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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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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, investigation on insecticide application equipment and packaging, the development of specifications for insecticides used in vector control, new and improved methods for the chemical control of arthropod vectors as well as on alternate noninsecticidal techniques, the detection and measurement of arthropod populations, the resistance of insects to pesticides, and molluscicides and rodenticides.

The laboratory is staffed by 26 professional scientists in the biological, chemical and engineering categories with 59 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 involving 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 3,600 samples of DDT powder representing 40.7 million pounds of product which had been in storage for 12 months. Suspensibility analyses were run on 2,269 samples to determine if they met the requirements of the warranty clause of Specification PHS/NCDC-I-102. Of the samples tested, 78 failed to meet the minimum suspensibility requirement. The failing samples represented 0.84 million pounds of powder which was about half the quantity reported failing in FY-1968. Unfortunately, most of the failures occurred in samples from companies which furnished the smallest fraction of the total powder purchased. For example, Central Chemical Company furnished only 1.9 million pounds of powder yet had 42 failures which represented almost one-fourth of their total production. Difficulty was also experienced with the Lebanon Company which had 23 failures out of 6.1 million pounds of production. The Montrose Company had the best record for the year with only 10 failures out of 32.2 million pounds of production.

Unlike the problems experienced last year which were primarily a result of surfactant failure, most of the recent failures appeared to be caused by particle agglomeration. Only one complaint was received from the field and that was from the mission in Ethiopia. Samples were returned from Ethiopia and compared with

reference samples held in Savannah. The surfactant content of all samples was high and suspensibility values were all above 1.1, however, results of sieve tests were not good which indicates the difficulty was due to particle agglomeration. The consignment was furnished by the Chem Air Company. Further studies of their production are in progress.

B. Development of Micro Analytical Methods for Determination of Pesticides

In the course of biological research on larvicides, micro methods for determination of trace quantities of a number of experimental compounds were required. The most difficult to determine was Abate for which analyses down to 0.001 ppm were necessary. Methods of extraction and gas chromatographic determination of Abate were developed which provided the necessary sensitivity for this work. Modifications of conventional gas chromatographic equipment were required to permit analysis of Abate. These modifications as well as the gas chromatographic method have now been published.

In addition to Abate, gas chromatographic methods for determination of trace quantities of Gardona, fenitrothion, malathion, Bayer 77488 and CIBA 9491 have been developed. In the case of Gardona and malathion, the methods are being used to study the stability of these compounds in test kits for determination of resistance. Methods for Bayer and CIBA experimental compounds were used to determine concentrations produced in water after application of the compounds in various experimental formulations.

C. Development of Macro Analytical Methods for Determination of Pesticides

The development of a titrametric method for determination of the DDT content in aliquots taken from suspensions prepared for testing the suspensibility of DDT water dispersible powders was reported last year. During the past year, the new method has been employed in the surveillance program. Parallel tests by the conventional procedure have been conducted and a statistical evaluation of the new method has been made. No statistically significant difference was found in results obtained by the two methods.

In the new procedure, the DDT content is determined by titration of liberated chloride ion after alkaline dehydrohalogenation. This method had not been used previously because the o,p'-isomer in technical grade DDT dehydrohalogenates at a slower rate than the p,p'-isomer. In the new procedure, quantitative results are obtained by careful selection of conditions including solvent, time, and temperature.

In addition to the development of improved chemical methods for testing DDT powders, a number of mechanical devices have been developed for manipulating samples in suspensibility testing. One of the most useful devices is a multi-mixer which enables an operator to invert 8 samples simultaneously through 30 cycles and cuts off automatically. Another device automatically pipets the sample aliquots and eliminates mouth pipetting with considerable saving in time. Comparison of results obtained by conventional and automated techniques has been made and statistical evaluation of the data showed no significant difference in the results.

With the new automated techniques, the time required for running a suspensibility test is cut in half.

D. Study on the Effect of Moisture on the Storage Stability of DDT Powders

DDT, 75% water dispersible powder from commercial production and from laboratory scale preparation, was analyzed for moisture content and additional water was added to make a series of samples containing 1.0 to 4.0% water. The samples were divided and half of each was stored at room temperature while the other half was placed in an oven at 50° C. The samples were removed from storage periodically and tested for suspensibility and surfactant content. After 4 months in storage, none of the samples have shown a significant decrease in surfactant content. The suspensibility values of samples run immediately after the addition of moisture were essentially the same for all samples. Even with 4.0% moisture, both laboratory and commercially ground samples suspended as well as corresponding samples with only 1% moisture. With time and, in particular, with time at elevated temperatures, the samples with high moisture content exhibited lower suspensibilities than those with low moisture. Samples with more than 1.5% moisture showed a serious loss of suspensibility at elevated temperatures. Studies at lower temperatures will be continued for at least a year.

E. Development of Specifications for p,p'-DDT, 90%, Water Dispersible Powder

Following favorable results obtained in laboratory scale tests with 90% p,p'-DDT powder, it was decided to conduct a limited field test with the product in Central America; the test to be carried out by CAMRS. The field experiment will require 50,000 pounds of powder, and it was necessary to develop a specification in order to purchase this quantity of material. Commercial samples from two suppliers were available for study for establishment of minimum requirements in a specification. In view of the relative ease of grinding p,p'-DDT, a 90% powder can be expected to have a very high suspensibility and long storage life. The minimum requirement was set at 2.0 (80% suspensibility) after heating for 2 hours at 70° C. Several methods for determination of the p,p'-DDT isomer content were considered. It was finally decided to establish a minimum melting point as a criteria for determination of isomer content. The specification requires an extract of the powder to melt within 2° C. of pure recrystallized p,p'-DDT.

One of the problems associated with the manufacture of p,p'-DDT powder is the disposal of the by-product material which is essentially o,p'-DDT. Experiments are now in progress to find ways to utilize this waste material. Results of these experiments will be reported later.

F. Research on Improved Formulations of Organophosphorous Compounds

Although DDT is unquestionably superior to all other known insecticides for world malaria eradication programs, future programs may be forced to employ alternate chemical compounds. This could be brought about by (1) development of resistance of some Anopheline strains to DDT, or (2) political pressure to reduce the contamination of the environment with DDT. In anticipation of a possible changeover from DDT to organophosphorous insecticides, research on formulation of several of the most promising compounds has been initiated. In view of the fact that cost will be a primary consideration in the choice of alternate compounds, our investigations have been confined to chemicals which are not tightly controlled by patents. The most promising candidates at this time are malathion and fenitrothion.

During the year, an opportunity arose to study water dispersible powders of fenitrothion produced by four different manufacturers. These powders were manufactured for the World Health Organization which was preparing to purchase 4,000 kg of the powder for field trials to be conducted in Kenya. The studies here included suspensibility tests before and after accelerated storage treatment, analysis of carrier composition, storage stability at ambient and elevated temperatures, and analysis of extracts of the powders for trace quantities of impurities. In addition to these studies, research has been initiated on improved fenitrothion formulations.

Some of the problems which need to be resolved are selection of carriers and surfactants to improve storage stability, ways to increase the bulk density of presently available formulations, and improvement of test methods presently available for evaluation of the formulations. A summary of the results of these investigations is given below.

