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TECHNICAL DEVELOPMENT LABORATORY

Herbert F. Schoof

Center for Disease Control
Atlanta, Georgia

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RESEARCH FINAL
PROJECT REPORT

JUNE 30, 1972

TECHNICAL DEVELOPMENT LABORATORY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
Health Services and Mental Health Administration
Center for Disease Control
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16. Abstracts

The Technical Development Laboratory studies have been oriented toward problems encountered in the many national malaria eradication programs. The findings on methods, materials and procedures have enabled the programs to improve the efficiency and economy of their operations. Results of some studies such as those dealing with specifications for and testing of pesticides, spray equipment, and pesticide containers, have benefited all those purchasing and using these items. Reductions in cost have come both through lower initial prices and through more uniform and longer lasting products. Likewise, research on new insecticide formulations, improved insecticide delivery systems, biologic control methods, and vector dynamics has benefited the world-wide effort in general. In addition, specific requests from developing countries for assistance relating to mosquito resistance, chemical testing, mosquito rearing, equipment testing, and local insecticide formulation procedures have been answered with solutions to immediate problems and frequently have led to training nationals to deal with similar problems in the future.

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REPORT SUMMARY

A. 1. *Project Title:* World Wide Malaria Eradication

Contract Numbers: PASA RA (HA) 7-00
PASA RA (HM) 7-00

2. *Principal Investigator:* Herbert F. Schoof, Ph.D.

Contractor: Technical Development Laboratories
Malaria Program
Center for Disease Control
1600 Clifton Rd., NE
Atlanta, Georgia 30333

3. *Contract Period:* September 1955 to June 1972

4. *Period covered by Report:* September 1955 to June 1972

5. *Total A.I.D. Funding of Contract to 30 June 1972:* $ 5,310,794

6. *Expenditures and Obligations through 30 June 1971:* $ 4,741,294

7. *Expenditures and Obligations for FY 1972:* $ 569,500

8. Estimated Expenditures for Next Contract Year: NA

B. Narrative Summary of Accomplishments and Utilization

During the life of the project TDL studies have been oriented toward problems encountered in the many national malaria eradication programs. The findings on methods, materials and procedures have enabled the programs to improve the efficiency and economy of their operations. Results of some studies such as those dealing with specifications for and testing of pesticides, spray equipment, and pesticide containers, have benefited all those purchasing and using these items. Reductions in cost have come both through lower initial prices and through more uniform and longer lasting products. Likewise, research on new insecticide formulations, improved insecticide delivery systems, biologic control methods, and vector dynamics has benefited the world-wide effort in general. In addition, specific requests from developing countries for assistance relating to mosquito resistance, chemical testing, mosquito rearing, equipment testing, and local insecticide formulation procedures have been answered with solutions to immediate problems and frequently have led to training nationals to deal with similar problems in the future.

A. General Background

The first contractual agreement between ICA-AID and CDC was established for FY-1956, effective September 1, 1955. Its purpose was to develop new or improved formulations of residual insecticides as possible substitutes for DDT to other chlorinated hydrocarbon insecticides in overseas vector control operations. Funding was $20,000. The fundamental reason for initiating investigations in this field was the fact that anopheline populations in certain areas in Greece had developed resistance to DDT. In anticipation that resistance to DDT would occur in populations of other anopheline vectors throughout the world, it was critical that research be undertaken to provide possible substitutes for DDT and related compounds. In subsequent years the problem of resistance among anopheline vector populations increased, which led to an expansion both in the objectives and fundings of the agreements. The changes in scope and direction of the research work on the biological, chemical, and engineering phases have been in response to field needs and to new developments in the control approach. Througnout the period of the contractual agreement, the prime purpose has been that of backstopping the global effort of AID to eradicate malaria through suppression of anopheline populations.

B. Statement of Project Objectives as Stated in the Contract

The objectives of the contract have changed from year to year. The same general objective of improving the efficiency and economy of AID supported malaria eradication programs through developing new or improved control methodology remained, but the elements composing it were altered as new items were added or substituted. The specific objectives over the 18 years have included the following:

1. Develop hand compression sprayers and accessories to provide increased reliability, wearability, ease of use, and safety.

2. Develop methods and equipment for testing sprayers and accessories for vector control use.

3. Develop and revise specifications for sprayers and components.

4. Test sprayers and nozzles for compliance with specifications.

5. Develop and evaluate flow control devices to improve the uniformity of spray deposits.

6. Develop improved packaging and shipping containers for water-wettable insecticide powders.

7. Develop new or improved formulations of residual insecticides for application inside houses for control of anopheline mosquitoes.

8. Develop and revise specifications for pesticide formulations which could be used as a basis for purchase of large quantities of materials for field programs.

9. Develop field tests for performance of insecticides which had been delivered and stored in recipient countries for long periods of time.

10. Develop tracer compounds as indicators which would be used to determine if a wall surface had been sprayed.

11. Determine the quality of insecticide powders after long-term storage through surveillance of batch samples.

12. Certify commercial laboratories which were under contract to analyze samples of products purchased by the program.

13. Investigate residual fumigants for possible use in malaria eradication programs. Develop formulations which could be used to maintain air concentrations of the vapor insecticide over long periods of time. Develop chemical methods of measurement of air concentrations.

14. Conduct basic studies on water-dispersible powder formulations including such problems as surfactant-carrier and carrier-insecticide interaction, the effect of fineness of grind and the effect of compaction on the final product.

15. Develop micro methods of analysis for determination of insecticides on wall and insect surfaces, in water and in air.

16. Improve macro methods of analysis used in pesticide specifications to reduce the unit cost of analyses of samples offered to the Government on contracts.

17. Conduct biochemical studies on new compounds such as chemosterilants, antimetabolites, and hormones (especially ovicides and larvicides) which might be used as alternatives to DDT in malaria programs.

18. Develop methods for detection of resistance to insecticides in adult and larvae mosquito populations.

19. Laboratory and field assessment of new chemical formulations synthesized by industry as adulticides and larvicides against malaria and filariasis vectors.

20. Toxicological evaluation of health hazards associated with potential use of new insecticides or of operational procedures.

21. Study effectiveness of the residual fumigant technique against *Anopheles gambiae* in Africa and against *A. albimanus* in Haiti.

22. Study alternative methods and supplementary techniques for mosquito control (e.g., insect pathogens).

23. Colonize malathion resistant *A. albimanus*.

24. Evaluate aerial application of ultra low volume applications of malathion and Abate to determine the most effective technique (i.e., spray equipment, droplet size, aircraft speed, etc.) for obtaining satisfactory kills of adult and larval mosquitoes.

25. Assess ground-applied ULV treatments, using caged adult mosquitoes, in comparison to fog applications by standard fogging or misting equipment.

26. Study the effect of sublethal exposure of mosquito adults to residues in relation to longevity, reproduction, blood feeding, and other responses.

27. Evaluate physical and chemical attractants as tools in surveillance and control of vector populations.

28. Study the feeding habits of anopheline adults and larvae.

29. Investigations on the influence of light, motion, temperature, and other factors on the behavior of anopheline larvae.

30. Develop improved methods for measuring mosquito densities.

C. Continued Relevance of Objectives

Through the early years of the world-wide malaria eradication program and into a time when the problems confronting the eradication effort have led to adoption of a new strategy by the World Health Assembly, TDL has continued to work toward objectives reflecting the field needs. The initial objective of the laboratory, to develop substitutes for DDT, has even greater relevance today as malaria is found to be refractory in many areas; a wide range of alternative methods will be necessary to overcome the multifarious problems.

D. Accomplishments to Date

1. Development of hand compression sprayers and accessories

As a result of TDL's continuing research and collaboration with manufacturers and field programs, the hand compression sprayer and components have been improved so that the estimated field life of sprayers has been substantially increased with little increase in the initial cost.

Improvement of the spray nozzle tip has been particularly significant. In the early years of malaria programs, the brass nozzle tips used were found to increase in flow rate (by erosion) by about 44% following discharge of 250 gallons of 5% DDT slurry. By working closely with suppliers, TDL has been instrumental in progressive improvement of materials, first to stainless steel, then to a hardened stainless steel, so that the useful life of a nozzle is now many times the former life. In addition to reduced replacement costs for nozzles, the reduction in erosion rate substantially reduces waste of insecticide.

Collaboration with PAHO in the field evaluation of sprayers and nozzle tips has resulted in substantial improvements in design.

2. Testing sprayers for vector control use

The methods and equipment for testing hand sprayers and nozzle tips for use in vector control spraying developed by TDL have been generally adopted by WHO and others involved with such equipment.

3. Development and revision of specifications for sprayers and components.

Specifications for the hand compression sprayer and nozzle tips were first prepared by TDL for ICA in 1956. The specifications have been revised periodically for AID to reflect changes and improvements resulting from laboratory research, field operational use, and combined laboratory-field trials. The specifications used by WHO for this equipment have resulted primarily from TDL's publications and its participation in expert committee meetings.

4. Testing sprayers and nozzles

Qualification and compliance testing of nozzle tips under the specifications revised by TDL in 1969 has thus far eliminated delivery of substandard tips to field programs, a problem that has been occurring for several years.

5. Development and evaluation of flow control devices on sprayers

A simple flow control device known as the disc flow regulator was developed by TDL. Laboratory and field evaluation of this device demonstrated that its use results in improved uniformity of spray deposits and increased nozzle tip life, but in some cases results were erratic and flow rates were low. Further laboratory evaluation of the disc flow regulator revealed reasons for the minor deficiencies in performance and demonstrated that the device is desirable in spite of these deficiencies.

6. Improved shipping containers for insecticide powders

In the late 1950's, water-wettable insecticide powder shipping containers became a serious economic problem to AID because of the loss of insecticide resulting from damage to the containers in transit and because the inefficient design of the container had increased shipping costs. TDL's investigations on the containers resulted in an improved container design which reduced shipping costs by about 25% while at the same time greatly decreased the loss due to damage or moisture penetration of the container. Surveillance has shown satisfactory performance of the improved containers.

7. New and improved formulations of residual insecticides

Two of the more promising alternatives to DDT are propoxur and malathion. TDL has carried out studies on formulations of these compounds and developed analytical methodology and physical test procedures for water-dispersible powders of both compounds. Laboratory-scale quantities of propoxur water-dispersible powders were prepared and its stability in storage studied. Data for minimum performance requirements for malathion and propoxur powders are now available. Test procedures and performance requirements have been recommended to WHO for use in specifications for pesticides for international health programs, and specifications for malathion and propoxur powders for AID programs can be drafted whenever required.

8. Development of specifications for pesticide formulations

The first specification for DDT powder was drafted at TDL in 1954 at the request of AID (then FOA). Since that date the specification has undergone 13 revisions (Appendix H.5). Each revision was made to strengthen the specification as improvements were made in manufacturing techniques or improved test methods were developed. During the period 1955-1971, some 824 million pounds of DDT powder were bought on specifications drafted by TDL at a total cost of over $178,000,000. Although DDT powder is by far the most important insecticide purchased for AID programs, specifications have been drafted for a number of other products (Appendix H.6). Also research on formulations of alternative pesticides is in progress and specifications could be made available on short notice.

Prior to 1954, serious problems were encountered in malaria programs when DDT powders were received which had very poor suspensibility characteristics and frequently stoppages occurred due to blocking of screens and nozzles in spraying equipment by large particles. Considerable improvement was observed when specifications were drafted for powders specifically designed for hand spraying equipment. Even then, some products failed after long-term storage in the field. In general, stopgap measures were taken to keep the quality of the powders up during the period 1954-1970 while basic studies on powder formulations were in progress at TDL. The first major breakthrough occurred in 1960 when a high temperature accelerated storage test was developed at TDL which was shown to correlate with long-term storage stability. This test was incorporated in the AID specification in 1961 and powders purchased under this specification exhibited much improved long-term storage characteristics. Later follow-up showed that many powders which failed the high temperature test performed poorly in long-term storage.

9. Field tests for performance of insecticides

One problem frequently occurring in the field is the determination of the quality of a powder. This is especially true in cases where powders have been stored for long periods under adverse climatic conditions. An improved quick visual suspensibility test was devised at TDL. This test was given to the World Health Organization and was published in the WHO Manual of Specifications for Pesticides. It has been widely used in the field as a guide for determining whether or not a powder is suitable for spraying.

10. Tracer compounds

Research on tracer compounds which could be used to determine if a surface had been sprayed was conducted at the request of AID. A kit was developed which could be used to detect a water soluble fluorescein dye on mud walls after spraying with insecticide to which the dye had been added. While this method could be used, it was considered to be impractical and was never pursued. Although the technique is not likely to be used in field programs, it might be useful in training.

11. Surveillance of batch samples of insecticide powders

With the introduction of a shelf-life requirement in the specifications, TDL

ccepted the responsibility for testing samples of DDT powder. Since 1964, hen the program went into effect, 2000 to 7000 samples have been kept under urveillance each year. (This represents up to 78.9 million pounds of the roduct a year).

12. Certification of commercial laboratories under contract

Along with the development of new methods for testing insecticide formulations, TDL has been responsible for surveillance over analytical laboratories which run tests on the products purchased. In the early days, commercial laboratories contracted for these services. These laboratories were certified by TDL and were inspected periodically to ensure that they were proficient. In more recent years, the analyses have been performed in GSA laboratories. These laboratories are also checked from time to time by collaborative testing programs.

13. Residual fumigants for use in malaria eradication programs

After the discovery that DDVP vapors were highly toxic to mosquitoes at low concentrations, the Chemistry Section was requested to develop a formulation which would release the compound into the atmosphere and maintain insecticidal concentrations over long periods of time. A number of formulations were developed which would release the DDVP vapors but due to the instability of DDVP and its sensitivity to moisture none of the conventional carriers were satisfactory for this purpose. In 1960, a stable formulation of DDVP in a wax base was discovered which was capable of a slow release of vapors over a long period of time. Prototype models of the DDVP dispenser were tested in experimental huts in Savannah and in villages in Africa. Later, field scale experiments were conducted in Africa and Haiti, and some 45,000 DDVP generators were produced at TDL and shipped to the field.

This project required not only research on the formulation of DDVP but also on analytical methodology in support of the chemical and biological phases of the work. Methods were developed for determination of DDVP in air at microgram levels and for macro determination of DDVP in vapor generators. Methods were also developed for determination of the breakdown products of DDVP which were useful in following the decomposition of the vaporizer units in storage.

14. Basic studies on water-dispersible powders

A study involving the effect of compaction on storage stability showed that compaction had an adverse effect on the storage stability of DDT powders. It appeared that the container was compacting the powder too severely and studies on container size were undertaken. Upon completion of these studies, a change in container volume was incorporated in the AID specification in 1966. This change again improved the quality of the products delivered to the field. Another factor, the surfactant system, was found to have an important influence on the suspensibility of DDT powders after long-term storage. Methods of analysis were devised which made it possible to accurately determine the surfactant content of a powder and to plot the rate of change with time in storage. A shelf-life requirement in the specification ensures that adequate surfactant is added during manufacture. The powders produced under the shelf-life requirement have been the best ever produced for the program.

15. Micro methods for pesticide analysis

In the course of investigations of new insecticides or new insecticidal formulations, the biologist frequently needs to know the concentration of the compound in question on the surface of a sprayed area or in treated water or air. Gas chromatography has proven to be a useful tool for this work and methods for determination of many pesticide residues have been developed. Among the most important are DDVP in air and water, Dursban and Abate in water, propoxur, DDT, dieldrin, and malathion on various surfaces. The method developed for propoxur has been used for biochemical research and also for determination of contamination of field samples of propoxur in El Salvador. A field test was developed for detection of DDT in propoxur powders. The TDL method for determining DDT in blood was used to assess the levels of DDT in blood of persons working in the malaria eradication program in El Salvador.

16. Macro methods of analysis

A continuing program of research on macro methods of analysis of pesticides has been in progress since 1956. Good analytical methods are essential for the development of sound specifications. When large quantities of products such as DDT powder are purchased on contract, the first consideration in method selection is accuracy; however, when thousands of samples must be run, some consideration must be given to the time required for analysis. In the case of DDT powders, the time required for analysis for DDT content and suspensibility has been reduced to less than half the time originally required as a result of improvements in methods made at TDL. This has resulted in large savings in cost of analysis by GSA. In the case of suspensibility analyses conducted at TDL on samples tested for compliance with the warranty clause of the specification, automated techniques have been developed which have reduced the time required for these tests by more than one-half.

In addition to development work on macro methods of analysis for DDT, research has also been conducted and improved methods have been developed for malathion and fenitrothion.

17. Biochemical studies on new compounds

The first biochemical studies undertaken under the AID contract were begun in FY 1963 when seven potential chemosterilants were evaluated by comparison of the rate of uptake of C^{14}-formate in insect testes as a measurement of chemosterilant activity. In subsequent investigations, techniques for chemosterilant evaluation were developed which were based on microscopic examination of insect gonads after treatment with the candidate compounds. After several years of work on the chemosterilant approach, emphasis was shifted from chemosterilants to other biologically active agents such as ovicides and larvicides because many of the chemosterilant compounds are alkylating agents and cannot be used without endangering higher forms of life. By FY 1968, biochemical studies had begun on relatively nontoxic compounds which affected the egg or larval forms of mosquitoes. Chemical work on larvicidal formulations progressed along two lines; namely, development of improved formulations of conventional pesticides and the search for new, biodegradable compounds. Some progress was made with slow-release formulations of methyl chlorpyrifos; however, the value of such formulations over existing granular

formulations is yet to be determined particularly in regard to malaria operations.

