only two (OMS-1169, 1345) approached

Abate or fenthion in efficacy. One (OMS-1169) had a much higher mammalian toxicity than either Abate or fenthion. Larvae of A. albimanus were found to be more difficult to kill with Abate than those of A. quadrimaculatus, a response which contrasts sharply with the adult stage of the two species; A. albimanus routinely succumbs to residues of candidate compounds that no longer are toxic to A. quadrimaculatus.

Formulations of propoxur or malathion when combined with specially prepared DDT did not show any marked improvement in length of effectiveness over either compound alone when evaluated in experimental huts. Earlier results on plywood panels under laboratory conditions had indicated that the addition of a DDT special preparation increased the residual life of both compounds.

Daytime tests of mosquito response to ultraviolet light with photoperiod entrained adults of A. stephensi and A. albimanus demonstrated much higher captures of the entrained strains than of the standard laboratory strains. The latter have their principal flight activity at night, whereas the entrained strains display this activity around noon. Studies with ultraviolet light of various wave lengths using A. stephensi indicate that in competition with incandescent

light, the adults showed their greatest response to three wave length bands (254-314, 365-413, and 640-765 mμ).

Field tests[5/] of an experimental ultraviolet light (UV) trap in El Salvador showed that the UV trap captured approximately four times as many total mosquitoes and six times as many Anopheles per trap hour as the standard CDC miniature trap. Either trap caught more mosquitoes inside dwellings than on the immediate exterior.

Two field investigations[5/] were conducted in El Salvador to study the biology of A. albimanus as it may relate to the potential of biological agents as a control tool. Larvae were collected in four specific types of breeding areas and examined for pathogens, particular attention being given to the detection of Coelomomyces. All specimens were negative for Coelomomyces infection. Water samples were positive for various nematodes and protozoa, none of which appeared as internal parasites. Ciliated protozoa were observed as ectoparasites but none appeared pathogenic.

Despite the initial promise of Coelomomyces as a fungal parasite of Anopheles, studies with this organism have met with little success in the

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5/ Studies performed in cooperation with the Central America Malaria Research Station.

laboratory. Consequently, investigations on this fungus have been stopped and will not resume unless the experimental work at the collaborating university succeeds in developing satisfactory methods for the propagation and maintenance of Coelomomyces.

In preliminary tests a fungus, Lagenidium, has been shown to be toxic to Culex larvae but not to the larvae of three species of Anopheles. Further efforts are being made to induce infection of anopheline larvae for the purpose of increasing the pathogenicity of the fungus to Anopheles by serial passage through several anopheline hosts.

Attempts to colonize a malathion-resistant strain from El Salvador have been unsuccessful to date. Since resistance to an organophosphorus compound has occurred in a species of Anopheles for the first time, it has become imperative that a colony be established and adults from it be included in the TDL insecticide evaluative system. Cross resistance to new candidate compounds by this strain is a distinct probability which would be adverse to the success of new compounds that are now considered to be ready for field testing based on their efficacy against organochlorine resistant strains.

Cooperation continues with CAMRS with the objective of expanding it from the biological-ecological phase to include the field testing of promising insecticides. Arrangements were made by TDL for the procurement and shipment of samples of several new compounds to CAMRS. Cooperative arrangements between CDC-MEP and TDL also have been initiated in regard to the evaluation of ultra low volume aircraft applications in operational areas of high malaria endemicity.