The fenitrothion content in the commercial samples varied from 40 to 50 percent. Percent active ingredient was determined by infrared spectrophotometry, nitrite titration and bromate titration. The infrared and nitrite titration methods were found to be satisfactory for the powders tested. The bromate method was rapid, but less reproducible than the other methods. Some modifications of the nitrite titration method were introduced which improved the accuracy. If a specification is required for fenitrothion powder, either the infrared or the nitrite method would be satisfactory.

Suspensibility values of all powders examined were quite satisfactory. The particle size was very fine and all powders easily passed through a 200 mesh screen. The bulk densities of the powders varied widely and even the most dense was rather bulky. Some attention will have to be given to this problem if a practical formulation is to be produced. Shipping costs for the bulky powders would be prohibitively high.

In previous work with malathion powders, good correlation between accelerated storage treatment at 90° C. and long term storage was obtained. This temperature was selected for short term tests with the experimental fenitrothion powders. All powders tested maintained their high suspensibilities when heated for up to 16 hours at 90° C. Two out of four samples, however, showed an excessive amount of decomposition of the active ingredient. In addition to the tests conducted at 90° C., longer term storage experiments were initiated at 50° C. and at ambient temperatures. Decomposition of the fenitrothion after 8 weeks at 50° C. was about equal to that found in corresponding samples heated at 90° C. for 16 hours. One sample failed the suspensibility test after 12 weeks at 50° C. All samples maintained satisfactory suspensibilities through 6 months storage at room temperature. These tests are still in progress. Although the results are preliminary, it appears that fenitrothion powders can be expected to have better storage stability than malathion powders.

The key to successful formulation of organophosphorous compounds lies in the proper selection of diluents and surfactants. Unfortunately, many of the highly sorptive carriers which are necessary to the formulation of powders with high percentages of active ingredient are catalytically active. A determination of the types of clays used in the commercial samples was made by infrared analyses. This was done by extracting the fenitrothion and surfactants from the powder and making KBr

pellets of the inert diluents. Spectra of these samples were compared with spectra of known diluents prepared in the same manner. From these data it was determined that the principal carriers used were Hi-Sil or Zeosyl and mixtures of these products with Micro-Cel.

Determination of the impurities in the commercial powders was made by gas chromatography and thin layer chromatography. Samples of the 2-methyl analog were obtained and resolved from fenitrothion by gas chromatography. None of this isomer was found in any of the samples tested. The oxygen analog of fenitrothion as well as free 4-nitro-m-cresol was resolved by thin layer chromatography. The oxygen analog was found in all samples, but the maximum concentration found in any samples was less than 0.08%. The free cresol was determined by a colorimetric method. The free cresol content in one sample was 1.1%. All other samples contained less than 0.5% free cresol.

In addition to experiments conducted on commercial samples, considerable developmental work was carried out on laboratory formulations prepared locally. Two series of tests were conducted to obtain basic information necessary to the development of a stable fenitrothion formulation. These experiments are described below.

In order to provide a basis for selection of carriers for fenitrothion formulations, a wide variety of clays and synthetic sorptive carriers were mixed with fenitrothion

and stored at ambient and elevated temperatures. Included in these tests were Barden (kaolinite) clay, Attaclay (attapulgite clay), Hi-Sil (precipitated silica), Micro-Cel (synthetic calcium silicate), and Celite (diatomaceous earth). The carriers were loaded to maximum capacity with fenitrothion and stored at 90° C., 50° C., and room temperatures. Samples were removed periodically and tested for decomposition. A summary of the results is given below.

	Maximum load, %	Rate of decomposition
Attaclay	25	high
Micro-Cel E	50	high
Barden clay	10	high
Hi-Sil	50	moderate
Celite 209	26	low
Celite SSC	36	very low

From the above data, it was obvious that the choice of carrier would be limited. The natural clays were eliminated by both low sorptivity and high rate of decomposition. Hi-Sil appears to be a good compromise, but its low bulk density may limit the percentage which can be used in a given formulation. The ultimate choice of carrier will probably be Hi-Sil mixed with one of the Celites.

A study of surfactants for fenitrothion powders was also initiated. Earlier work by Kihara, Chem. Abstracts, 60, 13816c (1964) indicated that certain dioctyl

sulfosuccinate esters were useful in reducing the catalytic decomposition of malathion in powder formulations. A series of tests were carried out in which one of these compounds, Aerosol OT, was added to fenitrothion formulations and samples were held at elevated temperatures. The samples were removed periodically and tested for fenitrothion decomposition. Some reduction in decomposition was observed in samples held at 90° and 50° C. as compared to control without the Aerosol OT, however, the differences were not as great as those observed by Kihara on malathion formulations. Studies on decomposition in samples held at ambient temperatures are in progress.

In summary, fenitrothion water dispersible powders with fairly good storage characteristics are now available from industry, however, considerable improvement in the products is necessary before they can be considered for large field programs. Both chemical stability and suspensibility are better in fenitrothion powders than in similar malathion powders. With some research, these can be improved. Additional research is needed to find a combination of ingredients which will reduce the bulk density. All present powders are too bulky. Finally, the insecticide content should be increased. Only one manufacturer was able to furnish a 50% powder. A practical powder for field use should contain a minimum of 50% active ingredient.

G. Biochemical Studies

In the course of investigations on new insecticides for use against *Aedes aegypti*, promising larvicidal activity of long chain aliphatic surfactant amines and good ovicidal activity of aqueous mixtures containing 0.1% of a surfactant amine and 1% of a polar water-soluble amine such as ethanolamine was observed. In view of the good results obtained, it was decided to study the effectiveness of these and related compounds on Anopheline mosquitoes and to investigate their mode of action. It is hoped that these studies will lead to a more rational approach to the attack on pre-adult forms of Anopheline and other mosquito species.

The mode of action of the ovicidal amine mixtures is being studied with the aid of ^{14}C-labeled ethanolamine and the preparation of yellow dinitrophenyl derivatives of the long chain amines for separation on thin-layer chromatograms. So far, the evidence indicates that the surfactant amine, although very toxic to hatched larvae, does not penetrate beyond the eggshell to reach the larva when tested in ovicidal mixtures. In ovicidal action the surfactant amine appears to increase the permeability of the shell to the polar ethanolamine, which can then be detected in abnormal amount in the larvae derived from such treated eggs. How the ethanolamine kills the egg is an interesting puzzle since this amine is a normal constituent of organisms and is relatively nontoxic to hatched larvae. In one experiment, eggs exposed to vapor of ^{14}C-ethanolamine took up a certain amount of

radioactivity, and when the eggs were pretreated with a surfactant amine the uptake was tripled. So far, only live larvae from treated eggs have been assayed. Perhaps some penetration of surfactant amines will be detected when a method has been worked out for the isolation of dead larvae from treated eggs. Progress in finding agents which alter the permeability of eggshells or cuticles should facilitate the development of effective insecticide formulations containing milder agents than the chlorinated hydrocarbons and organophosphates.

A large number of surfactant amines and other surfactants have been procured from commercial sources or synthesized in the laboratory, and tested against larvae and eggs of Aedes aegypti or adults of house flies. The order of activities of these compounds was similar for the Aedes larvae and house fly adults (topical application in benzene). The more promising of these compounds are now being tested against larvae, eggs, and adults of Anopheles albimanus.

The potential advantages of surfactant aliphatic compounds as insecticides or as ingredients of insecticidal formulations include low mammalian toxicity, biodegradability, low cost, good water solubility or emulsifiability, and in some cases high specificity for certain species resulting from hormone activity.