More than 50 new diamines have now been synthesized and tested against mosquito larvae in a variety of formulations. The most promising compound discovered has been didecyl putrescine. In certain oil formulations, it proved to be 1/25 as potent as Abate which is considered to be quite good in view of the sensitivity of mosquito larvae to Abate. Laboratory studies with combinations of didecyl putrescine and larvicidal oils showed that the addition of small amounts of the amine to the oil resulted in up to 95% reduction of the quantity of oil required for effective larval kill. Field testing of the more promising formulations has been recommended.

18. Detection of resistance to insecticides in adults and larvae

Certain components and procedures of the kit developed by TDL for use in detecting insecticide resistance were later adopted by WHO in developing the compact kit now in global use. TDL served as one of the two laboratories which conducted preliminary evaluation of procedures subsequently adopted by a WHO Expert Committee on Pesticides.

When a 50% decomposition in six months was found in the malathion larval test kits, TDL was able to reduce this to a 10% decomposition.

19. Laboratory and field assessment of new chemical formulations

a. Adulticides. New experimental compounds or formulations are received directly from industry or universities, or through the WHO Insecticide Evaluation Program, and each of these compounds or materials undergo a series of laboratory and simulated field tests to detect the materials of greatest promise as residual applications against anopheline vectors. More than 800 compounds have been tested at TDL.

Initially, these materials are tested in the laboratory at three dosage levels against resistant strains of *Anopheles quadrimaculatus*, *Anopheles albimanus* and/or *Anopheles stephensi*. Insecticide residues that provide satisfactory kills on the initial evaluation are reassessed at periodic intervals up to a maximum of 26 weeks. All tests are conducted with wettable powder formulations. During the process of formulation and spraying, observations are made on the characteristics of the compounds such as suspensibility, odor, etc.

Since deposits on plywood generally are much more effective against mosquitoes than those on painted or mud surfaces, any compound which does not perform satisfactorily in laboratory evaluation is discontinued. Materials that provide 12 or more weeks of satisfactory kills are then candidates for simulated field tests which are conducted out-of-doors under conditions of uncontrolled temperature and humidity. Two types of evaluation are employed -- the outdoor panel (clay, cement, plaster) test and the experimental hut test. The panel tests allow

several dosages to be evaluated concurrently and permit the comparison of various insecticides or formulations under the same fluctuating conditions of temperature and humidity. The experimental hut test is the final phase of simulated field aspect. For a promising compound, the evaluation period may be 1-2 years.

TDL conducted the initial field evaluations of malathion and fenthion as residual treatments against malaria vectors. The studies were conducted in El Salvador in cooperation with PAHO and Ministry of Health, El Salvador. Both compounds were subsequently evaluated further by WHO, and malathion is now the only organophosphorus compound accepted as safe and as effective in interrupting malaria transmission.

b. Larvicides. Testing compounds for use as larvicides involves exposure to serial concentrations of the insecticides to determine the concentration capable of producing 95% kill. Compounds that pass the laboratory criteria are then studied under field conditions using artificial ponds or small field plots.

Compounds tested as space sprays are applied as mist aerosols in test chambers and then promising materials are tested under simulated field conditions using standard, cold, or thermal fogging equipment.

Of nearly 100 compounds tested as larvicides between 1966-1972, ten showed some residual action and warranted further follow-up.

20. Toxicological evaluation of health hazards associated with potential use of new insecticides

In the process of testing new insecticides, new formulations, or new operational procedures, the assessment of mammalian toxicity is routine. If the toxicity is high, testing is discontinued.

21. Effectiveness of residual fumigant technique against malaria vectors

TDL discovered the residual fumigant effect of dichlorvos (DDVP) on mosquitoes and subsequently formulated the compound in a wax base. This formulation was then evaluated in mud huts in the Upper Volta, Africa, in cooperation with the Centre Muraz. Entomologically it proved highly effective for periods of three to five months, and from a toxicological viewpoint it could be used safely in occupied dwellings. Partial interruption of malaria was accomplished.

A special study of the dichlorvos residual fumigant was conducted in Haiti in cooperation with PAHO, AID, and the Government of Haiti. Approximately 5,000 houses were treated over a 2-year period at 3- to 4-month intervals. Treatment was biologically effective for three months. Although some suppressive effect on malaria transmission was noted, interruption was not achieved. Household treatment with DDT also failed to interrupt transmission, although

resistance was not present in the *A. albimanus* populations. Extradomiciliary transmission apparently was the factor that allowed continued transmission.

22. Alternative methods and supplementary techniques for mosquito control

Studies of insect pathogens indicated that *Anopheles* larvae frequently are infected with the fungus of the genus *Coelomomyces*. Field collections revealed five different species attacking *A. quadrimaculatus* larvae. Cooperative studies were established involving the University of North Carolina and Central America Malaria Research Station. Despite the initial promise of *Coelomomyces* as a fungal parasite of *Anopheles*, work was discontinued in FY 1971 on the cooperative investigation with the University of North Carolina of *Coelomomyces punctatus* as a pathogenic agent. Repeated attempts to locate natural infections and to culture this organism have been unsuccessful.

The fungal parasite, *Lagenidium culicidum*, has been cultured *in vivo* and procedures developed for mass rearing. Infections have been obtained of eight species of culicine mosquitoes with no loss of infectivity or pathogenicity between species. Determination also has been made of the effect of host age upon infection and induced mortality. Although initial attempts to infect *A. albimanus* with *L. culicidum* were unsuccessful, larvae of this species succumbed to the fungus when exposed to extremely high concentrations of zoospores. An extensive survey of the literature on protozoan parasites of mosquitoes has been completed. Onsite visits were made to Boyce Thompson Institute, New York; Entomology Research Institute for Biological Control, Canada; and Ohio State University, Ohio, to obtain detailed background on certain newly isolated fungal, nematode, and protozoan parasites of mosquitoes.

Poison baits have been developed primarily for controlling anopheline larvae by a method which would not be dependent on concentration (parts per million) for the applied dosage but on a volume per acre basis. Abate and fenthion were equally effective against larvae when applied as baits that contained 1/125 the amount of toxicant used in conventional applications. Assuming that subsequent field data support laboratory findings, the use of poison baits could result in a higher level of control with a lower amount of toxicant.

Techniques were developed for evaluating hormones against mosquito larvae. Among the synthetic hormones tested against four species of mosquito larvae, some compounds were highly effective at concentrations of .0025 ppm. Certain compounds were toxic to the larvae; one material stopped development at the prepupal stage. Preliminary field tests of three promising hormones have been conducted in El Salvador in cooperation with CAMRS. The compounds continued to show promise but densities of *A. albimanus* were not sufficient to provide conclusive data.

23. Colonization of malathion-resistant *A. albimanus*

Since resistance to an organophosphorus compound was first documented in a species of *Anopheles* in 1970, it was imperative that a colony of the malathion-resistant *A. albimanus* be established and that adults from it be included in the TDL insecticide evaluation system. Successful colonization was achieved during FY 1971.

24. Evaluation of ultra low volume applications

The ULV technique in which undiluted insecticides are applied from aircraft as larvicides and adulticides, at rates of 3-6 ounces per acre, is of interest as a supplementary treatment in malaria programs especially in epidemic situations, in areas of high population concentration, and where outdoor transmission is prevalent.

In November 1968 TDL participated in a WHO-sponsored ULV spray program in Ethiopia. In 1969 a surplus C-45 twin-engine Beechcraft Airplane was transferred to TDL. The TDL aircraft was equipped and tested for effective delivery of malathion as an ultra low volume (ULV) application. A cooperative study was arranged with the Government of Haiti and the Pan American Health Organization to evaluate the effects of malathion ULV treatments on malaria transmission in the highly endemic Miragoane area of Haiti. Preliminary tests completed in June 1972 indicated the treatments were highly effective against caged *A. albimanus*. Biting rates also declined, but it was not possible to determine conclusively that the decrease was due primarily to the spray applications. The large-scale trial is scheduled for the Fall of 1972.

25. Assessment of ground-applied ULV treatments

Field evaluations were conducted in 1971 to determine the relative efficacy of ground applied thermal fogs versus nonthermal ultra low volume (ULV) aerosols. Malathion was the insecticide used and three species of mosquitoes, including *A. albimanus* and *C. p. quinquefasciatus*, were the test insects used. Results indicated that at a vehicle speed of 5 mph, a flow rate of 3.9 fl. oz./min. gave 100% kills of caged *A. albimanus* at "open" sites up to 600 feet. At 10 mph, a flow rate of 5.6 fl. oz./min. produced mortalities of 96% to 100% to the same distance. At "open" sites, the effectiveness of the thermal fogs was equal to or superior to that of the ULV treatments. At wooded sites, the ULV treatments were less effective than the fog applications. However, the ULV method has several advantages over thermal fogs in that a fuel oil diluent is not required, the cost per treatment is less, and a greater coverage of an area can be obtained with the same amount of insecticide.

26. Effect of sublethal exposure of mosquito adults to residues

Certain compounds which do not inflict a high mortality on adults have been shown to exert an adverse influence on longevity, reproduction, and blood feeding.

27. Evaluation of physical and chemical attractants in surveillance and control of vector populations

Studies of the response of ultra violet light against four anopheline species indicated that it was highly effective in trapping *A. stephensi* and *A. albimanus* but not *A. sundaicus* and *A. quadrimaculatus*. Age had little effect on response of *A. albimanus* to UV light. This work led to the development of UV light trap.

Studies on the response of *Anopheles* to varying wavelengths of monochromatic light showed that *A. stephensi* exhibits significant preferences for certain

of the test colors. Light traps equipped with BLB-type fluorescent lamps, which peak in the region of 360 mμ, appear to have excellent potential for a surveillance tool with this species.

28. Feeding habits of anophelines

Attempts to isolate the factors involved in food selection by anopheline larvae have required much work on methodology. A colorimetric method for quantitative determination of ingestion rates has been developed with *A. albimanus* larvae. The influence of particle size on efficiency of ingestion has been demonstrated. These studies show a preference for particle sizes 88-105 microns. Preliminary tests incorporating methoxychlor on this size particle have given good kills demonstrating the possible application of this new approach to anopheline control. A method has been devised for differentiating between toxicity from contact and from ingestion through the use of anesthetized larvae.

29. Influence of light, motion, temperature, and other factors on the behavior of anopheline larvae

Studies on the response of *Anopheles* larvae to environmental stimuli have led to the development of the prototype of a larval trap. This trap utilizes the behavioral response of larvae to seek the intersection line where an object breaks the water surface. Many of the factors influencing the efficiency of the early model traps have been evaluated.

30. Improved methods of measuring mosquito densities

The TDL-developed UV light trap designed and tested in the dark chambers has demonstrated that the capture of mosquitoes by means of a fan-generated updraft is superior to the conventional downdraft type trap. Tests have been run with both *A. albimanus* and *A. stephensi*. The initial tests have disclosed that high mortalities were sustained after one hour when tests were carried out under standard test conditions (i.e., 70° F. temperature, 80% humidity). By reducing air flow rates, it was possible to lower the mortalities without affecting the capturing efficiency. The updraft UV light trap was demonstrated to be highly effective in capturing *A. albimanus* under field conditions in Haiti.

E. Dissemination and Utilization of Research Results

Information derived from this project is disseminated by publication, presentation of findings at meetings and conferences, visits to TDL of personnel from national governments, AID missions, etc., correspondence with AID missions, field visits of TDL personnel to operational areas, and by direct contacts with WHO, PAHO, and national governments.

Pertinent research findings are presented at meetings of scientific societies each year. The data likewise are documented in the various scientific journals such as the Bulletin of the World Health Organization, American Journal of Tropical Medicine and Hygiene, World Health Organization releases in the WHO/MAL and WHO/VBC series, etc. A list of the publications which include data obtained in part or wholly from AID-sponsored research is attached

(Appendix H.3). Reprints of these articles are distributed to AID and to personnel throughout the world. TDL maintains a mailing list of approximately 1,000 names, many of which represent health agencies, scientists, research laboratories, and libraries in foreign countries.

Annually, a report on the work accomplished under the AID PASA is submitted to AID-Washington and in turn is transmitted to AID missions.

Staff members act as consultants to WHO and PAHO and serve as members of the Expert Panel on Insecticides. At the Expert Committee Meeting there is a ready exchange of information on research at TDL between TDL participants and representatives from the various governments, as well as the WHO or PAHO secretariat. Working papers which have been prepared by TDL staff members for WHO meetings are listed in Appendix H.4.

One of the chief means of exchanging information is TDL's role as WHO Reference Center on Insecticide Evaluations. At the annual meeting of laboratory directors in Geneva there is a pooling of technical data on research on vector control, the majority of which relates to the world-wide malaria eradication program.

Another feedback of technical data is through correspondence with AID missions, AID-W, WHO, and national governments, or by personal contacts between persons from these agencies and the TDL staff either at Savannah or on field visits to project areas.

F. Statement of Expenditures and Obligations and Contractor Resources

Fiscal Year	Main PASA	Amendment to PASA	Supplemental PASA
1956	20,000		
1957	50,000		
1958	35,000		
1959	90,000		
1960	140,000		
1961	155,000		
1962	235,000		30,000
1963	235,000		100,000
1964	285,000		70,000
1965	285,000		
1966	356,000		
1967	420,000		
1968	480,000		
1969	504,268		
1970	607,026	34,000	
1971	610,000		
1972	569,500		

FY-1972 AID Expenditures

Personnel Costs	$471,070
Travel & Transportation	207
Contractual Services (Central Payroll)	1,909
Supplies & Materials	1,226
Equipment	171
Overhead	94,917
	$569,500

G. Work Plan and Budget Forecast for Coming Year

Not applicable.

H. Appendices

1. Activities Report, July 1, 1971-June 30, 1972.

2. Report of Activities for the year ending June 30, 1969.
   Report of Activities for the year ending June 30, 1970.
   Report of Activities for the year ending June 30, 1971.
   Available on request

3. Publications and Presentations.

4. Working Papers Prepared by TDL Staff for WHO Meetings.

5. AID Specifications for DDT Water-dispersible Powder, 1954-70.

6. Specifications for Pesticides other than DDT.

APPENDIX 1

Activities Report, July 1, 1971-June 30, 1972

ACTIVITIES REPORT

JULY 1,1971 thru JUNE 30,1972

TECHNICAL DEVELOPMENT LABORATORY

under Participating Agency Service Agreement Number RA(HM) 7-00 (Amend. No. 13)
PIO/T Number 931-17-511-033-72-3127700 with U.S. Agency for International Development

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
Health Services and Mental Health Administration
Center for Disease Control
Atlanta, Georgia 30333

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INTRODUCTION

The Technical Development Laboratories were established in Savannah, Georgia in 1943. The current staff consists of 24 professional scientists in the biological, chemical, and engineering categories with 56 persons at the supportive level. The primary function of the Laboratories is to conduct investigations on the control of arthropod- and rodent-borne diseases. Studies involve disease vectors such as mosquitoes, flies, fleas, and triatomids as well as rodents. 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 rodenticides.

Although research is conducted along the various professional lines, an outstanding quality of the station is its ability to apply a team approach to most of its investigations. 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.

The Laboratories have been designated as a Reference Center of the World Health Organization for the evaluation and testing of new insecticides.

RESEARCH ON METHODS AND MATERIALS FOR IMPROVING THE RESIDUAL TREATMENT OF HOUSES

Insecticide Studies*

Evaluation of new insecticides and/or formulations

Seven compounds were tested on plywood panels against three species of adult mosquitoes - dieldrin-resistant *Anopheles albimanus*, chlorinated hydrocarbon resistant *Anopheles quadrimaculatus*, and *Culex pipiens quinquefasciatus*. Standard procedures were used wherein 150 3-day-old adult mosquitoes were exposed for one hour to treated panels. Mortalities were determined at 24 hours. Evaluation ceased at 26 weeks or whenever two consecutive tests failed to produce greater than 70% mortality. Only one of the seven compounds (OMS-1558) failed to meet criteria for further tests (Table 2). The BAY compounds more than met criteria for further evaluation; but, unfortunately, these compounds were recently reported to be highly irritating to mucous membranes and subsequently were dropped by the manufacturer. Mobam also was dropped by its company because of limited marketing prospects. An eighth candidate compound, OMS-1483, was not tested because of its relatively high toxicity to rats (acute oral LD-50 on female rats, 50 mg./kg.) and its poor sticking quality as a residue. Only two compounds, BASF 2351 and OMS-1349, remained for further evaluation.

Comparisons were made of the efficacy of methoxychlor and malathion at two dosages against the Tecomatal and Melara strains of *A. albimanus*. The former is primarily resistant to dieldrin, while the Melara strain is resistant to both DDT and dieldrin and to some extent malathion. The data for the two strains (Table 3) indicated that the Melara strain of *A. albimanus* is somewhat more tolerant to both compounds. However, methoxychlor (1 g/m^2) gave mortalities above 90% against the Melara strain for 26 weeks except for the first four weeks. Against the same strain, malathion (1 g/m^2) yielded kills of 83% to 100% for 16 weeks.

OMS-1424 (methyl pirimiphos) was evaluated on outdoor panels. Three different surface materials (clay, plywood, and cement plaster) were treated with suspensions of the compound at 1 and 2 g/m^2. The panels (1' x 4') of the structural surfaces were treated and held under a peaked roof, outdoor shed (120' long). Each panel was 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 virgin *A. albimanus* females to the treated surfaces for 60 minutes under plastic cones. All specimens were held 24 hours for mortality counts. Results of the outdoor panel tests are shown in Table 4.