Many surfactants were tested as aqueous space sprays against adult Aedes aegypti. Promising results were obtained with certain nonionic surfactants as well as with amine surfactants. In preliminary testing against adult Anopheles albimanus,

100% mortality was achieved with 0.0025% malathion and 60% mortality with 0.05% of the nonionic Aerosol OT, the most active of the surfactants tested. To find a surfactant for space sprays having one-tenth the activity of malathion appears to be an attainable goal. Combinations of surfactants with other compounds will also be tested. A nonionic surfactant derived from dinonylphenol has been reported by Maxwell and Piper to be active against Culex pupae at 1 ppm.

A group of selected compounds were also studied for their ovicidal activity against Anopheles albimanus. In this experiment, male and female mosquitoes were placed in breeding cartons for three days. The insects were fed during the period and the eggs were collected in paper lined petri dishes. The test compounds were dissolved in alcohol and serially diluted to the desired concentration, then added to water for the experiment. The eggs were then exposed to the ovicidal solutions for fixed periods, then transferred to petri dishes containing water and observed for hatch for seven days.

Some of the more effective compounds studied were phenol and its alkyl and aryl derivatives, cinnamyl alcohol, derivatives of anisol, cyclopentanone derivatives, and certain polyhydric alcohol derivatives. The difference in effectiveness of the various compounds against melanized and unmelanized eggs was also noted. Anopheline eggs are unmelanized when newly laid and become melanized within a few hours, i.e., they are white when laid but darken soon after. Compounds which

prevent melanization usually prevent hatching. Observations made on the most promising groups of compounds are given below.

In the case of the phenols, the substituent groups on the ring exerted a significant influence on ovicidal effectiveness. The presence of an alkyl group on the phenolic ring enhanced the activity. For example, p-ethyl phenol is four times as effective as phenol. It was postulated that increased chain length facilitates penetration of the eggshell. The alicyclic substituted phenols were more effective than the alkyl substituted. Here again, the structural requirements for penetration of the eggshell as well as the enzyme substrate interaction are perhaps more closely met with the alicyclic than the alkyl substituted compounds. A chlorine group on the phenolic moiety increases the ovicidal effectiveness since 4-chloro-2-cylopentylphenol is 1.6 times more effective than cylohexylphenol against melanized eggs. The chlorinated compounds show their greatest effect against the unmelanized eggs. The aryl substituted phenols were the most effective ovicides tested. The steric effect of the aryl group is greater than the alkyl or alicyclic substituent. O-phenylphenol at 5 ppm produced a 60% hatch against melanized eggs. This compound is of interest because of its low mammalian toxicity. The LD_{50} for rats has been reported as 2.7 g/kg. O-phenylphenol is used in concentrations ranging from 0.1% to 6% for fruits and vegetables by dipping, spraying, and hydrocooling methods.

Tropolone, a cycloheptanone, is another promising ovicide. Tropolone at 2.5 ppm prevented 50% of Anopheles albimanus eggs from becoming melanized. Only 34% of the melanized eggs hatched. At a concentration of 25 ppm, the compound had ovicidal activity against fully melanized eggs with a 29% hatch. This difference in effectiveness against the two types of eggs indicates that the target enzyme for this compound is tyrosine hydroxylase.

The effect of increasing the alkyl side chain is more pronounced with polyhydric series than the phenolic series. In this class of compounds, N-hexyl resorcinol is of interest since it is a popular antiseptic. At 50 ppm, this compound gave a 4% hatch versus melanized eggs.

Butylated hydroxyanisole (BHA), a food additive, was the most effective antioxidant tested as an ovicide. Alcohols C_3-C_8 and substituted alcohols were also evaluated as ovicides. The presence of a phenolic substituent on the alcohol moiety increases the ovicidal properties of the alcohols. Of the alcohols and substituted alcohols tested, cinnamyl alcohol was the most effective killing 55% of the melanized eggs at 12 ppm.

Varying the exposure time of the eggs to the toxicant showed that the percentage of hatch decreased as contact time with the ovicide increased. An exposure time of 16 hours was necessary to kill all the eggs, using .02% BHA as ovicide. Cinnamyl alcohol was chosen to show the relationship between concentration and

exposure time. The results show that increasing the concentration of cinnamyl alcohol had no effect on embryonic development during the exposure period of eggs to toxicant for 4 hours. Absence of the evidence of abrupt inhibition of embryonic development indicates that cinnamyl alcohol acts slowly on the chorion gradually diffusing into the ovum. These results have been further confirmed in studies with Aedes aegypti eggs.

The study revealed that relationship between ovicidal efficiency and chemical structure is a complex one. Some of the compounds tested are highly effective ovicides and have relatively no toxic properties since they are used as food additives, germicides, and antimicrobials for fruits and vegetables. Proper formulations of these compounds offer an excellent method of control of the egg stage in the environment. This is currently being investigated.

APPLICATION EQUIPMENT AND PACKAGE INVESTIGATIONS

A. Hand Compression Sprayers and Nozzle Tips

1. Specifications.

New specifications for the hand sprayers and nozzle tips used in vector control programs have been finalized and issued. The sprayer specification, PHS/NCDC-S-101, incorporates changes and improvements made over the years as a result of test work at TDL as well as suggestions from the field. The nozzle tip specification, PHS/NCDC-S-P-200, reflects substantial improvements in nozzle tip manufacture and materials, and specifies the new Hi-Sil 233 standard abrasion test developed at TDL. A significant improvement in the new specifications is the requirement that manufacturers desiring the bid on contracts to supply these products to the AID-supported programs must first submit samples for qualification testing. Products which fail to meet the requirements will be rejected. TDL has received samples for qualification testing from two sprayer manufacturers. Of these, one type of sprayer failed to meet many of the basic requirements and the manufacturer was so notified. The other sprayer failed on several minor counts, but the manufacturer is correcting the faults and will resubmit additional samples.

Only one nozzle tip manufacturer responded to invitations to submit samples. Of two types of tips submitted, one was fully qualified and the other failed on one requirement. Samples of this type were resubmitted and were fully qualified.

2. Nozzle tip testing.

The nozzle tips presently in field use, and those referred to above, are made of an improved stainless steel alloy designated "C" by the manufacturer, and their orifices are machined to greater precision than previous tips. Extensive testing at TDL has shown that the "C" tip is more than four times as resistant to erosion by the spray suspension as the "B" alloy tip used until recently. The "B" tip was normally replaced after the passage of 250 gallons of the 5% water-wettable DDT suspension in field operations, but now the replacement of the "C" tip can be made after the passage of 1,000 gallons. The tip replacement cost can therefore be reduced about 75%. TDL is drafting a recommendation stating that the "C" nozzle tip should be replaced every two months of scheduled use, and that discarded units be examined in a central location to recover any units still serviceable.

The hardened stainless steel tips presently in use are excellent for the purpose but are relatively expensive. Tips of other materials have been evaluated in the past but were rejected for various reasons. TDL has recently tested several types of ceramic and plastic tips which show promise and would be considerably less expensive. Further testing is planned.

B. Disc Flow Regulator

The 8002 nozzle tip which is the standard used in malaria control programs has a discharge rate of 757 milliliters per minute (0.2 gal./min.) at 40 lb./sq. in. pressure, and the standard spraying techniques are based on this discharge rate. However, the normal operating range of the hand compression sprayer is 55-25 psi. Thus, in normal operation, the discharge rate of the 8002 tip is initially about 900 ml./min. and falls to about 600 ml./min. as the sprayer pressure decreases. This results in uneven application of insecticide because the spraymen are trained to maintain a constant rate of movement of the spray nozzle past the surface being sprayed. This deficiency has long been recognized, and some programs have utilized a mechanical pressure regulator on the sprayer. This device has not been satisfactory because of maintenance problems. For several years,

TDL has been working with a device known as the disc flow regulator, (DFR), which by means of a flexible rubber disc which has an orifice which changes diameter with changes in pressure, maintains a constant pressure at the nozzle tip and thus a constant flow through the tip.

In the laboratory, the DFR has performed very well and when used with the proper nozzle tip, usually a 9504 tip, the device results in an almost constant flow rate over the range of 25-55 psi. However, in field trials, performance has not been so successful, due apparently to absorption of insecticide by the disc, which results in erratic and lower rates of flow.

TDL has continued laboratory investigations of the DFR and a recent test, using 5% DDT under simulated field conditions, yielded encouraging results. Evaluations are continuing with the cooperation of the manufacturer, and it is hoped that improvements can be made which will permit recommendation of the DFR for field use in the very near future.

C. Ultra Low Volume Insecticide Application

The ULV technique in which insecticides in the undiluted form are applied for aircraft as larvicides and adulticides, at rates of 3-6 ounces per acre, is of great interest as a supplementary treatment in malaria programs especially where outdoor transmission is prevalent.

The Engineering Unit participated in a ULV spraying program in Ethiopia, sponsored by WHO, in November, 1968, and assembled a system to be used as a backup in case the Micronaire dispensers already installed in the Ethiopian aircraft should fail.

A variety of commercial aerial spraying devices used principally in crop protection have been acquired and a wind tunnel has been constructed for the testing of these devices. They are examined for capacities, droplet ranges obtained, and for adaptability to vector control in difficult terrain.

A surplus C-45 twin-engine Beechcraft airplane was transferred to TDL and modifications to the unit are underway to change it to accommodate experimental dispersal equipment for field evaluation.

D. Insecticide Shipping Containers

1. Corrugated box shipping containers.

Surveillance was continued during the year on corrugated boxes and plastic bags used for overseas shipment of insecticide. The performance of these containers remains quite satisfactory with no major problems reported.

E. Container Specifications

Corrugated box specifications are currently under study with the intent of eliminating or simplifying some of the test requirements. Records show that this could be accomplished without impairing box performance. This would be helpful to GSA in their inspection of boxes for acceptance and, likewise, of benefit to insecticide and container manufacturers. A revised specification will be issued upon completion of this study.

BIOLOGICAL INVESTIGATIONS

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

1. Evaluation of surface-applied residual insecticides.

a. Laboratory evaluation of new compounds as residual deposits.

Procedure:

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. On removal, the specimens were provided with food and water for 24 hours. Residues were from wettable powders and emulsifiable concentrates; 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 2 weeks after treatment and thereafter at 4-week intervals up to 26 weeks. The percent kill for each

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

Table 1

Compounds Tested, FY-1969

Name	Chemical Composition
Abate	O,O,O',O'-tetramethyl O,O'-thio-di-p-phenylene phosphorothioate
Akton	O-(2-chloro-1-(2,5-dichlorophenyl) vinyl) O,O-diethyl phosphorothioate
BAY 38799	O-cyclopentyl-N-methyl-phenylcarbamate
BAY 38800	O-cyclopentenyl-N-methyl-phenylcarbamate
BAY 77488	O,O-diäthylthiophosphoryl- α-oximinophenyl-essigsäurenitril
BAY 78182	O,O-diäthylthiophosphoryl- α-oximino-o-chlorphenyl-essigsäurenitril
BAY 79845	confidential
BAY 82231	confidential
BAY 85032	1,1-dimethyl-4-indanyl-N-methylcarbamate
BAY 85950	confidential
BAY 88991	confidential
BAY 91273	confidential
BAY 93820	confidential
BAY 99485	confidential

Table 1
(continued)

Name	Chemical Composition
Baygon	O-isopropoxyphenyl-N-methylcarbamate
bromophos	O,O-dimethyl O-(2,5-dichloro-4-bromophenyl) thionophosphate
carbaryl	1-naphthyl N-methylcarbamate
Cidial	ethyl mercaptophenylacetate, O,O-dimethyl phosphorodithioate
C-8353	2-(1,3-dioxolane-2-yl)-phenyl-N-methylcarbamate
C-8874	O,O-diethyl-O-2,5-dichloro-4-iodophenyl thiophosphate
C-9491	O,O-dimethyl-O-2,5-dichloro-4-iodophenyl thiophosphate
Chemagro-4641	confidential
Chlordecone-9287	confidential
DDF	dichloro diphenyl trifluoroethane
DDT ortho para	dichloro diphenyl trichloroethane
DDT para para	dichloro diphenyl trichloroethane
DDT	sodium salt of sulfonic acid derivative of p,p'
DDT	sulfonic acid derivative of p,p'
dichlorvos	2,2-dichlorovinyl dimethyl phosphate

Table 1
(continued)

Name	Chemical Composition
Dursban	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
fenitrothion	O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate
fenthion	O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate
Gardona	2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate
Hercules 9427	confidential
Hercules 14469	confidential
Hercules 16806	confidential
Landrin	3,4,5-trimethylphenyl methylcarbamate, 75%; 2,3,5-trimethylphenyl methylcarbamate, 18%
malathion	S-(1,2-dicarbethoxyethyl)-O,O-dimethyl-dithiophosphate
Mobam	4-benzothienyl-N-methyl carbamate
Neopynamin	N-(3,4,5,6 tetrahydrophthalimido) methyl dl-cis-trans-chrysanthemate
-	ortho phenyl phenol
-	para phenylphenol
Petkolin	petroleum distillate

Table 1
(continued)

Name	Chemical Composition
T-Det	ethoxylated alkyl phenol
OMS-1027	confidential
OMS-1074	confidential
OMS-1155	confidential
OMS-1202	confidential
OMS-1203	confidential
OMS-1244	confidential
OMS-1246	confidential
OMS-1247	confidential
OMS-1249	confidential
OMS-1262	confidential
OMS-1301	confidential
OMS-1302	confidential
OMS-1308	confidential
OMS-1310	confidential
OMS-1326	confidential
OMS-1342	confidential

Table 1
(continued)

Name	Chemical Composition
OMS-1343	confidential
RO 5-1923	confidential
TH-3971	confidential

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 data (Table 2) are based on the number of weeks of 70% kill produced by those insecticides evaluated as residu during FY-1969.

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

Procedure:

To determine the residual effectiveness of experimental compounds on various types of surfaces against *A. quadrimaculatus* females, panels (1 x 4 feet) of clay, whitewashed clay, plywood, thatch, and cement plaster were constructed and placed under the center of a peaked roof outdoor shed (120 feet long). These panels were treated in position by using a 2-gallon compression sprayer equipped with an 8002 nozzle and charged to 40 psi.