Results through the first five weeks indicate that OMS-1424 was effective on cement plaster and plywood against *A. quadrimaculatus* and *A. albimanus* at both dosages. However, on clay the 1 g/m^2 treatment was ineffective against *A. quadrimaculatus* but gave 96% kills of *A. albimanus*. At 2 g/m^2, OMS-1424 was effective on all surfaces against both species for five weeks, and was effective at both 1 and 2 g/m^2 for ten weeks on plywood.

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

TABLE 1
Compounds Tested, FY-1972

Name	Chemical Composition
BASF 2351	3,5-diethyl-phenyl-N-methyl-carbamate
BAY KUE 2302	Confidential
BAY KUE 2327	Confidential
BAY MEB 6046	Confidential
BAY ZUM 0673	Confidential
BAY ZUM 0676	Confidential
BAY ZUM 0677	Confidential
chlorpyrifos, methyl	O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate
DDT	dichloro diphenyl trichloroethane
DDT analog	DDT:CH_3:OC_2H_5
DDT analog	DDT:CH_3S:OCH_3
dieldrin	not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene
fenthion	O,O-dimethyl O-[4-(methylthio)-m-tolyl] phosphorothioate
GC-9150	ethyl 1,1a,3,3a,4,5,5,5a,5b,6-decachloroocta-hydro-2-hydroxy-1,3,4-metheno-1H-cyclobutal [cd]pentalene-2-levulinate
malathion	diethyl mercaptosuccinate S-ester with O,O-dimethyl phosphorodithioate
methoxychlor	1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane
Mobam	Benzo [b] thien-4-yl methylcarbamate (also 4-benzothienyl-N-methylcarbamate or 4-benzo-thienyl methylcarbamate)

(continued)

TABLE 1 (continued)

Name	Chemical Composition
NIA 24110	(5-benzyl-3-furyl)methyl 1R,2R-2-[(cyclopentylidene)methyl]-3,3-dimethylcyclopropanecarboxylate
OMS-1213	Confidential
OMS-1349	Confidential
OMS-1358	Confidential
OMS-1446	Confidential
OMS-1447	Confidential
OMS-1480	Confidential
OMS-1483	Confidential
OMS-1501	Confidential
OMS-1502	Confidential
OMS-1538	Confidential
OMS-1539	Confidential
OMS-1558	Confidential
OMS-1800	Confidential
Ortho 11775	3-(2-butyl) phenyl-N-methyl-N-(Phenylsulfenyl) carbamate
pirimiphos, methyl	2-Diethylamino-6-methylpyrimidin-4-yl dimethyl phosphorothionate
S-15053	Confidential

TABLE 2
Effectiveness of Experimental Insecticide Residues on Plywood Panels Against *A. quadrimaculatus*, *A. albimanus*, and *C. quinquefasciatus*

Insecticide	Dosage g/m^2	Weeks of 70% Kill		
		A. quad.	*A. alb.*	*C. quin.*
BASF 2351	0.5	2	6	2
	1.0	10	14	10
	2.0	22	>26	>26
BAY ZUM 0673	0.5	10	22	14
	1.0	>26	>26	>26
	2.0	>26	>26	>26
BAY ZUM 0676	0.5	6	14	10
	1.0	18	22	22
	2.0	>26	>26	>26
BAY ZUM 0677	0.5	2	18	10
	1.0	10	>26	>26
	2.0	>26	>26	>26
Mobam*	0.5	>22	>22	>22
	1.0	>22	>22	>22
	2.0	>22	>22	>22
OMS-1349	0.5	2	6	2
	1.0	6	>26	6
	2.0	>26	>26	>26
OMS-1558**	0.5	<2	<6	2
	1.0	<2	<6	2
	2.0	<2	10	10

* Dropped by Company. Not tested beyond 22 weeks.
** Still in progress.

TABLE 3
Effectiveness of Methoxychlor and Malathion Deposits on Plywood Panels Against Two Strains of *A. albimanus*, 60-minute Exposure

Age of panels (wk.)	*Anopheles albimanus*-Tecomatal*					*Anopheles albimanus*-Melara (El Salvador)**				
	Methoxychlor		Malathion			Methoxychlor		Malathion		
	50 mg/ft²	100 mg/ft²	50 mg/ft²	100 mg/ft²	Check	50 mg/ft²	100 mg/ft²	50 mg/ft²	100 mg/ft²	Check
2	85	100	100	100	14	69	83	74	96	1
4	88	100	100	100	5	45	76	72	97	1
6	85	100	98	100	3	-	95	65	100	1
8	60	98	50	100	2		98	56	96	1
10	-	100	-	100	1		96	-	86	1
12		100		100	8		91		86	1
14		100		100	2		98		83	0
16		100		100	1		94		90	1
18		100		94	1		98		68	1
20		100		92	1		98		59	<1
22		100		79	1		100		-	<1
24		99		71	3		98			5
26		99		48	<1		96			1

* Resistant to dieldrin.

** Resistant to dieldrin, DDT and malathion.

TABLE 4
Effectiveness of Residues of OMS-1424 on Outdoor Panels Against
Anopheles quadrimaculatus and *Anopheles albimanus*

		Weeks 70% and 90% Kill					
		Clay		Cement		Plywood	
Species	g/m²	70%	90%	70%	90%	70%	90%
A. quadrimaculatus	1	2	2	5	5	10	10
	2	5	2	5	5	10	10
A. albimanus	1	5	5	5	5	10	10
	2	5	5	5	5	10	10

OMS-1424 also was applied to the walls and ceilings of 6' x 6' experimental huts in which the ceilings and doors are plywood while the walls are lined with pressed clay blocks approximately 2" thick. The door (30" wide) opens onto a screened porch 30" wide extending across one side. A 2" opening is provided between the top of the front and back walls to give additional ventilation. Suspensions of OMS-1424 were applied by means of conventional hand sprayer equipped with an 8002 nozzle. The dosages were 1 and 2 g/m². Effectiveness was measured periodically by releasing 50 (3-day-old) female *A. albimanus* and *A. quadrimaculatus* (on different nights) inside the hut at sunset with the door to the hut open. After an overnight exposure of 12 hours, the mosquitoes were collected from porch and hut and held for 24-hour mortality determination. Cages of mosquitoes of both species were suspended inside the huts for the same 12-hour period to determine if the compound had any vapor toxicity. Approach of the winter season precluded evaluation beyond 11 weeks. Results given in Table 5 indicate that OMS-1424 shows promise both from the residual and fumigant aspects.

TABLE 5
Effectiveness of OMS-1424 against Caged and Free-flying
Anopheles quadrimaculatus and *Anopheles albimanus*
in Experimental Huts

		Weeks 70% Kill	
Species	g/m²	Caged	Free-flying
A. quadrimaculatus	1	>11	>11
	2	>11	>11
A. albimanus	1	>9	10
	2	>11	>11

Investigations on pesticide formulations and specifications

Studies on the chemistry and formulation of propoxur

Determination of propoxur in formulations: The carbamate insecticide, propoxur, is one of the more promising substitutes for DDT in malaria control. Work was begun on determining the stability of propoxur formulations and predicting critical factors in field usage in spray programs. Proposed methods of analysis were reviewed and tested to establish suitable procedures for testing propoxur formulations. The distillation method of Interim Specification WHO/IS/2.0033-4 (June 1970) was modified and satisfactory results were obtained. The method was shortened by omission of a preliminary acid digestion and substitution of vigorous stirring to improve physical contact between 40% NaOH and the insoluble propoxur. Also, the titration end point was easier to determine by back titration of a standard acid and omission of the proposed boric acid. Bracha[1] reported a colorimetric method for micro amounts of propoxur and this has been modified for the analysis of formulations.

The domestic supplier of propoxur, Chemagro Corporation, uses an infrared method for analysis of formulations which is similar to the official AOAC[2] method for carbaryl, another carbamate. This method was used to analyze four formulations repetitively and the standard deviation of results ranged from 0.7% to 2.5%. Any impurities present with similar infrared absorbencies would interfere.

Another colorimetric procedure studied involved the formulation of a color complex with aminoantipyrine[3]. The color measurement did not obey Beer's Law due to a solvent effect observed when aliquoting large volumes of standard for calibration.

This deviation was found to be due to addition of increasing volumes of methanol with large samples and linear response was obtained by limiting the amount of methanol present to 20% V. The method was found to be somewhat less reliable than the colorimetric Bracha[1] method.

In the colorimetric methods, propoxur is first cleaved to O-isopropoxyphenol with alkali which is subsequently reacted with the color forming reagent for 10 to 20 minutes[1,3]. The cleavage was found to take place in less than one minute at room temperature, so the procedure was shortened to five minutes for routine use.

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[1] Bracha, P., J. Agr. Food Chem., 12:461-463 (1964).

[2] Official Methods of Analysis of the Association of Analytical Chemists, Wm. Horwitz, Ed., 11th Ed., Section 6.206-8.

[3] Niessen, H. and Frehse, H., "Colorimetric Method for Determining Residues of the Insecticide Unden in Plant Material," Pflanzenschutz Nachrichten, "Bayer," 17:25-32 (1964).

A study of the color development step indicated maximum color was present after two minutes, so the specified 20 minutes was shortened for an additional time saving.

The primary decomposition product of propoxur in storage is O-isopropoxyphenol which is also the primary cleavage product. Attempts to measure the decomposition of formulations without interference from alkali cleavage were unsuccessful. Partial success was obtained with the aminoantipyrine method by adding the color reagent prior to triethanol amine, but this was not pursued after a chromatographic separation method was developed.

Separation of propoxur from its phenol was accomplished on a micro silica gel column of Kadoum[4]. Thin layer chromatography was used to establish the separation conditions. With a 1-inch silica gel column 10 to 50 μg. of phenol could be eluted with 4 ml. of benzene from 5 mg. of propoxur. The propoxur was quantitatively held and finally eluted with 5 ml. of benzene containing 20% ethyl acetate. Both the phenol and propoxur were then determined on the separated fractions.

Studies on the formulation of propoxur water-dispersible powders: Formulations of propoxur water-dispersible powders have been prepared and placed in storage to determine stability plus developing accelerated tests to predict long-term field performance. As part of the accelerated test study, samples were maintained at 45° C. for three months and analyzed for suspensibility and decomposition. The results are tabulated in Table 6. Compaction caused a significant reduction in suspensibility, whereas only a slight reduction was observed with uncompacted samples. The decomposition measured was insignificant.

TABLE 6
Laboratory Formulations of Propoxur after Three Months at 45° C

Sample Number	Suspensibility, %			Decomposition,2** %
	As Prepared	Uncompacted	Compacted	
101*	100	98	100	0.09
103*	87	85	60	0.01
105*	100	96	87	0.02
107	94	80	69	0.4
109	94	81	77	0.5
111	90	72	54	0.01

* Air milled and minimum compacted volume of 6 g./10 ml. (cf. 10 g./10 ml.)
** As free O-isopropoxyphenol on compacted samples.

4 Kadoum, A.M., Bull. Environ. Contamination and Toxicology, 3(2):65-70 (1968).

Several propoxur water-dispersible powder samples have been received from the Central America Malaria Research Station from batches used in spray programs at different times during the past two years. The content, suspensibility, and decomposition are tabulated in Table 7. The suspensibility of two samples received in 1971 were 33% and 39%, and are unsatisfactory for spraying. A recent sample completely flocked, and there was clearing in the top of the suspension. When 1% of a surfactant, Igepon T-77, was added to the powder, a suspensibility of 53% was obtained.

TABLE 7
Analysis of Propoxur Water-dispersible Powder from CAMRS

Sample Number	Date Received	Percent		
		Propoxur	Suspensibility	Decomposition*
TDL 9526a	Jun 70	50.1	--	0.01
TDL 9526b	Jun 71	83.7	--	0.02
TDL 9952a	Jun 71	74.3	88	0.02
TDL 9952b	Jun 71	54.8	33	0.03
TDL $\overline{X}$50	Sep 71	53.1	39	0.04
CAMRS 17342	May 72	48.4	70	--
CAMRS 67	May 72	46.5	66	--
CAMRS 68	May 72	45.5	82	--
CAMRS 69	May 72	45.1	flocked	--

* As free O-isopropoxyphenol.

The amount of decomposition present was insignificant. It was concluded that decomposition will not be a problem in the long-term storage of propoxur powders.

Studies on malathion formulations

Development of analytical methods: As a result of the loss of a patent position on malathion by the American Cyanamid Company, a number of foreign chemical manufacturers began to produce the compound for export to the United States as well as other countries. Analytical methodology which had served very well during the period when Cyanamid was the sole manufacturer of the product was not adequate for analysis of new lower quality products which reached the market. In view of the importance of malathion to health programs, every effort was made to improve the methodology so that meaningful specifications could be written which would rule out inferior quality products. The Cyanamid Company, which was also aware of the problem, initiated their own research program which was addressed to the problem of analytical methodology. American Cyanamid concentrated its efforts on a nonaqueous copper colorimetric method, while TDL pursued a silver titrametric procedure. Both methods were presented to the scientific community through the Association of Analytical Chemists, and much interest has been generated by the introduction of the new methodology. An international collaborative trial of

both methods is now in progress and one or the other will eventually be adopted as the official method. Several recent refinements of the silver titrametric method are described below.

A method was developed for removal of free dimethylphosphorodithioic acid from malathion formulations prior to the determination of malathion content. Since the determination is based upon conversion to the acid for measurement, any free acid initially present would interfere leading to high results. Removal is accomplished by percolation of a hexane solution of the malathion through a column of moistened cellulose.

A study was made of the effect of diethylmercaptosuccinate on the determination of malathion. Complete recoveries of malathion were obtained by the argentimetric method. With the nonaqueous copper colorimetric method, low and erratic results were obtained with mercaptosuccinate present in a concentration equivalent to malathion which was 14% decomposed.

Results of analysis of technical malathion by the two new methods were compared with those obtained by gas chromatography. Results by a gas chromatographic method, considered the most accurate with the lowest precision, were approximately 2% lower than by the argentimetric or anhydrous copper colorimetric methods. For multiple analyses of seven technical malathion samples, the range of standard deviations for each method was as follows: Argentimetric (automatic derivative mode) 0.51-0.14; argentimetric (manual mode) 0.55-0.21; and the nonaqueous copper colorimetric method 0.84-0.40.

A Metrohm Model 436 differential titrator was placed into operation for the argentimetric analyses of malathion after it was determined that an older Metrohm Model 336 had inadequate sensitivity to properly follow the differential titration.

The alkaline cleavage of malathion was studied in several solvent systems, and comparable results on high and low quality malathion. Complete cleavage was obtained in one minute with all of the solvent systems. A study was made of extractants for malathion from water-dispersible powder formulations. Hexane was the most suitable of the solvents tested, as complete malathion recoveries were obtained with a minimum of interference. Polar solvents such as acetone, ethanol, and acetonitrile extract polar impurities which interfere with the determination.

Preliminary collaborative trial of the argentimetric method for water-dispersible powders: The argentimetric method was tested collaboratively by General Services Administration, PPG Industries, and TDL. Three water-dispersible malathion formulations were prepared and a portion of each was heated for accelerated storage treatment by the procedure outlined in WHO/SIF/10.R2, paragraph 2.4, making a total of six samples for analyses. The results of the study are tabulated in Table 8, and the agreement is quite good. The two laboratories had no difficulty with the method even though they had no previous experience with it.

TABLE 8
Malathion, Water-dispersible Powder Collaborative
Analysis by Argentimetric Method[1]

Sample Number and (original composition)	Malathion, %		
	PPG[2]	GSA[3]	TDL[4]
263 (44.8)	43.36	43.4	42.4
	43.53	43.6	43.3
		43.5	
		43.7	
		43.7	
	43.4	43.6	42.8
264[5] (36.2)	35.38	35.2	35.2
	35.45	35.20	35.19
		35.16	
	35.4	35.2	35.2
265 (40.3)	39.14	39.55	38.92
	39.20	39.42	39.02
	39.2	39.5	39.0
266[5] (32.5)	31.52	31.88	31.87
	31.85	31.81	32.20
	31.50		
	31.7	31.9	32.0
267 (50.6)	49.07	49.28	49.06
	49.15	49.27	49.26
	49.1	49.3	49.2
268[5] (44.4)	42.90	43.43	42.91[6]
	43.14	43.28	43.29
			43.96[6]
			43.34
	43.0	43.4	43.4

1 Miles, Goette, Guerrant, and Churchill, JAOAC, July or September 1972. Prepared and sampled February 17, 1972; analyzed April 19 through 26, 1972. To end point 450 mv.

2 Agricultural Chemicals Formulations, PPG Industries, Barberton, O.

3 General Services Administration, Quality Control Division, San Francisco, California.

4 Technical Development Laboratories, Savannah, Georgia.

5 Preceding sample heated according to WHO/SIF/10.R2, para. 2.4.

6 Sampled without mixing, deviation possibly from inhomogeneity.

Establishment of the end point potential in the argentimetric titration: A number of titrations have been made on various malathion concentrates and some slight variations have been observed in the potential at which the end point inflection occurs in the manual argentimetric titration. Most of the observations have been made using a Beckman zeromatic pH meter and a Metrohm Model E-436 automatic titrator which gave comparable results. The end point potentials have been found to vary with purity in a pattern as tabulated in Table 9.