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.*
DDF WP	0.5	< 2	14	< 2
	1.0	< 2	> 26	6
	2.0	10	> 26	> 26
OMS-1244 WP	0.5	10	14	14
	1.0	14	18	18
	2.0	> 26	> 26	> 26
OMS-1246 WP	0.5	10	10	6
	1.0	22	22	14
	2.0	> 26	> 26	> 26
OMS-1247 WP	0.5	< 2	< 6	< 2
	1.0	6	6	6
	2.0	14	14	14
OMS-1249 WP	0.5	10	14	10
	1.0	18	18	> 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-1308 WP	0.5	< 2	-	< 2
	1.0	< 2	-	< 2
	2.0	< 2	-	< 2
OMS-1310 WP	0.5	6	14	6
	1.0	14	20+	14
	2.0	20+	20+	20+
BAY 85032 WP	0.5	10	22	18
	1.0	22	> 28	> 28
	2.0	> 26	> 28	> 28
carbaryl WP	1.0	> 26	> 26	6
	2.0	> 26	> 26	10
Neopynamin WP	0.5	6	6	6
	1.0	10	14	14
	2.0	> 26	> 26	> 26
RO 5-1923:carbaryl WP	1.5:0.5	6	> 26	10
	3:1	> 26	> 26	14
	6:2	> 26	> 26	19

Table 2
(continued)

Insecticide Formulation	Dosage g/m^2	Weeks 70% Kill		
		A. quad.	A. alb.	C. quin.
DDT*:malathion EC	0.25:0.25	8	24	-
	0.5:0.5	24	> 28	-
	1.0:1.0	> 28	> 28	-
malathion EC	0.25	1	4	-
	0.5	4	16	-
	1.0	16	20+	-
DDT* EC	0.5	< 2	4	-
	1.0	< 2	> 28	-
	2.0	< 2	> 28	-
DDT*:Baygon EC	0.25:0.25	1	12	-
	0.5:0.5	12	20+	-
	1.0:1.0	20+	20+	-
Baygon EC	0.25	1	4	-
	0.5	4	20+	-
	1.0	12	20+	-
DDT*:malathion WP	0.25:0.25	4+	4+	-
	0.5:0.5	4+	4+	-
	1.0:1.0	4+	4+	-

* Special DDT preparation

Evaluation was by exposure of dieldrin-resistant A. quadrimaculatus females to the deposits for 1 hour beneath a plastic cone at weekly intervals. In tests with DDT, a susceptible strain of A. quadrimaculatus was used. Each test represented 30 females, 10 in each of three cones, per surface. After exposure, the specimens were held under laboratory conditions for 24-hour mortality determination.

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.

Treatments were made with the same equipment used to treat the panel shed. After the spray application, the floors were lined with clean paper. Treatments were evaluated by (1) confining mosquitoes on the treated clay and wood surfaces, (2) releasing mosquitoes in the hut, and (3) suspending

caged mosquitoes near the center of the hut. Wall surface tests were made with plastic cones in the same manner as in the panel tests. 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 three types of tests were duplicated in an untreated hut. Records were also made on location of free-flying mosquitoes at the end of the tests. Unless otherwise noted, the mortalities given represent specimens found inside the hut and on the porch. Compounds tested on panels were C-9491, BAY 78182, BAY 38799, BAY 38800, Mobam, and DDT. Mobam and BAY 38800 were omitted from hut tests.

Results:

The results obtained from panel shed tests are given in Table 3. Results for BAY 78182, 38799, and 38800, and C-9491 indicate that C-9491 and BAY 38799 at 2 g/m^2 were

Table 3

Number of weeks that surfaces on panel shed treated with indicated insecticides gave mortalities of 90% or more of dieldrin-resistant *A. quadrimaculatus* females exposed for 1 hour[1/].

Toxicant	g/m^2	Clay	W. W. Clay	Plywood	Thatch	Cement
C-9491	2	10	> 16	> 16	> 16	> 16
BAY 78182	1	0	12[2/]	> 14	> 14	4
BAY 78182	2	12	10	> 14	> 14	0
BAY 38799	1	0	0	> 14[2/]	> 14	4
BAY 38799	2	6	> 16	> 16	> 16	14[2/]
BAY 38800	2	2	4	> 8	> 8[2/]	4
Mobam, 80%	2	6	> 16[2/]	> 16	> 16	14[2/]
Mobam, 50%	2	10[2/]	12	> 16	> 16	14
DDT, 75% tech.	0.5[3/]	7	> 13	9	> 11	7
DDT, 90% p,p'	0.5	11[2/]	11	9[2/]	11	> 13
DDT, 75% tech.	0.75[3/]	> 13[2/]	> 13	> 11	> 11	> 11
DDT, 90% p,p'	0.75	11	> 13	> 13	> 13	> 13
DDT, 75% tech.	1[3/]	> 13	> 13	> 11	> 11	> 11
DDT, 90% p,p'	1	11	> 13	> 13	> 13	> 13

1/ DDT-susceptible mosquitoes used in DDT tests.

2/ Indicates mortality fell below 90% during test.

3/ Dosage refers only to p,p' content.

superior if all surfaces are considered. Both gave greater than 90% mortality on all surfaces except clay for 14 or more weeks. Results show little difference between the two formulations of Mobam; each gave results equal to or better than BAY 38799 and C-9491. DDT was formulated to give equal amounts of p,p' DDT with each dosage. Results from tests show little difference between formulations or dosages. Most were still giving high kill at termination of tests (11 and 13 weeks). Table 4 gives the results obtained with the free-flying female mosquitoes in treated huts. C-9491 and BAY 78182 at 2 g/m^2 were superior to other compounds and gave 11 and 13 weeks, respectively, of 90% or greater kills. No marked difference was noted between results from huts treated with the two formulations of DDT.

In the untreated hut, an average of 94% of collected mosquitoes were found inside of the hut. With the BAY compounds and C-9491, the average percentages found in the hut ranged from 90 to 97. With the two DDT formulations, approximately 77% were found in the hut.

Table 4

Number of weeks that adobe-lined plywood huts treated with indicated insecticides gave mortalities of 90% or more of dieldrin-resistant *A. quadrimaculatus* females released in huts and exposed for 2 hours.

Toxicant	g/m^2	No. Weeks
C-9491	2	11
BAY 78182	1	7
BAY 78182	2	13 1/
BAY 38799	1	< 5
BAY 38799	2	7 1/
DDT, 75%	0.75	< 4
DDT, 90%	0.75	4

1/ Indicates mortality fell below 90% during test.

Table 5 gives the results obtained from exposure of female mosquitoes held under plastic cones for 1 hour on clay and wood surfaces inside the huts. None of the experimental compounds gave mortalities above 90% on clay. On wood, all compounds were effective for the duration of tests (9 or more weeks). The two formulations of DDT were highly effective on both surfaces.

Results from tests with suspended caged mosquitoes showed that only BAY 78182 and BAY 38799 at 2 g/m^2 gave kills of greater than 70% for any appreciable length of time; each was effective for 5 weeks.

2. Evaluation of space applications.

a. Laboratory evaluation of new compounds as space applications.

Procedure:

Adult 3-day-old female dieldrin-resistant *A. albimanus* and DDT-resistant *C. quinquefasciatus* were exposed 30 seconds to a mist spray discharged within a modified Hoskins-Caldwell chamber. The toxicant, T-Det, was formulated at a strength of 5% in a glycerol (15 parts) 95% ethanol (85 parts) carrier. One, 2, 3, 4, and 5 milliliters of the formulation were

Table 5

Number of weeks that surfaces in experimental huts treated with indicated insecticides gave mortalities of 90% or more of *A. quadrimaculatus* females exposed for 1 hour.

Toxicant	g/m^2	Clay	Wood
C-9491	2	0	> 9
BAY 78182	1	0	> 9
BAY 78182	2	0	> 13
BAY 38799	1	0	> 9
BAY 38799	2	0	> 13
DDT, 75% tech.	0.75 1/	> 15 2/	> 15 2/
DDT, 90% p,p'	0.75	> 15 2/	14 2/

1/ Dosage refers only to p,p' content.

2/ Indicates mortality fell below 90% during test.

atomized into the spray chamber and the spray particles exhausted through the exposure carton. Three replicates of each dosage were used. After exposure, the insects were removed and provided with food and water for 24 hours at which time the percentage kill was determined.

Results:

The data are given in Table 6.

b. Field evaluation of new materials as thermal fogs.

Procedure:

Evaluation of malathion, Gardona, C-9491, BAY 38799 and BAY 78182 as aerosols was conducted in an urban area near Savannah, Georgia, in 1968. A Leco 120 thermal fog generator mounted on a 3/4-ton truck was used in these tests. In all evaluations, malathion was formulated at a rate of 6 oz./gal. of finished spray; the other compounds were tested at 4 or 6 oz./gal. The solvent was No. 2 fuel oil. Tests were conducted at night between 7:00 and 11:00 p.m. using dieldrin-resistant *A. albimanus* and DDT-resistant *C. quinquefasciatus*. Approximately 100 females of each species were placed in each screen-wire cage (3-1/4 inches

Table 6

Laboratory space spray tests with T-Det.

Dosage (ml.)	Percent Kill	
	A. albimanus	*C. quinquefasciatus*
1	92	4
2	99	68
3	100	95
4	100	99
5	100	98
Control (ethanol:glycerol)	2	< 1
Check	1	0

diameter by 6 inches in length). Caged specimens were exposed at sites, 150 and 300 feet, on each of three streets, 270 feet apart and perpendicular to the line of fog release. The cages were hung 6 feet above the ground at each exposure station. The length of the application run was 1,300 feet; the travel time was 3 minutes at about 5 m.p.h. At a discharge rate of 40 gal./hr., 2 gallons of the formulation were discharged during each run.

Runs were conducted only when wind velocities permitted a drift of the fog over the test area. Each night's testing began with the malathion fog and was followed by two runs of the test material. Approximately 15 minutes after each test run, the cages were removed to the laboratory and the insects transferred to clean holding cages, given food, and held for 24-hour female mortality counts. The cages containing the check insects also were transported to the study site prior to each test and then returned to the laboratory grounds where they were suspended outdoors in the same manner as previously described. Subsequent handling was the same as that for the treated mosquitoes.

Results:

Tests with Gardona, C-9491, BAY 38799, and BAY 78182 at 4 or 6 oz./gal. showed BAY 78182 and BAY 38799 to be superior to C-9491 and Gardona against both *C. quinquefasciatus* and *A. albimanus* at distances up to 300 feet from the point of discharge of the fogs (Table 7). Both BAY 78182 and BAY 38799 were essentially equal to the standard, malathion, at 6 oz./gal. The results with BAY 78182 at 4 oz./gal. were only slightly superior to those achieved with 2 oz./gal. in FY-1968.

Temperatures ranged from 72 to 82°F. during the 7:00 and 11:00 p.m. test period; the average relative humidity was from 85 to 100%. Wind conditions varied from 0 to 500 feet per minute (0-6 m.p.h.); however, over 90% of the time the wind velocity was less than 1 m.p.h.

3. Evaluation of new compounds applied as larvicides.

a. Laboratory evaluation of new compounds as larvicides.

Procedure:

The LC-95 level in parts per million was determined for each species using the standard WHO test method which

Table 7

Percent kill of adult female mosquitoes 150 and 300 feet from the point of discharge of thermal fogs.

Insecticide	Oz./Gal.	Test Runs	A. albimanus		C. quinquefasciatus	
			150'	300'	150'	300'
Gardona	6	5	30	9	25	5
BAY 38799	6	4	100	100	81	81
C-9491	6	16	68	55	70	54
BAY 78182	4	4	100	97	97	78
malathion	6	11	96	96	97	96

involves a 24-hour exposure of 25 third instar larvae in 250 ml. of water with the toxicant introduced 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 Petkolin and T-Det: Petkolin 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:

The LC-95 levels for the various compounds against larvae of dieldrin-resistant *A. quadrimaculatus* and DDT-resistant *C. quinquefasciatus* are in Table 8.

b. Field evaluation of larvicides.

Procedure:

Evaluation of candidate insecticides for control of *Anopheles* and *Culex* larvae was continued in the field plots at Fort Stewart, Georgia. The insecticides, made up as granular, wettable powder and emulsifiable concentrate formulations,

Table 8

Concentration of insecticide required for the LC-95 of

A. quadrimaculatus and *C. quinquefasciatus*.

Insecticide	*A. quadrimaculatus* ppm	*C. quinquefasciatus* ppm
BAY 79845	0.1	0.02
BAY 82231	0.5	0.02
BAY 85032	2.5	2.5
BAY 85950	0.1	0.004
BAY 88991	0.5	0.1
BAY 91273	0.5	0.1
BAY 93820	0.5	0.5
BAY 99485	0.5	0.1
Chemagro 4641	2.5	0.5
Chlordecone-9287	0.1	0.1
DDT, sodium salt of sulfonic acid der. of p,p'	> 2.5	> 2.5
DDT, sulfonic acid der. of p,p'	> 2.5	> 2.5

Table 8
(continued)

Insecticide	*A. quadrimaculatus* ppm	*C. quinquefasciatus* ppm
Neopynamin	2.5	0.5
OMS-1202	2.5	0.5
OMS-1203	> 2.5	> 2.5
OMS-1246	2.5	0.5
OMS-1247	0.5	2.5
OMS-1249	0.5	0.5
OMS-1262	> 2.5	> 2.5
OMS-1301	0.5	0.1
OMS-1302	0.5	0.5
OMS-1308	> 2.5	> 2.5
OMS-1310	0.5	0.5
OMS-1326	0.1	0.1
OMS-1342	0.02	0.004
OMS-1343	0.004	0.02
O-phenylphenol	> 2.5	> 2.5
p-phenylphenol	> 30	30

Table 8
(continued)

Insecticide	*A. quadrimaculatus* ppm	*C. quinquefasciatus* ppm
TH-3971	> 2.5	> 2.5
Petkolin	0.02 (ml)	0.08 (ml)
T-Det:Petkolin	0.08 (ml)	> 0.08 (ml)

were applied to these plots, and their residual life evaluated. Three-hundred-square-foot grassy plots were treated with 50 different insecticide formulations. The soil in those plots was of a sandy loam type with clays beginning from 18 to 22 inches down. The treatments were made to damp but not flooded plots. Materials were applied at rates of from 1 to 4 pounds actual per acre. The granules were hand broadcast, while the wettable powders and emulsions were applied with a 3-gallon compressed air sprayer (40 psi - #8002 nozzle).