TABLE 9
Malathion End Point Potential in the Argentimetric Titration of Various Malathion Concentrates

Sample Type	Inflection, mv
0,0'-dimethylphosphorodithioic acid potassium salt	400
Technical LVC (new)	400
Technical (old)	415-430
Water-dispersible powder	400-450
Technical, foreign	435-448
Technical plus mercaptosuccinate	450

A highly purified potassium salt of 0,0'-dimethylphosphorodithioic acid and also technical malathion have a large potential break at the end point with the inflection at 400 mv. The addition of impurities shortens the potential break and the inflection occurs at higher millivoltages. For greatest accuracy, the end point inflection can be determined for the type of sample being analyzed. When a wide variety of sample types are being analyzed, it is desirable to have an end point that can be used with minimum error without the necessity of determination of the inflection. In previous work, 450 mv. was selected as the end point from observations made on a foreign malathion, and also technical mixed with mercaptosuccinate, the decomposition product. Also the difference observed on a series of fresh technical malathion was only 0.06% between 400 and 450 mv. end points. Subsequent results have indicated greater differences, such as the average relative error on one-year-old water-dispersible powder was 0.7% in the presence of decomposition products.

The determination of end points on technical malathion in field use and some recent water-dispersible powders established an end point compromise at 425 mv. Results on badly decomposed and poor quality foreign products can be high by as much as 0.5%, but better results will be obtained on most samples.

Studies on storage stability of malathion formulations: One-year storage stability tests were completed on six malathion 50% water-dispersible powders. The results are tabulated in Table 10, along with the analyses performed on the samples as received one year previously. The original samples were subjected to heating at 90° C. for 20 hours as an accelerated storage treatment (WHO/SIF/10.R2) as a prediction of the shelf-life storage after one

year. In a previous study (VBC/EC 19/71.15), good correlation was obtained of the malathion content on the accelerated-storage-treated samples and after-shelf storage. The results of this study show poor correlation as the shelf-life samples are lower in malathion content than initially found by the accelerated storage test.

TABLE 10
Analysis of Malathion, 50% Water-dispersible powder

Sample Number, TDL	Malathion, %			Suspensibility, %		
	As rec'd.	20 hr.* at 90° C	1 year storage	As rec'd	20 hr.* at 90° C	1 year storage
9811	51.8	50.4	46.2	81	82	82
9812	52.6	49.9	46.7	79	80	80
9813	52.3	50.4	47.1	78	80	80
9814	52.4	50.4	47.6	81	80	80
9815	51.8	49.2	45.9	71	73	73
9816	51.1	50.2	45.6	72	66	72

* By WHO/SIF/10.R2, paragraph 2.4.

One factor was different in the two tests; i.e., the samples in the tests (Table 10) were freshly prepared when tested, while those of the previous study had aged two to six months prior to testing. Another indication that freshly prepared formulations show a more rapid decline in concentration was observed in the powders prepared for collaborative analysis by the argentimetric method as presented in Table 10. The average decrease in concentration in the first two months was 2.7% relative.

Further evidence of an initial rapid decline in concentration was observed in production samples formulated for UNICEF. The powders were purchased as 50% powders, and the contents when analyzed four months after manufacture ranged from 45.3% to 48.5% malathion, as reported in VBC/EC 19/71.15.

A similar observation has been made on a 50% Cythion water-dispersible powder (Accession No. $\bar{X}$ 109) which contained 48.1% malathion two months after receipt of sample.

Surveillance of DDT powders held in storage

During the fiscal year 1972, 1,388 samples of DDT, 75% water-dispersible powder, representing 30,006,825 pounds, were held in surveillance. Of this number, 463 samples were tested without failures. Additional tests were made on stored 90% p,p' DDT powder, and formulations of DDT pasta samples for U.S.A.I.D. Brazil.

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TABLE 11
Gasket Materials in Malathion Soaking Test (24 Hr)

Material	TDL Acc. No.	Dimensions, in Flat	Dimensions, in Edge	Weight, g	Durometer
Nozzle Assembly	9879-A	.050**	.095	0.1011	96
		.050***	.095	0.1003 -.0008	96
Shut-Off Packing	9879-B	.125	.095	0.3447	80
		.131 +.006	.100 +.005	0.3751 +.0304	73 -7
Cylinder Gasket	9879-C	.137	.192	3.3101	75
		.140 +.003	.177 -.015	2.7540 -.5561	85 +10
Cover Gasket	9879-D	.250	.190	12.5542	62
		.243 -.007	.182 -.008	11.2968 - 1.2574	74 +12
Hose Connector	9879-E*	.070	1/2	0.1180	68
		.073 +.003	1/2	0.1302 +.0122	69 +1
Winged Valve Body Cap	9879-F*	.100	5/8	0.2986	71
		.104 +.004	5/8	0.3170 +.0184	73 +2
Strainer Assembly	9879-G*	.095	11/16	0.3182	72
		.100 +.005	11/16	0.3302 +.0120	78 +6
Supply Tube	9879-H*	.100	13/16	0.3998	69
		.100	13/16	0.4160 +.0162	69
Winged Valve Body Cap	9879-I*	.067	3/8	0.1112	65
		.070 +.003	3/8	0.1132 +.0020	64 -1
Extension Assembly	9879-J*	.100	1/2	0.2289	71
		.103 +.003	1/2	0.2433 +.0144	71
Check Valve Assembly	9879-K*	.067	1/4	0.0690	64
		.069 +.002	1/4	0.0722 +.0032	70 +6

TABLE 11 (cont'd.)
Gasket Materials in Malathion Soaking Test (24 Hr)

Material	TDL Acc. No.	Dimensions, in Flat	Dimensions, in Edge	Weight, g	Durometer
Crude Rubber	9880-A	.160	.288	8.3836	66
		.165 +.005	.288	8.9238 +.5402	71 +5
GRS (Butadiene Styrene)	9880-B	.200	.260	7.0972	71
		.186 -.014	.245 -.015	6.1645 -.9327	84 +13
Ethylene Propylene	9880-C	.054	.135	0.2806	66
		.054	.125 -.010	0.2706 -.0100	68 +2
Hypalon	9880-D*	.140	2-9/16	3.1473	67
		.142 +.002	2-9/16	3.2772 +.1299	65 -2
Natural Rubber	9880-E	.120	.125	1.0875	57
		.126 +.006	.123 -.002	1.1206 +.0331	58 +1
Styrene Butadiene	9880-F	.127	.220	4.8155	62
		.121 -.006	.200 -.020	4.0773 -.7382	79 +17
Viton	9880-G*	.210	2-1/8	6.6004	66
		.210	2-1/8	6.6712 +.0708	70 +4
Viton	9880-H*	.135	2-1/2	3.4473	65
		.138 +.003	2-1/2	3.5267 +.0794	64 -1
Ethylene Propylene	9880-I*	.135	2-1/2	2.2750	73
		.124 -.011	2-1/4 -1/4	1.8058 -.4692	92 +19

* O-Ring.
** Before soaking.
*** After soaking.

TABLE 11a (cont'd)
Gasket Materials in Malathion Soaking Test (1 week)

Material	TDL Acc. No.	Dimensions, in Flat	Dimensions, in Edge	Weight, g	Durometer
Crude Rubber	9880-A	.156	.291	8.6560	65
		.150 +.006	.287 +.004	8.3836 +.2724	66 +1
GRS (Butadiene Styrene rubber)	9880-B	.200	.257	7.2795	71
		.200	.257	7.0972 +.1823	71
Ethylene Propylene	9880-C	.054	.126	0.2925	66
		.054	.126	0.2806 +.0119	66
Hypalon	9880-D*	2-9/16	.140	3.3102	66
		2-9/16	.137 +.003	3.1473 +.1629	67 +1
Natural Rubber	9880-E	.120	.125	1.2144	56
		.120	.125	1.0875 +.1269	57 +1
Styrene Butadiene	9880-F	.125	.218	4.8627	65
		.125	.215 +.003	4.8155 +.0472	62 -3
Viton	9880-G*	2-1/8	.208	6.7761	71
		2-1/8	.205 +.003	6.6004 +.1757	66 -4
Viton	9880-H*	2-1/2	.140	3.5821	66
		2-1/2	.135 +.005	3.4473 +.1348	65 -1
Ethylene Propylene	9880-I*	2-3/8	.130	2.1432	81
		2-1/2 -1/8	.135 -.005	2.2750 -.1318	73 -8

* O-Ring.
** Before soaking.
*** After soaking.
**** Outside diameter.

TABLE 11a
Gasket Materials in Malathion Soaking Test (1 week)

Material	TDL Acc. No.	Dimensions, in Flat	Dimensions, in Edge	Weight, g	Durometer
Nozzle Assembly	9879-A	.050**	.090	0.1063	95
		.050***	.090	0.1011 +.0052	96 +1
Shut-Off Packing	9879-B	.135	.430****	0.4246	72
		.125 +.010	.405 +.025	0.3447 +.0799	80 +8
Cylinder Gasket	9879-C	.145	.215	4.2511	65
		.137 +.008	.195 +.020	3.3101 +.9410	75 +10
Cover Gasket	9879-D	.255	.200	14.6105	60
		.250 +.005	.185 +.015	12.5542 +2.0563	62 +2
Hose Connector	9879-E*	5/8	.085	0.1925	61
		1/2 +1/8	.070 +.015	0.1180 +.0745	68 +7
Winged Valve Body Cap	9879-F*	11/16	.110	0.4272	65
		5/8 +1/16	.100 +.010	0.2986 +.1286	71 +6
Strainer Assembly	9879-G*	13/16	.105	0.4790	61
		11/16 +1/8	.095 +.010	0.3182 +.1608	72 +11
Supply Tube	9879-H*	1"	.120	0.6695	57
		13/16 +3/16	.100 +.020	0.3998 +.2697	69 +12
Winged Valve Body Cap	9879-I*	3/8	.069	0.1247	68
		3/8	.066 +.003	0.1112 +.0135	65 -3
Extension Assembly	9879-J*	9/16	.115	0.3475	61
		1/2 +1/16	.100 +.015	0.2289 +.1187	71 -10
Check Valve Assembly	9879-K*	1/4	.072	0.0797	71
		1/4	.066 +.006	0.0690 +.0107	64 +7

Equipment Investigations

Compatibility of gaskets and other nonmetallic components with various insecticides

Because of the increasing use of insecticides other than DDT in malaria control programs, TDL began a study to determine compatibility of various components of the hand compression sprayer with some of these insecticides. Both presently used materials and potential replacements for them were tested. A baseline study was first run by soaking samples of the materials in xylene for varying periods of time. Weight and dimensions before and after testing were used as evaluation criteria. This was followed by a more extensive test using malathion (Table 11) and a limited test with propoxur. In general, the materials presently used in the sprayer were found to be satisfactory, although most were affected to some degree, especially by the malathion.

Evaluation of modified sprayer components

The H.D. Hudson Manufacturing Company, in collaboration with TDL, has developed several improvements to the sprayer used in U.S.A.I.D. malaria programs. Certain of these items have been tested in the laboratory and, after proving desirable, have been subsequently incorporated into a current field evaluation (see below). These include a much improved pressure gauge which is completely waterproof and rustproof, an all plastic pump plunger assembly which is of simpler design than the present metal assembly, and an all plastic strainer.

Field trial of modified sprayers

Phase II of the collaborative project with PAHO, H.D. Hudson Manufacturing Company, and the SNEM of Nicaragua was resumed and was scheduled for completion in the summer of 1972. This project was begun in FY-1970 to evaluate a number of modifications made to the sprayer in response to suggestions received in a worldwide survey by WHO. Phase I was carried out in Nicaragua and El Salvador during FY-1970. Following evaluation of the results, a number of further improvements and modifications were made and the newly modified sprayers were returned to the field for further testing. The new items included: a plastic discharge hose, plastic pump plunger assembly, plastic strainer, plastic carrying strap, improved pressure gauge, a redesigned tank top brace, and improved corrosion resistant material on certain parts. A preliminary report received from Nicaragua indicates that most of the modifications have been proven successful. A full report and recommendations for adoption of proven changes will be made after examination of the sprayers upon their return from the field.

SUPPLEMENTARY OR ALTERNATIVE METHODS OF CONTROLLING MALARIA VECTORS

Larvicide Studies

Insecticides*

Laboratory evaluation of new compounds

The LC-95 level in parts per million was determined for *A. quadrimaculatus*, *A. albimanus*, and *C. p. quinquefasciatus* 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. Seventeen compounds were tested and of these eleven met the criterion for further evaluation by giving an LC-95 of 0.1 ppm or less (Table 12). These were BAY KUE 2302, BAY KUE 2327, NIA-24110, OMS-1349, OMS-1446, OMS-1447, OMS-1480, OMS-1501, OMS-1502, OMS-1538, and OMS-1800.

Experimental field tests with larvicides

To evaluate the effectiveness of candidate insecticides against *Anopheles* and *Culex* larvae, field plots were set up at Fort Stewart, Georgia. Five insecticides, made up as wettable powder, granules, and/or emulsifiable concentrate formulations, were evaluated as to their residual life. The test plots were 300 square feet with the soil of a sandy loam type with clays beginning from 18 to 22 inches down. Each was covered with plant growth, principally grass. All treatments were made to damp but not flooded plots. Materials were applied at the rates of one or two pounds actual per acre. Wettable powders and emulsions were applied with a 3-gallon compressed air sprayer (40 psi - 8002 nozzle). At weekly or biweekly intervals, three sod samples (1/12 sq. ft. x 3 in. deep each) for each dosage and each 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 wire mesh baskets (2 in. x 2 in. x 2-1/2in.) which were lowered into the upper surface of the water; *A. albimanus* and *C. p. quinquefasciatus* were the larvae used routinely. Larval food was given and mortality observations were 48 hours after introduction. Each sample remained flooded for ten days at which time a second series of caged larvae were introduced into the jars and the mortality assessed after 48 hours. The results for the dosage of two pounds per acre are shown in Table 13. Against the two species, methyl chlorpyrifos (Dursban) was the most effective of the five compounds tested; methyl pirimiphos (OMS-1424) gave good results against *C. p. quinquefasciatus* but was much less effective against *A. albimanus*. At a dosage of one pound per acre, the methyl chlorpyrifos emulsion was ineffective against both species.

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

TABLE 12
Concentration in Parts Per Million of Insecticide Required for the LC-95 of Three Mosquito Species

Toxicant	*Anopheles quadrimaculatus*	*Culex pipiens quinquefasciatus*	*Anopheles albimanus*
BAY KUE 2302	0.1	0.1	0.1
BAY KUE 2327	0.1	0.1	0.02
BAY MEB 6046	2.5	0.5	>2.5
DDT:CH_3S:OCH_3	>2.5	0.5	>2.5
DDT:CH_3:OC_2H_5	>2.5	0.1	0.1
NIA-24110	0.1	0.1	0.1
OMS-1800	0.02	0.004	0.02
OMS-1349	0.1	0.1	0.1
OMS-1358	>2.5	2.5	2.5
OMS-1446	0.1	0.02	0.1
OMS-1447	0.1	0.1	0.1
OMS-1480	0.5	0.1	0.1
OMS-1501	0.02	0.1	0.1
OMS-1502	0.5	0.1	0.5
OMS-1538	0.5	0.5	0.5
OMS-1539	2.5	0.5	0.5
S-15053	>2.5	>2.5	>2.5
fenthion standard	0.02	0.005	0.02

TABLE 13
Weeks of 90% Mortality of Larvae of Two Species of Mosquitoes in Field Plots Treated at a Rate of Two Pounds Active Ingredient Per Acre

Insecticide	Formulation	Weeks 90% Kill	
		A. alb.	*C. quin.*
GC-9160	Emulsifiable concentrate 2#/gal.	<1	<1
GC-9160	50% wettable powder	<1	<1
methyl chlorpyrifos	Emulsifiable concentrate 2#/gal.	4	4
OMS-1213*	40% Emulsifiable concentrate	1	1
OMS-1213	25% wettable powder	1	<1
Ortho 11775	Emulsifiable concentrate 2#/gal.	1	1
Ortho 11775	5% granules	2	1
methyl pirimiphos	25% wettable powder	1	4

* Confidential compound.

Formulation studies

Three slow-release formulations of methyl chlorpyrifos were prepared and tested as mosquito larvicides in outdoor tanks, each containing 200 liters of water. Each of the formulations contained 10% active ingredient and was designed to float on the surface of the water. One formulation was prepared with polyvinyl chloride (PVC), the others were made with polyolefin and Montan wax. The formulations were applied at dosage rates of two or three ppm. The new preparations were compared with a conventional commercial 1% granular methyl chlorpyrifos formulation and a laboratory-prepared powder containing 1% methyl chlorpyrifos on Micro Cel E. The granular and powder formulations were applied at dosage rates of 0.1 and 0.3 ppm, respectively. The concentration of methyl chlorpyrifos in each of the tanks was monitored over a period of 25 weeks by extraction of samples with organic solvent and determination of the active ingredient by gas chromatography. The results of these tests are given in Table 14.

TABLE 14
Methyl Chlorpyrifos Content of 200-liter Tanks Located in the Open

Formulation	Treatment	Concentration, ppm			
		2 weeks	5 weeks	16 weeks	25 weeks
Dow 1% granular P. Peak Clay	0.1	0.0007	0.004	0.00014*	0.00018*
	0.3	0.0012	0.004	0.00018	0.00016
TDL 1% Micro-Cel E powder	0.3	0.001	0.004	0.00010*	0.00016*
TPX Polyolefin + dibutyl phthalate (floating)	2	0.009	0.003	0.0011	0.00013
Montan wax (floating)	3	0.12	0.012	0.0015	0.0015
PVC (floating)	3	0.0007	0.007	0.0016	0.0011

* Naturally infested with mosquito larvae at this time.