Two weeks after treatment and biweekly thereafter, three sod samples (1/12 sq. ft. x 3 in. deep each) for each dosage and mosquito species to be tested were taken at random from the treated plots. The samples were placed in battery jars (three per jar) and flooded 72 hours prior to introduction of 25 third instar larvae per jar. The larvae were placed inside a wire mesh basket (2 in. x 2 in. x 2-1/2 in.) which was lowered into the upper surface of the water; dieldrin-resistant A. albimanus and DDT-resistant C. quinquefasciatus larvae were used. Larval food was given and mortality observations

were made 24 and 48 hours after introduction. Each biweekly sample remained flooded for 10 days at which time a second series of caged larvae were introduced into the jars, larval food given, and mortality counts made. Eleven of the formulations gave 6 or more weeks of residual kills of 90% or better (Table 9).

Dursban was the most effective compound in all formulations. Abate and fenthion as emulsifiable concentrates were effective for greater than 12 weeks and 8 weeks, respectively, against both of the test species.

Akton granular and carbaryl wettable powder were ineffective against C. quinquefasciatus but effective against A. albimanus. Emulsifiable concentrates of Cidial and bromophos were effective for 10 and 6 weeks, respectively, against both test species. Mobam granules were effective for 12 weeks or better on both of the test species.

Table 9

Weeks of 90% kill based on 48-hour exposure of third instar larvae in 3-day flooded soil samples.

Insecticide	# Actual/ Acre	Granular		Wettable Powder		Emulsifiable Concentrate	
		A. alb.	*C. quin.*	*A. alb.*	*C. quin.*	*A. alb.*	*C. quin.*
Abate	4	4	-	> 12	> 12	> 12	> 12
Akton	2	> 12	< 2	-	-	> 12	2
BAY 38799	4	-	-	4	2	-	2
BAY 78182	2	4	2	-	2	2	2
BAY 77488	2	-	-	< 2	< 2	2	2
Baygon	2	6	-	6	-	12	2
bromophos	2	-	-	-	-	6	6
C-8874	4	-	-	6	6	-	-
C-9491	4	< 2	< 2	4	2	-	-
carbaryl	4	2	-	> 12	-	-	-
Cidial	2	4	2	< 2	< 2	10	10

Table 9
(continued)

Insecticide	# Actual/ Acre	Granular		Wettable Powder		Emulsifiable Concentrate	
		A. alb.	C. quin.	A. alb.	C. quin.	A. alb.	C. quin.
DDF	4	-	-	4	< 2	-	-
dichlorvos	2	< 2	-	-	-	-	-
Dursban	1	> 12	-	> 12	-	> 12	-
fenitrothion	2	4	-	6	-	4	-
fenthion	2	-	-	-	-	8	8
Gardona	2	-	-	< 2	< 2	< 2	< 2
Herc 9427	2	-	-	2	2	-	-
Herc 14469	2	2	6	< 2	2	-	-
Herc 16806	2	-	-	< 2	2	-	-
Landrin	2	-	-	< 2	6	-	-
Mobam (50 & 80%)	4	> 12	12	4	2	-	-
Neopynamin	4	-	-	< 2	< 2	< 2	< 2

Table 9
(continued)

Insecticide	# Actual/ Acre	Granular		Wettable Powder		Emulsifiable Concentrate	
		A. alb.	C. quin.	A. alb.	C. quin.	A. alb.	C. quin.
OMS-1027	4	-	-	< 2	< 2	-	-
OMS-1074	2	< 2	< 2	< 2	< 2	< 2	< 2
OMS-1155	2	< 2	2	-	-	-	-

B. Studies on the Development of Alternate Noninsecticidal Methods of Mosquito Control

1. Studies on attractants for adult mosquitoes.

An olfactometer was developed to study the behavioral response patterns of newly emerged adult *A. albimanus* to olfactory stimuli. It consists of two 31-inch long plexiglass tubes (I. D. 11-3/8 inches) joined at the center by a sliding panel which contains one screened panel and one unscreened opening. The screened door prevents the movement of specimens from one tube to another prior to the test. They are introduced into the olfactometer by an aspirator through a 1/2-inch circular hole in the holding portion of the olfactometer. The other section is divided into four quadrants each equipped with a small horizontal platform to hold the test materials. The quadrants taper to a point approaching the center of the olfactometer allowing the mosquitoes to make a choice as they ascent an odor gradient.

Test materials are injected by syringe through 3/8-inch diameter needle-puncture rubber stoppers inserted in the wall of the olfactometer, one per quadrant, above the test platform.

Outside air of the proper temperature and humidity is drawn into the olfactometer and passed over absorbent paper disks impregnated with test essences. Air speed is regulated by a variable transformer coupled to a squirrel cage fan.

After a period of equilibration allowing odors to pass into the holding chamber, the sliding panel is pulled to draw the screened door from the center of the olfactometer and replace it with an opening which permits the mosquitoes to enter the test area. Counts are then made of the numbers of mosquitoes in each quadrant giving an indication of the relative attractiveness of the odor being tested. This olfactometer is being designed as an aid in determining the odors that are the most attractive for young A. albimanus. Such attractants are to be used in conjunction with an insecticide treated food source as a control procedure or as means of improving assessment procedures.

2. Studies on the response of mosquitoes to ultraviolet light.

Investigations on the responses of adult A. albimanus to ultraviolet light have been continued. As described in the report for FY-1968, a commercially produced light trap equipped with a BLB fluorescent lamp and a pair of electric fans which produce an

air curtain to sweep attracted insects into a pan of detergent solution is used in these studies. All tests were done with insectary-reared mosquitoes liberated in 6-foot cubical chambers housing the traps. Recovery of untrapped specimens was made with a small vacuum cleaner instead of the pyrethrum knock-down procedure previously employed.

Under test conditions, A. albimanus has shown maximum activity and response to ultraviolet light in the early evening. Progress in the design of an efficient live-capture trap has been hampered by the need to conduct tests during this time period. Emphasis, therefore, has been placed on development of a more suitable testing procedure whereby more tests per day can be run with no increase in the testing facilities. Such a method has been found.

Four- and 5-day-old A. albimanus adults subjected from the pupal stage onward to an artificial photoperiod schedule of 10:30 p.m., light on - 10:30 a.m., light off, showed high level activity at midday when normally they are quiescent. In UV light trap tests run from 11:00 a.m. to 1:00 p.m., they responded as though sunset had occurred at 10:30 a.m. Table 10 compares the

Table 10

Capture of Anopheles albimanus conditioned under two light periods.

UV light trap operated from 11:00 a.m. to 1:00 p.m.

Six replicates.

Light Period	Males		Females	
	Number Released	Percent Trapped	Number Released	Percent Trapped
10:30 p.m. - 10:30 a.m.	344	92	426	90
6:00 a.m. - 8:00 p.m.	379	44	331	16

catch of A. albimanus exposed to the artificial photoperiod with that of others held under natural daylight conditions. These data demonstrate the feasibility of producing, through photoperiod manipulation, A. albimanus and other night-biting mosquitoes whose subjective sunsets would form a series and whose responses in light trap evaluations will be maximal at convenient times during the work day.

When conditioned adult A. albimanus were dusted with fluorescent ZnS powder to make them visible in the darkened test chamber, it was found that fully three-fourths of the total captures by an ultraviolet trap in 120 minutes operation occurred in the first half hour. Table 11 records this catch by 5-minute increments for 30 minutes and total capture at 120 minutes. Preliminary data from additional tests suggested by these results indicate that interrupted trap operation yields a substantially larger catch of A. albimanus than does continuous operation when total "on" time is the same. The catch obtained with an ultraviolet trap located near the ceiling (5 feet) of the test chamber was compared with catches in traps at 0, 3, and 4 feet. The results, summarized in Table 12, indicate the critical importance of trap location.