After 16 weeks the tanks treated with the powder and the granular formulations had natural infestations of mosquito larvae. After 25 weeks, samples of water were drawn from the six tanks and tested against mosquito larvae. Only the samples from tanks treated with the floating wax formulation and the floating PVC formulation showed any biological activity. Each gave 100% kill of late third and early fourth instar larvae of *C. p. quinquefasciatus* exposed for 24 hours. During the course of the study, the tanks accumulated a quantity of natural debris, primarily algae growth and tree leaves. The methyl chlorpyrifos concentration in samples containing a high debris content was a hundredfold greater than that of filtered water samples indicating preferential adsorption on the solid materials present.

Studies on nonpersistent biodegradable compounds

Syntheses of N,N'-dialkylalkylenediamines and related diamines

The method employed is typified by the procedure for preparation of N,N'-didecylputrescine. To 5 ml. (0.05 mole) of putrescine and 20 ml. of pyridine, heated to 85° C. and stirred, was added dropwise 20.7 ml. (0.1 mole) of 1-bromodecane over a period of four hours. The mixture was stirred with 200 ml. of water containing 4 g. (0.1 mole) of NaOH and excess NaCl, after which it was cooled and the product filtered off. After successive recrystallization from ethanol and water, ethanol and heptane, crystals of the N,N'-didecylputrescine were obtained which melted sharply and gave a single spot on a thin layer silica gel chromatogram developed in n-butanol, acetic acid, water (4:1:5, top layer) and sprayed with ninhydrin. Nitrogen analyses by Kheldahl method and melting point data indicated that the synthetic procedure provided dialkyl rather than mono, tri, or tetra-alkyldiamines. The calculated and found nitrogen percentages of N,N'-didecyl derivatives of the following alkylenediamines were: propylenediamine 7.89, 7.61; putrescine 7.59, 7.50; cadaverine 7.32, 7.16; and hexylenediamine 7.06, 6.83. Melting points found for N,N'-dioctyl and N,N'-didodecylethylene-diamines, N,N'-dihexyl-1, 3-diamino-2-propanol, and the lactate of N,N'-diheptylhexylenediamine agreed well with those reported in the literature. The N,N'-didecyl and N,N'-didodecylpropylenediamines melted at 51-52° C. and 58-61° C., respectively, much higher than the N-decyl and N-dodecylpropylenediamines, which were reported to melt at 13.2° C. and 29.6° C., respectively.

That the synthesized dialkyldiamines were symmetrical, $R\text{-}NH\text{-}(CH_2)_n\text{-}NH\text{-}R$, rather than unsymmetrical $R_2N\text{-}(CH_2)_n\text{-}NH_2$, was evident from results of color tests. Thus, very little, if any, color was given by the didecylalkylenediamines when pure spots of these compounds on thin layer chromatograms were sprayed with alcoholic p-dimethylaminobenzaldehyde and exposed to HCl vapor. The secondary amine, dihexylamine, also gave no color, but primary amines as well as fatty diamines with primary amino groups, such as Adogen 551, gave bright yellow spots.

Fatty diamines synthesized for larvicide tests

In Table 15 are listed 59 compounds prepared by reaction of an alkyl or arylalkyl bromide with an alkylenediamine or other type of diamine. The small changes in physical properties, such as melting point and R_f values, in going from one of a series of homologs to another is consistent with the

TABLE 15
Physical and Larvicidal Properties of Synthesized Diamines

N,N'-Dialkyldiamines	R group	Properties: M.p., °C	Properties: R_f*	Richfield larvicide oil formulations: % diamine	% oleic acid	48-hr LD_{50} µl/0.5 sq ft: *A. albimanus*	*C. p. quinquefasciatus*
Ethylenediamines	Octyl	47-8	0.63	4.3	5	3.6	6.9
R-NH-$(CH_2)_2$-NH-R	Nonyl	43-4	0.63	4.7	5	3.0	5.2
	Decyl	51.3	0.64	5.1	6	2.8	4.4
	Undecyl	47-9	0.64	5.5	6	3.0	30
	Dodecyl	55-8	0.65	6.0	6	8.7	37
	Benzyl	91-3		3.6	0	10	140
Propylenediamines	Hexyl	41-3	0.53				
R-NH-$(CH_2)_3$-NH-R	Octyl	47-9	0.58				
	Nonyl	49-50	0.60	4.9	5	2.6	3.4
	Decyl	52-3	0.61	5.3	5	2.1	2.4
	Undecyl	55-7	0.61	5.7	6	2.4	2.4
	Dodecyl	58-61	0.61	6.2	6	3.7	20
	Hexadecyl	64.5					
	Diphenylmethyl	73-7		6.1	0	>20	5
Putrescines							
R-NH-$(CH_2)_4$-NH-R	Butyl	52-4	0.27				
	Hexyl	42-4					
	Octyl	52-4	0.51	4.7	4	3.6	5.1
	Nonyl	52-4	0.52	5.1	4	2.3	2.1
	Decyl	51-2	0.52	5.5	4	1.9	2.5
	Undecyl	53-5	0.53	6.0	5	3.1	3.8
	Dodecyl	53-4	0.53	6.4	6	4.1	24

(continued)

TABLE 15 (cont'd)

N,N'-Dialkyldiamines	R group	Properties: M.p., °C	Properties: R_f*	Richfield larvicide oil formulations: % diamine	% oleic acid	48-hr LD_{50}, µl/0.5 sq ft: *A. albimanus*	*C. p. quinquefasciatus*
	Benzyl	127-9		4.0	6	>20	
	3,7-Dimethyl-octyl	Liquid	0.31	5.7	3	3.6	
	2-Ethylhexyl	Liquid	0.28	4.7	[illegible]		23
	11-Hydroxy-undecyl	75.9	0.29				
	10-Carboxy-decyl	140-6	0.27				
N,N'-Dimethyl-putrescines	Decyl	Liquid	0.23	6.4	5	13.3	3.6
R-N(CH$_3$)-(CH$_2$)$_4$-N(CH$_3$)-R	Dodecyl	Liquid	1.0	7.1	5	13.8	>3
N,N'-Dimethyl-1,4-diamino-2-butenes	Decyl	Liquid	0.37	6.4	5	6.4	14
	Dodecyl	30-1		6.75	0	15	35
R-N(CH$_3$)-CH$_2$-CH=CH-CH$_2$-N(CH$_3$)-R				6.75	4	12	23
N,N'-Diethyl-1,4-diamino-2-butynes	Decyl	Liquid	0.29	7.1	0	>20	
				7.1	4	>20	
R-N(CH$_2$-CH$_3$)-CH$_2$-C=C-CH$_2$-N(CH$_2$-CH$_3$)-R	Dodecyl	Liquid					

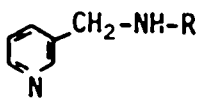
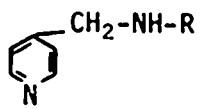
(continued)

TABLE 15 (cont'd)

N,N'-Dialkyldiamines	R group	Properties M.p., °C	Properties R_f*	Richfield larvicide oil formulations % diamine	% oleic acid	48-hr LD_{50}, µl/0.5 sq ft *A. albimanus*	*C. p. quinquefasciatus*
Piperazines	Decyl	36-7	0.75	5.7	0	>10	>20
$R\text{-}N(CH_2\text{-}CH_2)_2N\text{-}R$	Dodecyl	49-51	0.76	6.3	0	23	>40
	Benzyl	90-3		4.0	0	25	25
	Hexadecyl			5.3	2	>10	
Cadaverines	Heptyl	52-4	0.49	5.0	5		>10
$R\text{-}NH\text{-}(CH_2)_5\text{-}NH\text{-}R$	Octyl	53-5	0.50	4.9	5	4.4	7.6
	Nonyl	53-4	0.52	5.3	6	3.5	3.2
	Decyl	55-6	0.52	5.7	6	1.8	2.9
	Undecyl	57-8	0.53	6.2	6	2.8	4.2
	Dodecyl	61-2	0.53	6.6	7	4.0	19
Hexylenediamines	Hexyl	43-5	0.46				
$R\text{-}NH\text{-}(CH_2)_6\text{-}NH\text{-}R$	Heptyl	46-9	0.51				
	Octyl	48-50	0.52	5.1	5	4.1	2.7
	Nonyl	51-3	0.52	5.5	6	2.8	2.5
	Decyl	54-5	0.53	6.0	6	2.0	1.7
	Undecyl	57-8	0.53	6.4	7	3.1	5.8
	Dodecyl	60-1	0.54	6.8	9	4.2	25
	3,7-Dimethyl-octyl			6.4	10	>10	>10
	Benzyl	91-2		4.4	0	>20	40

(continued)

TABLE 15 (cont'd)

N,N'-Dialkyldiamines	R group	Properties M.p., °C	Properties R_f*	Richfield larvicide oil formulations % diamine	% oleic acid	48-hr LD_{50}, µl/0.5 sq ft *A. albimanus*	*C.p. quinquefasciatus*
1,3-Diamino-2-propanols	Hexyl	57-8	0.57				
R-NH-CH_2-CHOH-CH_2-NHR	Octyl	67-8	0.64				
	Nonyl	75-6	0.69	5.1	5	4.6	5.8
	Decyl	76-7	0.69	5.5	5	2.8	6.2
	Undecyl	79-81	0.70	6.0	7	3.4	
	Dodecyl		0.70	6.2	7	5	6.5
3-Aminomethyl-pyridine	Decyl	38-9		6.2	6		20
							
4-Aminomethyl-pyridine	Decyl	37-40	0.92	6.2	4		10
							
Control (oil only)					-	52	46
Control (oil plus acid)					4	39	36
					6	36	34

* Thin layer silica gel chromatogram developed in n-butanol, acetic acid, water (4:1:5), top layer.

assumption that these compounds are homologs. Most of the synthesized compounds were tested in two types of oil formulations. The oil was applied to the surface of 1 liter of water in a pan in which the area of the water surface was 0.5 sq. ft. Each pan contained 50 fourth instar larvae of *A. albimanus* or *C. p. quinquefasciatus*. Initial tests (not included in Table 15) were made with Richfield larvicide oil containing 2% fatty diamine and 1% of the surfactant-spreader Triton B-1956. The latter was needed also to enhance solubility of the diamine. In these tests, the activity in each series of alkylenediamines reached a peak with the didecylhomolog and fell off as the alkyl chains were lengthened or shortened. Against *A. albimanus*, the most effective formulation contained N,N'-didecyl-1,3-diamino-2-propanol and gave an LD-50 of 8 µl./0.5 sq. ft., 0.3 times that of the control oil containing surfactant only. Against *C. p. quinquefasciatus*, the best formulation contained N,N'-didecylputrescine and gave an LD-50 of 5.2 µl./0.5 sq. ft. which was 0.15 times that of the control.

In the second type of oil formulation tested, a larger amount of diamine was dissolved in the oil with the addition of oleic acid as solubilizer. Table 15 contains the results of such tests with Richfield larvicide oil containing 0.15 molar (4.3%-6.8%) fatty diamine and enough acid to maintain solubility. Larvicide data is lacking for those compounds which had only slight oil solubility or which, on the basis of results with other homologs, appeared less worth testing. The data show that in each series of homologs, activity reached a peak with the didecyl compound and diminished with longer or shorter chain homologs. Against *A. albimanus*, the most effective formulation contained N,N'-didecylcadaverine and gave an LD-50 of 1.8 µl./0.5 sq. ft., 0.05 times that of the control containing 6% oleic acid. Against *C. p. quinquefasciatus*, the best formulation contained N,N'-didecylhexylenediamine and gave an LD-50 of 1.7 µl./0.5 sq. ft., 0.05 times that of the 6% acid control.

Water dispersions of diamines and Abate

Using Abate as a basis for comparison, some of the synthesized N,N'-dialkyldiamines showing highest activity in oil formulation tests were found to give even higher activity against *Anopheles* and *Culex* when dispersed in water by a special method. Data for the most active water-dispersed dialkyldiamines is presented in Table 16, along with data for two commercial monoalkyldiamines and the organophosphate Abate. The diamines were dispersed in water by two methods; the standard WHO method for measuring susceptibility of mosquito larvae to pesticides which involves dissolving the toxicant in alcohol, and a method involving emulsification of the diamine by heating and shaking. The latter method provided, on cooling, floating crystals which appeared smaller and more needlelike or fiberlike than those provided by the WHO method. The data in Table 16 show that the heat and shake method generally provided much higher activities than the WHO method. Against *Anopheles* and *Culex*, N,N'-didecylputrescine, the most effective diamine gave LC-50's of 41 and 14 parts/billion, respectively, by the heat and shake method. These values were only 21.6 and 35 times, respectively, those given by Abate dispersed by the WHO method.

Additives in diamine-oil formulations

Long-chain acids not only enhanced solubility of fatty diamines in the

TABLE 16
Larvicidal Activities of Fatty Diamines
or Abate dispersed in Water

		Avg. 48-hr LD_{50} (ppm) after dispersion by either of two methods			
		A. albimanus		*C. p. quinquefasciatus*	
Diamine or Abate	R	WHO	Heat & shake	WHO	Heat & shake
Propylenediamines,	Nonyl	0.21	0.13	0.65	0.095
$R\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}R$	Decyl	0.28	0.71	0.54	0.019
Putrescines, $R\text{-}NH\text{-}(CH_2)_4\text{-}NH\text{-}R$	Decyl	0.49	0.041	0.70	0.014
Cadaverines,	Nonyl	0.80	0.067	2.2	0.21
$R\text{-}NH\text{-}(CH_2)_5\text{-}NH\text{-}R$	Decyl	0.40	0.41	0.35	0.10
Commercial propylenediamines, $R\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$					
Adogen 551		0.5	0.8	1.1	2.0
Redicote E-9		0.5	0.4	0.9	2.3
Organophosphate					
Abate		0.0019		0.0004	

Richfield larvicide oil, but enhanced larvicide activity. A number of oil solutions containing 5% Adogen 551 and 5% acid, applied at a rate of 5 µl./0.5 sq. ft. to a water surface were tested against *A. albimanus* larvae. The acids used in these formulations, listed in decreasing order of potency, gave 48-hour percent mortalities as follows: oleic, 74; linoleic, 72; dimerized linoleic, 70; linolenic, 66; dodecyl phosphate, 60; isomerized linoleic, 58; phenylundecanoic, 50; and decanoic, 26. In a series of tests against *A. albimanus* of oil containing 5% Adogen 551 and different levels of oleic acid, the mortality reached a maximum with 5% acid.

A variety of surfactants were tested in Richfield larvicide oil containing 3.69% (0.1 molar) N,N'-didecylputrescine against *Culex* larvae. Formulations containing various surfactants listed in decreasing order of potency gave the following 48-hour LD-50's in µl./0.5 sq. ft.: 2% Aerosol TR, 3; 3% Sarkosyl O, 3.3; 4.5% Triton B-1956, 3.6; 2% Aerosol OT, 3.8; 2% T-det N-12, 4.5; 2.5% Plurafac RA-20, 5.4; 2% T-det N-20, 5; 2% T-det DD-1, 6; 2.5% Pluronic L-121, 5.4; 2% T-det N-6, 8; and 2% T-det DD-5, 8.8.

Evaluation of solvents and polar amines in larvicidal oil formulations

Various amounts of isooctane, n-hexanol, or n-butanol were tested as a constituent in Richfield larvicide oil formulations containing the larvicidal fatty diamine N,N'-didecylputrescine, and the surfactant Triton B-1956. It was expected that the use of volatile or somewhat water-soluble solvents in oil formulations might speed up larvicidal effects by facilitating quick release of the didecylputrescine into the water soon after the oil formulation was applied to the water surface. Such formulations were tested against fourth instar larvae of *C. p. quinquefasciatus*. Results presented in Table 17 reveal that none of the solvents appreciably enhance larvicidal activity of the 2% didecylputrescine formulations and decreased activity of the more concentrated formulations. With the latter formulations, isooctane or n-butanol were needed to solubilize the diamine in the absence of a high concentration of surfactant.

TABLE 17
Various Solvents Tested as Constituents of Larvicidal Oil Formulations

Oil formulation			% mortality *C. p. quinquefasciatus* 48-hr after application of formulation to water surface	
% N,N'-Didecyl-putrescine	% Triton B 1956	% Solvent	5 µl/0.5 sq ft	10 µl/0.5 sq ft
2	1	--	50	95
2	1	50% Hexanol	57	92
2	1	25% Isooctane	64	88
2	1	50% Isooctane	44	80
3.7 (0.1 M)	4.5	--	70	95
5.5	1	40% Isooctane	8	--
5.5	1	30% n-Butanol	25	--
5.5	1	50% n-Butanol	10	--

In studies with ovicidal formulations, it had been found that combinations of short-chain amines with long-chain amines gave much better results than either type of amine used alone. Therefore, larvicidal formulations containing the long-chain diamine, N,N'-didecylputrescine, with and without the addition of the shorter chain amine, octylamine, were tested. The data (Table 18) indicate that octylamine had much less activity than the longer chain diamine. Two formulations were tested against fourth instar larvae of *C. p. quinquefasciatus*. The LD-50's were taken from plots of log dosage vs. probit mortality. The results indicate some synergism between the amines in the case of the formulation employing Aerosol TR as surfactant, but no synergism with the other formulation containing Triton B-1956.

TABLE 18
Larvicidal Effects of Oil Formulations Containing Two Amines having Different Chain Lengths on Larvae of *C. p. quinquefasciatus*

Richfield larvicide oil ingredients			48-hr LD_{50},
% N,N'-Didecylputrescine	Surfactant	% Octylamine	µl/0.5 sq ft
2	1% Aerosol TR	-	6.5
2	1% Aerosol TR	2	3.9
-	1% Aerosol TR	2	13
2	1% Triton B 1956	-	6.6
2	1% Triton B 1956	2	7.2
-	1% Triton B 1956	2	30

Rate of deterioration of larvicidal oil films

Immediately and one hour after application of larvicidal oil formulations to the surface of water in a pan, fourth instar larvae of *C. p. quinquefasciatus* were added. Mortality data presented in Table 19 show that the oil films lost much of their activity during the first hour following application. It was observed that such films broke up into larger drops during this period, a process which may be facilitated by partition of the surfactant from the oil to the water.

If fatty diamine oil formulations lose their effectiveness soon after application to a water surface, a brief exposure of larvae to a fresh film should produce about as high mortality as a long exposure. To test this hypothesis, larvae were retrieved from pans of water at various time intervals after application of an oil film, transferred to fresh water, and mortality determined 48 hours after the time of film application. A simple method was developed for pipetting out the larvae without contacting the oil film. Prior to film application, an inverted plastic beaker with bottom removed was placed in the pan. The oil formulation was applied to the water surface outside of the cylinder and, therefore, did not contaminate water enclosed by the cylinder. To remove a larva, the cylinder was moved carefully over the larva, which was then pipetted out through the cylinder. In two experiments, fourth instar larvae of *C. p. quinquefasciatus* were exposed to oil formulations for various periods of time. For each exposure period, three

pans were used with a different oil dosage for each so that LD-50 values could be determined from plots of log dosage vs. probit mortality. The data in Table 20 indicate that long exposures to the 2% diamine formulation gave little improvement in results over those obtained after four hours exposure. With the 5.5% diamine formulation, results were almost as good with 1- to 2-hour exposure as with any longer exposure.

TABLE 19
Larvicidal Activities of Fresh and Aged Oil Formulations
Against Larvae of *C. p. quinquefasciatus*

Richfield larvicide oil ingredients			Film age (hr)	% Dead in 48 hr with volumes oil/0.5 sq ft	
Fatty amine	Surfactant	Acid		5 µl	10 µl
2% Oleylamine	1% Triton B 1956		0	60	
			1	2	
2% N,N'-Didecyl-putrescine	1% Triton B 1956		0	20	66
			1	0	36
2% N,N'-Didecyl-putrescine	1% Triton B 1956	1% Oleic acid	0	96	
			1	56	

TABLE 20
Lethal Dosage as a Function of Time of Exposure of
C. p. quinquefasciatus Larvae to Oil Formulations

Richfield larvicide oil ingredients		Exposure hr	48 hr lethal dosage, µl/0.5 sq ft	
Fatty diamine	Surfactant or acid		LD_{50}	LD_{90}
2% N,N'-Didecyl-putrescine	1% Triton B 1956	1	14	40
		2	9.4	24
		4	8	13
		6	7.2	20
		24	7.4	12
5.5% N,N'-Didecyl-putrescine	4% Oleic acid	1	3.0	5.8
		2	2.9	5.8
		4	3.1	7.0
		6	3.2	10
		24	2.6	6.3

Stimulation of larval respiration by N,N'-didecylputrescine

Most insecticides, including the chlorinated hydrocarbon and organophosphates, are known to stimulate insect respiration. N,N'-didecylputrescine (DP) has now been found to be no exception in this respect. Respiration of treated and control larvae of *C. p. quinquefasciatus* was measured in a simple apparatus consisting of two Warburg flasks attached to a differential syringe manometer. In one flask was placed 25 treated or untreated larvae in 5 ml. of water, and in the other flask was placed 5 ml. of water with or without control or untreated larvae. Both flasks were immersed in a water bath maintained at 78-79° F. The center well of each flask contained 0.3 ml. of 20% KOH to absorb expired CO_2. Larvae were treated in pans containing 1 liter of water and a small plastic cylinder through which larvae could be inserted or retrieved from the water without contacting the surface film of oil formulation or floating crystals of toxin. Formulations were applied to the water area outside of the area enclosed by the cylinder. To prepare a water dispersion of N,N'-didecylputrescine, the diamine in 200 ml. of water in a 250-ml. graduated cylinder was heated in a water bath to liquefy the diamine, after which it was emulsified by shaking the stoppered cylinder. The emulsion was poured onto the surface of 800 ml. of hot water outside the cylinder in a pan. After cooling, larvae were inserted below the surface film of floating crystals of diamine by means of the cylinder.

Results of the respiration studies are presented in Table 21. The oxygen uptake for 25 fourth instar *C. p. quinquefasciatus* larvae was 1.8 µl./min. when the larvae were untreated. The rate was increased to 2.9 µl./min. after exposure of larvae to a 5 µl. oil film containing 2% DP and 1% Triton B-1956 for one hour. With a 2-hour exposure, the rate was reduced to below normal. In the last five experiments in which flask #2 contained control of untreated larvae, it can be seen that although treatment with only Triton B-1956 in oil gave an increase in respiration, there was a further increase when DP was added. It can be seen that DP stimulated respiration even when used alone in water at a level of 0.2 ppm. With the use of the organophosphate Abate in oil, there was an initial very large stimulation in respiration followed by a much lower rate. These dosages of oil formulations containing DP or Abate are one to two times the 48-hour LD-50's in µl./0.5 sq. ft.

Aqueous larvicidal emulsions

N,N'-Didecylputrescine showed highest activity after dispersion in water by a technique involving emulsification by heating and shaking. Since such a procedure would be impractical in the field, other methods of emulsification were explored. A fairly stable aqueous emulsion of this diamine could be sprayed onto a water surface to provide a floating layer of highly larvicidal crystals such as that provided by the heat and shake technique.

Richfield larvicide oil containing 2% N,N'-didecylputrescine and 1% surfactant was homogenized with water by means of the Virtis "45" Hi-Speed Homogenizer. This machine was operated for one minute at about 60% of its maximum power. With only 4 ml. of mixture in the flask, the sharp blades were probably turning at almost the maximum rate of 45,000 rpm. Best results were obtained with 1% dinonylphenol in the emulsion, as shown by data of Table 22.

TABLE 21

Effects of N,N'-Didecylputrescine (DP) and Abate on Respiration of *Culex* Larvae

Additions to 5 ml water in each Warburg flask: Flask #1	Flask #2	Hr exposure of larvae to toxin	Average rate of oxygen uptake in flask #1 less that in flask #2, µl oxygen/min/25 larvae: 10-20 min (interval)	20-50 min (interval)
Untreated larvae	None		1.8	1.8
Larvae exposed to 5 µl oil with 2% DP & 1% Triton B 1956	None	1	2.9	2.9
		2	1.6	0.5
Larvae exposed to 10 µl oil with 1% Triton B 1956	Untreated larvae	1	1.4	1.4
Larvae exposed to 5 µl oil with 2% DP & 1% Triton B 1956	Larvae exposed to 5 µl oil with 1% Triton B 1956	0.5	0	0
		1	1.2	1.2
Larvae exposed to 0.2 ppm DP dispersed in water	Untreated larvae	1	1.8	0.93
Larvae exposed to 5 µl oil with 0.02% Abate, 1% Triton B 1956	Larvae exposed to 5 µl oil with 1% Triton B 1956	1	10	1.9

TABLE 22
Lethal Effects Against *Culex pipiens quinquefasciatus* Larvae of 2% N,N'-Didecylputrescine and Surfactant in Oil Emulsified with Water

Surfactant or acid in the oil before emulsification	Volume water / Volume oil	48 hr LC_{50}, ppm
1% Aerosol TR-70	500	0.28
	200	0.25
	100	0.22
	40	0.24
1% Triton B 1956	400	0.43
	100	0.47
	40	0.25
1% Oleic acid	100	0.38
1% Dinonylphenol	100	0.17

Larvicidal activities of mono- and di-alkyldiamines compared with Abate

Many of the synthesized N,N'-dialkylalkylene-diamines and a few commercial N-alkylpropylenediamine mixtures were tested in Richfield larvicide oil with either the surfactant, Triton B-1956, or oleic acid. In the case of the dialkyldiamines, the main reason for adding the surfactant or oleic acid was to maintain solubility of the diamine in the oil. Although oil solubility was less of a problem with the commercial amines, the additives were still employed in cases where they enhanced larvicidal activity. The most effective of the different types of formulations are listed in Table 23 on the basis of lowest 48-hour LD-50 in µl. oil/0.5 sq. ft. For each formulation, the LD-50 for the most active ingredient was calculated in mg. toxin/0.5 sq. ft. On this weight basis, it can be seen that against *A. albimanus* N,N'-didecylputrescine and N,N'-didecylcadaverine with LD-50 of 0.1 mg./0.5 sq. ft. were the most active diamines used with oleic acid, and Adogen 551 and N,N'-didecyl-1, 3-diamino-2-propanol with LD-50's of 0.15 and 0.16 mg./0.5 sq. ft., respectively, were the most active diamines used with Triton B-1956. It is to be noted that 1/50 as much Abate was required in the formulation with Triton B-1956 to achieve the same results as the didecylputrescine in the formulation with oleic acid. Against *C. p. quinquefasciatus*, the dialkyldiamines were more effective than the monoalkylamines, the LD-50 being 0.1 mg./0.5 sq. ft., 192 times that for Abate, with the use of either didecylputrescine with Triton B-1956 for didecylhexylenediamine with oleic acid.

TABLE 23

Larvicidal Activities of Mono and Dialkyldiamines Compared with the Organophosphate Abate

Oil formulation ingredients		Avg. 48-hr LD_{50} in µl formulation/ 0.5 sq ft or mg toxin/0.5 sq ft			
		Anopheles		*Culex*	
Toxin	Additive	µl	mg	µl	mg
N,N'-Dialkylalkylenediamines					
2% N,N'-Didecyl-1,3-diamino propanol	1% Triton B 1956	8	0.16	8	0.16
5.7% N,N'-Didecylcadaverine	6% Oleic acid	1.8	0.10	2.9	0.17
6% N,N'-Didecylhexylene-diamine	6% Oleic acid	2.0	0.12	1.7	0.10
2% N,N'-Didecylputrescine	1% Triton B 1956	10	0.20	5.2	0.10
5.5% N,N'-Didecylputrescine	4% Oleic acid	1.9	0.10	2.5	0.14
Commercial monoalkylamines					
2% Adogen 551	1% Triton B 1956	7.9	0.15	12	0.24
5% Adogen 551	5% Oleic acid	2.8	0.14	8.1	0.40
2% Redicote E-9	1% Triton B 1956	9.8	0.19	13	0.26
5% Redicote E-9	5% Oleic acid	2.6	0.13	7.9	0.40
4% Oleylamine		6.7	0.27	6.4	0.26
4% Oleylamine	4% Oleic acid	4.1	0.16	14	0.56
Organophosphate					
0.02% Abate	1% Triton B 1956	10	0.002	2.6	0.00052

Investigations on poison baits for larvae

Investigation of the potential of floating insecticide baits[5] as larvicides for anophelines has been continued, using larvae of a dieldrin-resistant strain or a DDT-dieldrin-resistant strain of *A. albimanus* as the test organism. The larvae, in lots of 50, are given access for 24 hours to measured amounts of whole-wheat flour treated with candidate, water-insoluble, insecticides and floated on the water surface in glass dishes. Nineteen insecticides have been tested in this manner at a number of concentrations and application rates. Results of these tests are summarized in Table 25 which also shows the per acre equivalent amounts of technical grade insecticide which produced the recorded larval mortalities.

All tabular data pertain to tests with third instar larvae offered food within the 88-105μ size range. In addition, 0.1% methoxychlor bait containing particle sizes less than 149μ has been tested against larval stages 2, 3, and 4. Applications of 0.05 and 0.1 mg./cm.^{2} caused 24-hour mortalities of 88% and 97%, respectively, of third instar larvae. The same applications gave 97% and 100% mortalities of fourth instar larvae. The maximum mortality of second instar larvae was 81%. A comparison of 0.05% DDT and methoxychlor baits at various application rates from 0.1 to 0.01 mg./cm.^{2} showed methoxychlor to be the more effective material against third instar larvae, both alone and in the presence of competing, nontoxic food. In general, competing food seemed to have little influence on the 24-hour mortalities caused by insecticide baits. The larvae apparently feed until they acquire a toxic dose, although, presumably, this takes longer when nontoxic food is also present. The potential effectiveness indicated for this method of larvicide application can be appreciated by taking Abate and fenthion as examples. The recommended application rate for both these materials is 0.05-0.1 lb. per acre (actual)[6]. When used in baits, however, they caused mortalities exceeding 95% at rates equivalent to 0.0004 and 0.0011 lb. per acre (technical), respectively (Table 25). While it is unwise to rely on extrapolation from a 6-inch dish in the laboratory to an acre in the field as a certainty, the test results indicate, nevertheless, a considerable potential for poison baits in anopheline larval control. Investigation of the stability in water of the more effective bait preparations was made. In these tests, the toxicities of baits freshly applied were compared with those of the same baits aged on the water surface for various periods after application. In addition, the toxicities of filtrates from the aged applications were assessed. Present indications are that some of the insecticides, although stated by the manufacturers to be insoluble in water, nevertheless, have sufficient solubility to cause larval mortality. Table 26 shows the results of the stability tests conducted thus far and indicates the variability encountered in the level of 24-hour filtrate activity. Coumaphos and fenthion baits are noteworthy for the rapidity with which they lose their toxicity to anopheline larvae.

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[5] Table 24 lists the compounds and their chemical names.

[6] Public Health Pesticides, Pest Control, April 1972.

TABLE 24
Compounds Tested as Poison Baits Against Larvae, FY-1972

Name	Chemical Composition
Abate	0,0,0',0-tetramethyl 0,0'-thio-di-p-phenylene phosphorothioate
allethrin	2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one ester of 2,2-dimethyl-3-(2-methyl-propenyl)-cyclopropanecarboxylic acid
carbaryl	1-naphthyl N-methylcarbamate
chlordecone	decachlorooctahydro-1,3,4-metheno-2H-cyclobutal[cd]pentalen-2-one
chlorpyrifos	0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate
chlorpyrifos, methyl	0,0-dimethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate
coumaphos	0-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl 0,0-diethyl phosphorothioate
DDT	dichloro diphenyl trichloroethane
fenitrothion	0,0-dimethyl 0-4-nitro-m-tolyl phosphorothioate
fenthion	0,0-dimethyl 0-[4-(methylthio)-m-tolyl] phosphorothioate
methiocarb	4-(Methylthio)-3,5-xylyl methylcarbamate
methoxychlor	1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane
Mobam	Benzo [b] thien-4-yl methylcarbamate (also 4-benzothienyl-N-methylcarbamate or 4-benzothienyl methylcarbamate)
phoxim	phenylglyoxylonitrile oxime 0,0-diethyl phosphorothioate
propoxur	Ortho isopropoxyphenyl methylcarbamate
resmethrin	(5-benzyl-3-furyl)methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate
tetramethrin	2,2-dimethyl-3-(2-methylpropenyl) cyclo-propanecarboxylic acid esters with N-(hydroxymethyl)-1-cyclohexene-1,2-dicarboximide
toxaphene	chlorinated camphene containing 67-69% chlorine
Zectran	4-dimethylamino-3,5-xylyl methylcarbamate

TABLE 25

Mortalities of Third Instar *A. albimanus* Larvae given 24 Hours Access to 88-105μ Whole Wheat Flour Treated with Indicated Target Concentrations of Technical Grade Insecticides (Carbamate=C, Chlorinated Hydrocarbon=CH, Organophosphorus=OP, Synthetic Pyrethroid=SP

Insecticide	% Conc.	Application (mg/cm²)	No. Larvae	Mean % 24-hr. Mortality	Equivalent Amts. Tech. Insecticide/Acre Grams	Lb.
carbaryl(C)*	1.0	0.046	200	100	19.3	0.0426
		0.012	200	97	4.8	0.0106
	0.5	0.023	300	1	4.8	0.0106
methiocarb	1.0	0.046	100	100	19.3	0.0426
(C)*		0.023	100	99	9.7	0.0214
	0.5	0.023	300	77	4.8	0.0106
Mobam(C)*	1.0	0.046	200	52	19.3	0.0426
propoxur	1.0	0.046	100	48	19.3	0.0426
(C)**	0.1	0.092	100	3	3.9	0.0086
Zectran(C)**	1.0	0.092	100	25	38.6	0.0851
chlordecone (CH)*	1.0	0.046	200	100	19.3	0.0426
DDT(CH)**	0.1	0.092	100	99	3.9	0.0086
		0.046	200	97	1.9	0.0042
		0.023	100	81	1.0	0.0022
		0.012	100	80	0.5	0.0011
	0.05	0.092	300	99	1.9	0.0042
		0.046	300	91	1.0	0.0022
		0.023	300	82	0.5	0.0011
		0.012	300	54	0.2	0.0004
methoxychlor	0.05	0.092	300	92	1.9	0.0042
(CH)**		0.046	300	98	1.0	0.0022
		0.023	300	97	0.5	0.0011
		0.012	300	86	0.2	0.0004
toxaphene (CH)**	1.0	0.092	100	10	38.6	0.0851
Abate(OP)**	0.1	0.023	200	100	1.0	0.0022
	0.05	0.023	300	100	0.5	0.0011
		0.012	300	96	0.2	0.0004