Table 11

Cumulative capture of A. albimanus by ultraviolet light trap in 120 minutes. Four replicates.

Minutes	Number	Percent
5	220	40
10	104	59
15	43	67
20	23	71
25	13	73
30	8	75
120	137	100
	548	

Table 12

Catch of Anopheles albimanus by ultraviolet light traps at various heights. Values based on six replicates each.

Trap Height (ft.)	Males		Females	
	Number Released	Percent Trapped	Number Released	Percent Trapped
0	469	67	360	57
5	546	96	201	93
3	794	70	741	68
5	881	91	768	85
4	1004	84	1071	81
5	852	89	1051	86

Reduction of the attraction of UV light for A. albimanus due to extraneous illumination is presently under investigation to determine the effect of moonlight or other illumination on the number of mosquitoes trapped.

Initial field trials of ultraviolet traps as survey instruments for A. albimanus were begun in El Salvador in late June.

3. Studies on biological agents for control of anopheline species.

a. Coelomomyces punctatus, C. indicus, and C. spp.

During the current year, cooperative efforts with Dr. John Couch, University of North Carolina (UNC), were continued in an effort to develop methods for the laboratory propagation of the fungus C. punctatus. This fungus is an obligate parasite of A. quadrimaculatus that produces the death of the larva except in cases of light infestations. Essentially, nothing is known concerning the mode of infection or factors influencing the initiating sporangial germination and subsequent larval penetration by the motile zoospores. Efforts to date by Dr. Couch have been directed toward determining these factors. Recently, the fungus has been successfully propagated at UNC and arrangements have

been made to supply infective material necessary to conduct laboratory and field tests at TDL. The Technical Development Laboratories presently provide twice weekly shipments of Anopheles eggs to UNC as a source of suitable host specimens for the production of the infective material.

Larvae and pupae of A. crucians have been collected from the field during the past year and used in an unsuccessful attempt to establish a laboratory colony. This species harbors a Coelomomyces which is similar to C. punctatus and affords an excellent parasitic association for evaluating techniques and methods that may have potential value in future control methods.

Attempts to obtain infection of C. indicus in A. stephensi using dried clay from buffalo wallows in India have been unsuccessful.

A field survey was begun in mid-June in El Salvador to detect larvae of A. albimanus parasitized by Coelomomyces and/or other organisms.

4. Studies of oviposition attractants for *C. p. quinquefasciatus*.

Ovipositional attractants for *C. p. quinquefasciatus* are presently being isolated and identified from the following:

Cynodon dactylon	-	Bermuda grass
Phleum pratense	-	Timothy grass
Paspalum notatum	-	Bahia grass
Medicago sativa	-	Alfalfa
Arachis hysogala	-	Peanut hay

Each of the above plants was first ground in a Wiley mill and 60 grams of the resultant powder extracted with 600 ml. of distilled water in a Soxhlet apparatus.

Aqueous extracts of these plants were assessed as ovipositional attractants in 24 in. x 24 in. x 24 in. cages containing approximately 600 males and 600 blood fed females which were ready to oviposit. One-pint black glass jars containing 100 ml. of test solution or distilled water were used to conduct the test. One jar was placed in each corner of the cage. The test fractions were placed in the containers diagonally from one another. Laboratory tests employed a dilution of the extracts of one part to 19 parts distilled water. Results (Table 13) showed that all these materials were highly attractive to ovipositing females.

Table 13

Percent of egg rafts of *C. p. quinquefasciatus* laid on refluxed plant extract and on distilled water. Results are based on four replicates.

Test Material	Percent Egg Rafts Deposited	
	5% Extract Mixture	Distilled Water
Bahia	89	11
Peanut	82	18
Alfalfa	82	18
Bermuda	81	19
Timothy	77	23

When the pure extract was tested, however, the material lost its attractiveness and the above figures were essentially reversed, apparently due to an increase of attractive substances to the point that they become repellent.

Extensive chemical testing of these extracts show that in addition to refluxing they may withstand autoclaving, dialysis, lyophilization and sublimation without losing attractiveness.

One of the refluxed extracts (Bermuda) was tested as a 5% concentration in a large outdoor cage containing approximately 4,800 male and 4,800 blood-fed female C. p. quinquefasciatus. During the 7-day test period, 84% of all egg rafts were deposited in the aqueous extract and 16% in the distilled water control despite two heavy rains (3.29 and 4.20 inches) each of which flooded all containers during the test period.

Studies are continuing in an attempt to further isolate and identify these plant fraction(s).

C. Summary of Activities on Biological Studies

The past year's data from laboratory evaluation has revealed nine new compounds of maximum potential as residues for use in house treatments.

Each of the materials (DDF, OMS-1244, 1246, 1247, 1249, 1308, and 1310, BAY 85032, and Neopynamin) produced greater than 26 weeks of effective kills of A. albimanus and A. quadrimaculatus at the dosage of 2 g/m^2. BAY 85032 was the most promising; it was highly effective at 0.5 g/m^2.

Certain compounds offer particular promise because of their low mammalian toxicity (i.e., acute oral LD-50 for rats ranges from 500 to 10,000 mg/kg).

In the advanced tests, compound C-9491 has displayed unusual promise because of its prolonged activity on mud and on other surface materials that normally cause an adverse effect on the life of the deposit.

The high degree of efficacy of DDT 90% p,p' as a residue on different surface materials has resulted in its inclusion in the field testing program of the Central American Malaria Research Station.

A development of much potential is the effectiveness of chemical mixtures of malathion or Baygon with specially prepared DDT formulations. The combined formulations of the chlorinated hydrocarbon and organophosphorus compounds have, in several instances, shown a much longer residual life than either component alone.

These findings on insecticide residues suggest several new compounds of promise and also indicate a promising lead in developing ways of extending the life of the organophosphorus compounds as residues. The latter may have special value from the standpoint that it will reduce the amounts of insecticides required, thereby decreasing the cost and environmental contamination factors.

As a space application against adults, two experimental compounds, BAY 38799 and BAY 78182, offer considerable promise.

Of the more than 30 new materials tested against anopheline larvae, only one, OMS-1343, showed a level of toxicity equivalent to Abate or Dursban. Since this compound has a much greater toxicity to mammals than either of these materials, it does not warrant further testing. In the evaluation of 26 compounds as residual larvicides, Dursban showed the longest residual life at the lowest dosage. Although Abate gave equivalent biological activity, it required four times the dosage. However, lower mammalian toxicity of Abate has made it the insecticide of choice in further experimental work with larvicides that are now underway at the Central American Malaria Research Station.

Preliminary work with the ultraviolet light traps indicates that this method has much promise as a means of evaluating adult densities of

A. albimanus. At present, the practical value of these traps points more toward their use as a surveillance tool than as a possible control method under urban conditions. These traps are currently under study in the field in El Salvador.

The work with biological agents has shown slow progress because of the lack of information on the use of such methods for controlling anopheline mosquitoes. The parasite fungus Coelomomyces potentially offers the most promise against anopheline mosquitoes, and the effort in this area primarily relates to this group. However, close observations are being made on field specimens for any other type of parasitic organism that could be adapted for control purposes.

In the area of studies on mosquito behavior and tropisms, close co-operation between TDL and the CAMRS is in effect so that preliminary leads detected at TDL can be evaluated in the field as soon as feasible.