(cont'd)

TABLE 25 (cont'd)

Insecticide	% Conc.	Application (mg/cm^2)	No. Larvae	Mean % 24-hr. Mortality	Equivalent Amts. Tech. Insecticide/Acre Grams	Lb.
	0.025	0.023	300	99	0.[illegible]	0.0004
		0.012	300	92	0.1	0.0002
chlorpyrifos	0.05	0.023	200	100	0.5	0.0011
(OP)*		0.012	200	100	0.2	0.0004
	0.025	0.023	300	100	0.2	0.0004
		0.012	300	100	0.1	0.0002
coumaphos	1.0	0.046	100	100	19.3	0.0426
(OP)*		0.023	100	100	9.7	0.0214
	0.5	0.023	200	100	4.8	0.0106
		0.012	200	100	2.4	0.0053
	0.1	0.023	300	99	1.0	0.0022
		0.012	300	98	0.5	0.0011
	0.05	0.023	300	85	0.5	0.0011
		0.012	300	70	0.2	0.0004
fenthion(OP)*	1.0	0.023	100	100	9.7	0.0214
		0.012	100	100	4.8	0.0106
	0.5	0.023	100	100	4.8	0.0106
		0.012	100	100	2.4	0.0053
	0.1	0.023	300	99	1.0	0.0022
		0.012	300	89	0.5	0.0011
	0.05	0.023	300	97	0.5	0.0011
		0.012	300	74	0.2	0.0004
fenitrothion	0.5	0.023	300	100	4.8	0.0106
(OP)*		0.012	300	99	2.4	0.0053
	0.1	0.023	300	13	1.0	0.0022
methyl chlor-	0.1	0.023	300	100	1.0	0.0022
pyrifos		0.012	300	94	0.5	0.0011
(OP)*	0.05	0.023	300	98	0.5	0.0011
		0.012	300	86	0.2	0.0004
phoxim(OP)*	0.5	0.023	100	100	4.8	0.0106
	0.1	0.023	100	100	1.0	0.0022

(cont'd)

TABLE 25 (cont'd)

Insecticide	% Conc.	Application (mg/cm²)	No. Larvae	Mean % 24-hr Mortality	Equivalent Amts. Tech. Insecticide/Acre Grams	Lb.
		0.012	100	100	0.5	0.0011
	0.05	0.023	100	100	0.5	0.0011
		0.012	300	100	0.2	0.0004
		0.006	300	94	0.1	0.0002
	0.025	0.023	300	100	0.2	0.0004
		0.012	300	94	0.1	0.0002
allethrin (SP)*	0.5	0.023	100	23	4.8	0.0106
resmethrin (SP)*	1.0	0.023	100	100	9.7	0.0214
	0.5	0.023	200	100	4.8	0.0106
		0.012	300	91	2.4	0.0053
	0.1	0.023	300	54	1.0	0.0022
tetrameth-rin(SP)*	0.5	0.023	200	2	4.8	0.0106

* DDT-dieldrin resistant strain used in these tests.

** Dieldrin resistant strain used in these tests.

TABLE 26
Percent Mortalities of Third Instar *A. albimanus* Larvae Exposed to Insecticide Baits at 0 and 24 Hours after Application, and to 24-hour Filtrates

Toxicant	% Conc.	Application (mg./cm.²)	Hours Aging 0	24	24-hr. Filtrate
Abate	0.05	0.023	99	98	3
	0.025	0.023	100	83	<1
chlorpyrifos, methyl	0.1	0.023	95	34	13
coumaphos	0.1	0.023	99	11	-
fenthion	0.05	0.023	90	0	-
phoxim	0.05	0.023	100	65	54
		0.012	100	98	-
resmethrin	0.5	0.023	100	99	-

Hormone studies

Laboratory and simulated field tests, Savannah, Georgia

All laboratory tests were conducted in 600 ml. glass beakers containing a total of 250 ml. well water. Twenty-five third instar larvae were introduced not less than 1/2 hour after the treatment was applied. Food was provided within two hours thereafter with additional amounts provided daily until approximately half the specimens had pupated. The number of dead larvae and pupae were removed daily and recorded. Live pupae also were removed daily and placed in untreated water in paper cups for observation of subsequent emergence. Mortality was based on the number of "presumed normal" adults emerged in relation to the number of larvae per beaker. Partially emerged adults or those found completely emerged but dead or dying on the water surface were not counted as emerged specimens. No further observations of normality such as proper rotation of the male genitalia or ability to successfully mate, feed, or oviposit were made. All tests were conducted at 76°-80° F. with approximately 40-60% R.H. and a 12:12-hour photoperiod. The compounds tested and their chemical compositions are given (Table 27). Stock solutions were prepared and serially diluted with acetone so that the addition of 1 ml. of solution gave the desired concentration in the water. Control beakers were similarly treated with solvent only. The test species were *C. p. quinquefasciatus* (DDT-dieldrin resistant), *A. albimanus* (dieldrin resistant), and *Anopheles stephensi* (susceptible). The results (Table 28) indicate Herc. 24734, RO 20-3600, ZR-0485, and ZR-0515 at dosages of 0.1 ppm or less gave at least 95% reductions in adult emergence (LC-95) of one or more of the test species.

All compounds were characterized by their inhibition of adult emergence, i.e., development proceeded normally (comparable to controls) to pupation but emergence of imagoes was adversely affected. However, in contrast to the other compounds tested to date, the Hercules compounds at slightly increased concentrations caused marked mortality of larvae.

RO 20-3600 was notably more effective against *C. p. quinquefasciatus*, producing 95% or higher mortality at 0.005 ppm, than the other test species.

ZR-0515, in general, was slightly more effective than ZR-0485 and was markedly more effective against *A. albimanus* than the other test species; it produced 95% or higher mortality at 0.0025 ppm. The manufacturer is actively pursuing the development of ZR-0515 (Altosid) as a mosquito larvicide.

Herc. 22948, 22949, and 24108 were uniformly active against the test species at 0.25 ppm.

Herc. 24734 was highly effective against *C. p. quinquefasciatus* at 0.1 ppm but higher concentrations were required for the anopheline species.

Several tests were conducted in which *C. p. quinquefasciatus* third instars were exposed for specific intervals to ZR-0515 treated water before being transferred to untreated water to complete their development. The results (Table 29) indicate that exposure for 24 hours to 0.1 ppm or two hours or longer to 1.0 ppm was necessary to produce approximately 90% mortality in

TABLE 27
Chemical Composition of Experimental Insect Development Inhibitors

Designation	Chemical Composition
RO 20-3600	6,7-Epoxy-3-methyl-7-ethyl-1-[3,4-(methylenedioxy)-phenoxy]-2-*cis/trans*-octene
ZR-0485	Isopropyl 3,7,11-trimethyldodeca-2,4-dienoate
ZR-0515	Isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate
Herc. 22948	Confidential
Herc. 22949	Confidential
Herc. 24108	Confidential
Herc. 24734	Confidential

TABLE 28
Concentration (ppm) of Compounds with JH-like Activity Required for 95% Mortality* of Mosquito Species Exposed as 3rd Instars

Compound	*C. p. quinquefasciatus*	*Anopheles albimanus*	*A. stephensi*
RO 20-3600	0.005	0.05	0.05
ZR-0485	0.025	0.025	0.1
ZR-0515	0.025	0.0025	0.05
Herc. 22948	0.25	0.25	0.25
Herc. 22949	0.25	0.25	0.25
Herc. 24108	0.25	0.25	0.25
Herc. 24734	0.1	0.25	0.5

* Mortality based on number of "normal" adults emerged in relation to the number of larvae used.

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this species. The activity of the compound in such exposures resulted in responses similar to that previously described for continuous exposure of larvae to lower concentrations. Thus, an apparently irreversible response or reaction to the treatment occurs with ZR-0515, as has been shown previously with MON 585.

TABLE 29
Percent Mortality of *C. p. quinquefasciatus* from Exposure of 3rd Instars to ZR-0515 Treated Water for Specified Intervals

Conc. (ppm)	1 hr.	2 hr.	4 hr.	6 hr.	24 hr.
0.1	-	-	-	-	88
0.25	-	-	39	55	99
0.5	-	62	70	80	-
1.0	70	88	98	99	-
2.5	91	97	100	-	-

Studies involving the feeding of specific amounts of graham flour (Particle size 53-125 μ) treated with JH-type compounds were continued. The treated food was provided on the initial test day only with all other subsequent daily feedings being made with untreated ground lab chow. Tests were conducted in stainless steel pans (H_2O surface area - 177 cm.^{2}) with fifty third instar larvae per pan. Other procedures followed during the test were as outlined previously for laboratory LC-95 determinations. The results (Table 30) indicate promising levels of mortality were obtained against *C. p. quinquefasciatus* and *A. albimanus* with flour treated with 0.05% or 0.1% MON 585. In contrast to these species, an increase in concentration to 0.2% MON 585 and an increased amount per pan were required for similar levels of mortality of *A. stephensi*. ZR-0515 at 0.1% and 0.5% on graham flour was notably more effective against *A. stephensi* than against *C. p. quinquefasciatus*. Flour treated with 0.5% Herc. 24108 gave promising results against *C. p. quinquefasciatus* and *A. stephensi*. In general, the effect on specimens from ingestion of food treated with these compounds was similar to that obtained by exposure of larvae continuously in water treated with the compound at concentrations which would produce comparable levels of mortality. Thus, these compounds are active by ingestion as well as by exposure of larvae in treated H_2O. The use of food with JH-like compounds shows promise as an alternate means of control with such materials.

Determination of MON-585 in water

A gas chromatographic method previously developed for determination of MON-585 in concentrated solutions was modified for determination of MON-585 in water in the parts per million range.

An acetone solution of MON-585 was prepared to contain 500 μg./μl. This solution was used to spike distilled water samples at 0.5 ppm, 1.0 ppm, and 2.0 ppm. Aliquots of 500 ml. were taken from each of the treated samples and

placed in 1000-ml. separatory funnels. The funnels were placed in a semi-automatic extraction apparatus. To each funnel was added 1 ml. conc. HCl and 100 ml. of n-hexane. The samples were then extracted by tumbling action for three 1-minute intervals allowing the phases to separate completely between each extraction period. The aqueous phase was discarded. The n-hexane phase was then dried by passing through a plug of cotton and collected in a 250-ml. beaker. The funnels were then rinsed twice with 20 ml. of n-hexane and the rinses added to the above extracts. The hexane extracts were then evaporated down to about 5 ml. on a steam bath and transferred to screw-capped graduated centrifuge tubes. Appropriate aliquots were then subjected to GLC as described. Recoveries ranged from 82% to 102%, with an average of 92% when samples were extracted on the same day as treated. However, when the treated water was allowed to stand at room temperature for two weeks before extraction, recoveries from the 0.5 ppm, 1.0 ppm, and 2.0 ppm samples were 14%, 30%, and 50%, respectively. The reason for the low values obtained on the aged solutions is unknown at present, but may be due to hydrolysis in aqueous solution.

Biological control investigations

Life cycle of *Lagenidium culicidum*

In the initial investigations with *L. culicidum*. the fungus parasitized all the culicine larvae tested but none of the anopheline larvae. As the studies progressed, there appeared to be a host specificity with none of the anophelines being susceptible to infection. However, subsequent work proved this assumption incorrect. When small numbers of *A. albimanus* were first infected, it was thought that such infection was the result of mechanical injury to the test larvae at the time they were placed with the inoculum. Other tests, however showed that small numbers of healthy larvae could be successfully infected when placed in inocula having a very high concentration of motile zoospores. Observations of the fungus in these larvae (about 1% of those exposed) indicated the life cycle as described below. The portal of entry for the biflagellate, motile zoospores is unknown. Microscopic observations of test larvae exposed to the fungal inoculum revealed: (a) mycelial growth usually occurred first in the head, and grew toward the posterior, infecting the anal segment and gills last; (b) mycelial growth, at least in the early stages of infection, was most abundant in the head and neck. These observations suggest that the motile zoospores normally are ingested and probably enter the tissues of the host larvae through the anterior portion of the digestive tract. Hyphae were usually first observed about 72 hours after placing the test larvae with the inoculum. The hyphae appeared to grow via the haemocoele and had usually spread to the anal segment and gills by the end of 96 hours. The hyphae were then spread throughout the body, and about 18 to 24 hours later formed the reproductive sporangia. Sporangial formation and larval death were usually simultaneous. By the next day, the motile zoospores were being produced by the infected cadavers. See Figure 1.

It now appears that the reason for the low numbers of *A. albimanus* that become infected lies in the behavior of the larvae and zoospores. Young *A. albimanus* larvae feed almost exclusively on material floating as a surface film on the water and tend to ignore any material below this surface film. It is only during the third and fourth instar that they begin to graze the sides and bottom of the container to any great extent. The fungal zoospores, on

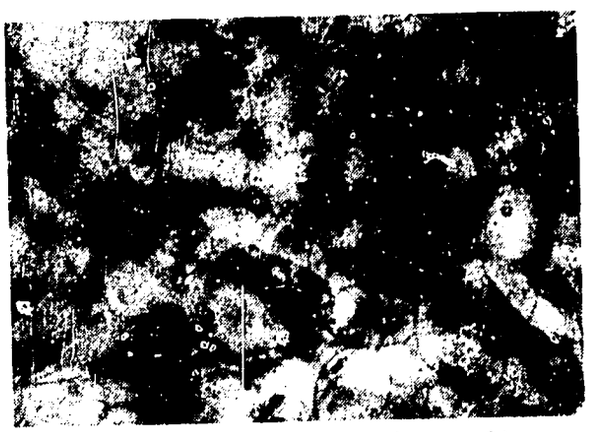
FIGURE 1

Lagenidium culicidum in *Anopheles albimanus* larva

1. Immature sporangia.

2. Mature sporangia.

3. Mature sporangia beginning to form exit tubes.



4. Enlarged view of forming exit tubes.

5. Exit tube of *L. culicidum* outside body of *A. albimanus* prior to forming zoospores.

the other hand, never become a part of the surface film. Though they apparently move freely about the water they are unable to break through the top layer and become potential food particles. This is not because of some peculiarity of the zoospores, but is a natural phenomenon characteristic of practically all minute particles completely immersed in water. Thus, the zoospores are not available as ingestible particles until late in the life of the larvae, and then for only a relatively small percent of its feeding time. Preliminary tests to demonstrate the above hypothesis have been unsuccessful, primarily because the fungus cannot withstand dessication. A few tests in which the water depth was maintained at approximately 1/4" and no food was provided as a floating material forced the third instar larvae to graze the bottom and has resulted in increased infections. Such an approach, however, offers little likelihood of a practical method for field use.

Several approaches are being considered in which oospores (resting spores) may be incorporated in a floating material. Laboratory data indicate that resting spores are capable of withstanding dessication for short periods of time, although the factors necessary for the germination of such spores are presently unknown.

Effect of temperature on infection with *L. culicidum*

Tests to determine the minimum, optimum, and maximum temperatures in which *L. culicidum* will effectively invade and kill test larvae were conducted with culicine larvae. These data revealed that infection and growth of the fungus within the larval host was satisfactory between 75 and 90° F., temperatures well within the normal range for *A. albimanus*. Infection and development did occur up to 100° F., though the percentage of larvae infected in the tests was less than 90%. Infection at 60° F. was erratic although those larvae becoming infected died and the fungus was able to complete its life cycle.

Effect of *L. culicidum* on aquatic, nontarget organisms

Because *L. culicidum* was being considered as a biological control organism in the larval habitats of mosquitoes, several naturally occurring aquatic organisms were collected and brought into the laboratory for testing. Copepods (*Cyclops*), Cladocera *Daphnia*, *Scapholeberis*), Polycheatae, Dytiscidae, and Chironominae were all tested by placing approximately equal numbers in test and control containers with *L. culicidum* being added to the test material daily. Equal quantities of brewers' yeast, algae and mosquito larvae were periodically added to all containers. The culicine mosquito larvae used to monitor infectivity in all inoculated containers became infected and died. Tests were conducted for periods as long as two weeks. Population estimates and actual counts revealed no greater survival among the control than in the test organisms. Microscopic examination of young and adult organisms revealed no fungal infections. Adults were observed daily with eggs; abundant young specimens, from newly hatched through all stages, were routinely observed. Microscopic examination of centrifuged detritus revealed no fungal infection of any organism except the culicine mosquito larvae.

In outdoor tests with culicine larvae in 55-gallon drums, chironomids and dytiscids thrived throughout an entire summer in tests in which test mosquito larvae were becoming infected and dying daily.

TABLE 30
Percent Mortality of Mosquitoes from Feeding of Treated Graham Flour on Initial Test Day Only*

Treatment	Amount (mg.)	*C. p. quinquefasciatus*	*A. albimanus*	*A. stephensi*
0.025% MON 585	50	51	54	-
	75	86	-	-
0.05% MON 585	25	60	-	-
	50	96	90	-
	75	96	97	-
0.1% MON 585	10	75	76	-
	15	94	100	13
	25	-	90	35
	50	-	98	85
0.2% MON 585	5	72	74	-
	10	98	98	-
	15	100	-	63
	25	-	-	79
	50	-	-	100
0.1% ZR-0515	15	24	-	78
	25	26	-	96
0.5% ZR-0515	15	23	-	98
	25	55	-	99
	50	-	-	100
1.0% ZR-0515	25	-	-	100
0.5% Herc. 24108	10	-	-	-
	25	100	-	93
	50	100	-	100

* Untreated lab chow provided on all subsequent daily feedings. Fifty larvae per stainless steel pan (H_2O surface area - 177 cm.^{2}).

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In test ponds constructed during the summer of 1971 and inoculated with *L. culicidum*, natural populations of dytiscids, hydrophilids, chironomids, cyclops, daphnia, tadpoles, frogs, rotifers, Eubranchiopoda sp., and Ostracoda sp. have continued to flourish. Periodic collections and microscopic examination of hundreds of specimens have not yet revealed a single infected individual.

Adulticiding Investigations

Space spray applications

Laboratory tests of new insecticides

A. albimanus and *C. p. quinquefasciatus* were exposed to a mist spray discharged within a modified Hoskins-Caldwell chamber. Each toxicant was formulated at concentrations from .01% to .0025% in a glycerol (15 parts): 95% ethanol (85 parts) carrier. Two milliliters of each formulation were atomized through the exposure cartons containing approximately 50 specimens. After exposure, the insects were held 24 hours with food and water after which the percent mortality was determined. Three replicates were made per concentration. Results are given in Table 31. The most effective materials against *A. albimanus* were the two synthetic pyrethroids, OMS-1800 and NIA 24110, each of which gave 100% kill at .0025%. Only OMS-1075 was equally effective against the two species.

TABLE 31
Percent Mortality of *A. albimanus* and *C. p. quinquefasciatus* Exposed to Mist Applications at Concentrations of 0.025, 0.01, 0.005 and 0.0025 percent

	Percent Mortality							
	0.025		0.01		0.005		0.0025	
Toxicant	*A. alb.*	*C. p. quin.*	*A. alb.*	*C. p. quin.*	*A. alb.*	*C. p. quin.*	*A. alb.*	*C. p. quin.*
BASF 2351	99	11	47	0	0	0	1	0
BAY ZUM 0673	100	100	99	94	97	5	28	0
OMS-1075	100	100	100	100	86	83	4	30
Mobam	100	75	99	39	75	0	1	0
NIA 24110	100	100	100	100	100	94	100	49
OMS-1800	100	100	100	100	100	98	100	65

Aerial, ultra low volume studies

During the first two quarters of FY-1972, TDL's aircraft was used extensively to evaluate various techniques and equipment for application of ULV malathion. Performance of the equipment was generally satisfactory and it was established that the aircraft produced consistently acceptable spray coverage with good insect kills under most conditions with an effective swath of 200 feet when flown at 140 mph at an altitude of 150 feet. Several nozzle types were used for these trials. Techniques used to evaluate the spray included oil red dye cards to determine the number of spray droplets per unit area, silicone coated glass slides to determine droplet size, filter papers to determine dosage by chemical analysis, and caged mosquitoes for bioassay of the spray. The evaluation stations included sites which were completely open, partially covered (light brush), covered (light woods), and heavily covered (dense woods). Good

insect mortality and spray coverage were usually obtained (except in the dense woods) provided that atmospheric conditions, particularly wind, were within suitable limits.

During the fourth quarter, plans for the proposed preliminary trial of aerial ULV as a possible malaria control technique in Haiti were finalized. In preparation for this trial it was decided to standardize on one or two types of nozzles which could generate acceptable spray, depending on the dosage required. Also, because of the large areas to be sprayed in Haiti, it was desirable to attempt to increase the effective swath width. After a number of tests, the following conditions were established:

Application Rate, fluid oz./acre	Nozzle		Effective Swath, feet
	Type	No. Required	
3	M-6	12	300
6	8002E	12	300
8	8002E	12	200

The maximum practicable application rate with the existing system is 8 fluid ounces per acre. This is limited by the capacity of the 24 VDC pump which is rated at 3-1/2 gallons per minute.

Ground ULV power equipment

Four gasoline-powered, back-pack mist blower type sprayers have been obtained for evaluation in ground ULV application. Three of the units are commercial ones made in W. Germany and one is a prototype made by a United States manufacturer. The units have all been tested using a glycerine-water mixture which simulates technical malathion. The units all were found to deliver much higher flows than are required for ground ULV and will have to be modified before testing with malathion can begin. One unit suffered a broken starting rope, and a number of other minor malfunctions have occurred. Problems such as these would be much more troublesome in field use, so careful observations of mechanical performance are being made.

Cooperative study on aerial ULV in Haiti

In cooperation with Government of Haiti and Pan American Health Organization, a study was organized to determine the effects of ultra low volume aerial spray on malaria transmission in the Miragoane Petit Guave area of Haiti. Special funding in support of the study as to insecticide, per diem, and equipment was provided by AID through the Haiti mission.

Project activities were scheduled as follows:

1. Advance visit in the Spring of 1972 to acquaint officials with the design of the study to establish prespray procedures for collection of epidemiologic and entomologic data, to initiate necessary clearance for aircraft use and ground maintenance, and to select the test areas.

2. Preliminary tests to be conducted in early summer when *A. albimanus*

densities were adequate to permit the evaluation of treatment and entomologic procedures, and to measure the effect of malathion ULV applications on mosquito population.

3. Provided the results in phase 2 were of sufficient promise, the third and large-scale test will be conducted in the Fall of 1972. In this test, 20,000 acres would be treated six times over a 50-day period. The effectiveness of the treatment will be evaluated epidemiologically and entomologically. Such evaluations were begun early in 1971 and will continue until the completion of phase 3.

As of July 1972, phases 1 and 2 have been completed. The preliminary spray tests were conducted in June. ULV treatments of malathion were applied at rates of six and eight fluid ounces per acre in selected areas of 8 km^2.

Results of light trap and biting collections are shown in Table 32. There was a decline in *A. albimanus* populations following the spray applications, but the data did not provide conclusive evidence that the population decline was due solely to the ULV spray. Caged *A. albimanus* were placed in the test area during spray applications in both protected and unprotected sites. Some cages were well protected under various types of vegetation including mango trees, banana plants, and sugar cane, but sufficient penetration of malathion occurred to cause 100% mortality in all cages within two hours. High mortalities were observed in mosquitoes captured during biting collections (after spray) that were held for 24 hours.

Resistance tests conducted by SNEM on *A. albimanus* females captured in the area before spraying indicated that these mosquitoes are susceptible to malathion. At the 0.8% concentration, 90% mortality was observed with no mortality in the controls.

The pilot was able to fly the TDL aircraft at 150' above the terrain in the study area and the spray applications were exceptionally good. Droplet size was close to 25 microns (MMD) and approximately 40 droplets per square inch impinged on dye cards in the treated area. The TDL updraft UV light trap proved to be a highly effective means of capturing *A. albimanus*.

The full scale experiment will be initiated when data from two successive inspection cycles show a significant increase in malaria cases in the study area.

TABLE 32
Anopheles albimanus Females Captured in the
Miragoane Lakes Study Area

Date	Cuperlier		Source Pinaud	
	Biting Collections[1]	Light Trap[2]	Biting Collections[1]	Light Trap[2]
29-30 May	127	37	59	97
30-31 May	103	75	67	138
31- 1 June	[3] 138	35	62	967
1- 2 June	91	111	59	504
2- 3 June	57	55	51	897
3- 4 June	[4] 53	23	58	983
4- 5 June	16	34	67	564
5- 6 June	21	15	[5] 57	715
6- 7 June	9	6	43	87
7- 8 June	6	3	24	141
8- 9 June	22	3	36	40
9-10 June	1	-	22	122
10-11 June	10	-	20	59
11-12 June	0	-	3	58

[1] Total females collected by four collectors from eight human baits.
[2] Females collected by TDL updraft UV light trap.
[3] ULV malathion application at 6 fl. oz./acre, 8 km.^{2} (a.m.).
[4] ULV malathion application at 6 fl. oz./acre, 8 km.^{2} (p.m.).
[5] ULV malathion application at 8 fl. oz./acre, 8 km.^{2} (a.m.).

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INVESTIGATIONS ON DEVELOPING IMPROVED METHODS FOR DETERMINING MOSQUITO DENSITIES

Studies on Developing Improved Light Traps

Laboratory tests of a 12-volt, battery-powered, ultraviolet, updraft light trap designed for field evaluation have been completed. The trap (Figure 2) was constructed to permit operation in either the updraft or the conventional downdraft position. A two-blade fan in the throat moves air through the trap into the holding cage which is made from hardwood embroidery hoops with surgical tube gauze stretched over them. No sewing is required and the tube gauze is easily replaced when it becomes torn or dirty. Results of a comparison of the two modes of operation made with insectary-reared *A. albimanus* strongly confirmed the superiority of the updraft principle for capture of adult *Anopheles*. Updraft catches of females exceeded downdraft catches by more than 2-1/2 times and yielded twice as many live specimens per 100 at risk. Male captures were similar to those of females.

The TDL updraft trap was used in the Miragoane Lakes area of Haiti to sample *A. albimanus* populations. At one sampling site, captures of female *A. albimanus* ranged from 504 to 967 per trap night over a 6-day period. During the same period, biting collections at four sampling sites within approximately 100 yards from the light trap ranged from two to 31 per bait during a 1-hour sampling period each evening.

A modified updraft light trap in which the captured specimens are not drawn through the fan is under development. Its purpose is to produce catches with a maximum of live mosquitoes.

A study of the response of blooded *A. albimanus* females to ultraviolet light has been completed with an 8-watt, fan-equipped, commercial, black light trap. Blood meals were given at predetermined intervals before testing by placing the mosquitoes' holding carton against the shaved back of a rabbit and allowing them to feed for approximately 15 minutes through the carton's nylon net cover. Immediately after feeding, the mosquitoes were lightly anesthetized, examined under an illuminated magnifier, and any females which failed to take blood were discarded. All mosquitoes were of equal age at the time of trapping. A low but rather consistent level of attractiveness was demonstrated during the first hours following a blood meal. With time, catches increased until, at 48 hours after the blood meal, they equalled those of nonblooded females. The relation of catch to time after blood feeding is shown in Figure 3.

FIGURE 2
Portable black light trap developed by
Technical Development Laboratories for mosquito surveillance

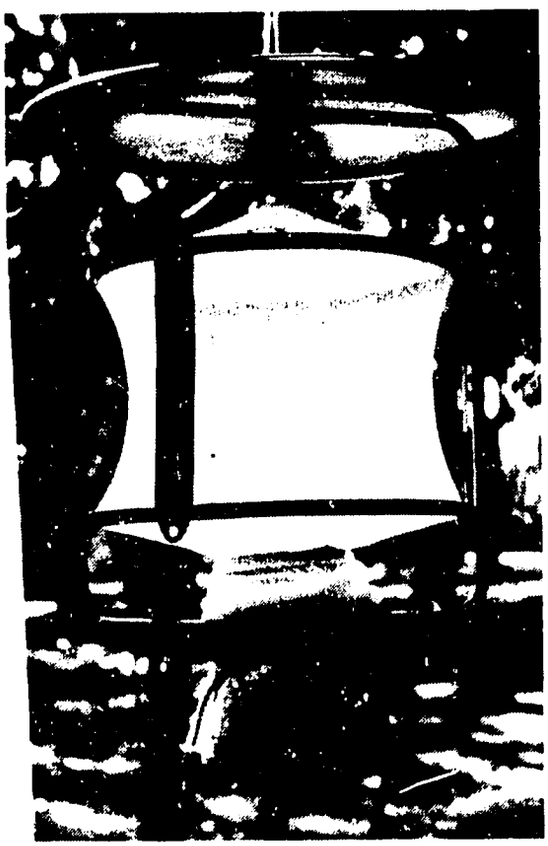
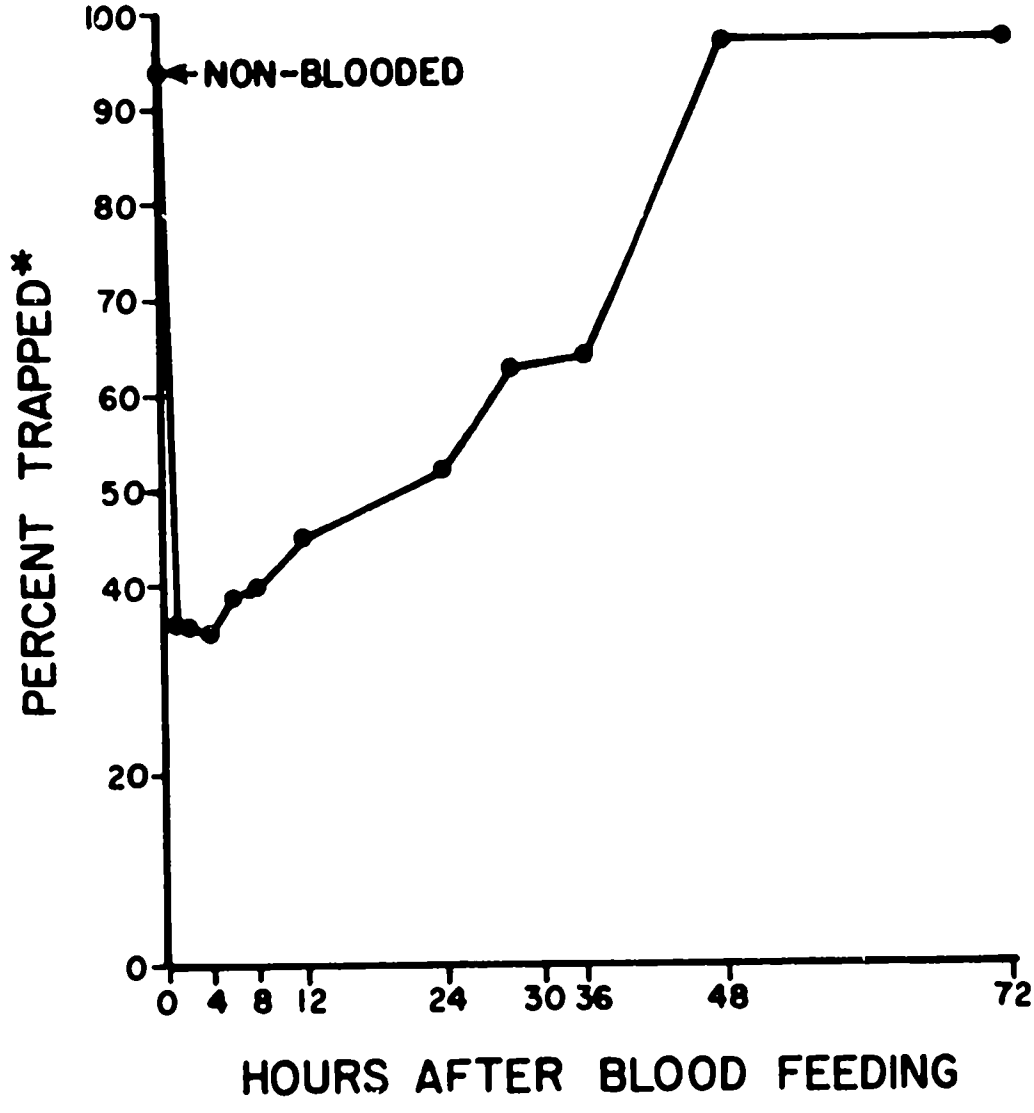


FIGURE 3

TWO-HOUR CAPTURES OF 5-6 DAY OLD *ANOPHELES ALBIMANUS* FEMALES BY ULTRAVIOLET LIGHT TRAP AT VARIOUS INTERVALS AFTER BLOOD FEEDING.



* PERCENTAGE TRAPPED BASED ON A MINIMUM OF 477 INDIVIDUALS AT EACH TIME PERIOD.

SPECIFICATIONS, CONSULTATION, AND OTHER SERVICES

Equipment Services

At the request of the Chief Malaria Advisor in Brazil, TDL obtained and calibrated a nozzle flow rate tester similar to the one used here. TDL equipped this device with a housing and the necessary fittings, and also fabricated a stainless steel nozzle pattern test sheet. The equipment was shipped to Brazil in May.

When in Haiti on the preliminary ULV tests, it was learned from SNEM personnel that a fairly large number of sprayers were out of service because of damaged threads on the discharge assembly fitting and the pressure gauge port. Attempts to repair by SNEM had been unsuccessful. The problem is similar to one encountered several years ago in Thailand. At that time, TDL and the Hudson Company developed a repair kit which may be available at reasonable cost to repair the sprayers in Haiti. The manufacturer has agreed to furnish several repair kits, and these kits will be demonstrated to SNEM in Haiti when TDL personnel return in the fall.

Comparative Evaluation of Susceptibility Test Procedures for Adult Mosquitoes

Because of the short life of papers impregnated with organophosphorus and carbamate insecticides in the WHO susceptibility kits for adult mosquitoes, the test procedure was modified at the 1968 WHO Meeting of the Expert Committee on Insecticides. In the modification, the procedure for use with organophosphorus and carbamate compounds was changed from a fixed exposure period (one hour) and variable dosages to one in which the exposure period varied (0.5, 1.0, 2.0, and four hours) and only two concentrations were used. Data had shown that with the chlorinated hydrocarbon insecticides and malathion, a doubling of the concentration for a standard exposure time gave the same results as doubling the time of exposure for a fixed concentration.

The WHO requested TDL to make a comparison of the time and concentration responses of papers of three other insecticides. Included in these tests were papers impregnated with fenthion, propoxur, fenitrothion, and malathion. *Aedes aegypti* females were used for all tests. A total of 100 females (four replicates of 25) were used for each concentration tested. Results are shown in Tables 33 through 36. With this species and these compounds, the LT-50 appears to be a useful measure for the comparison of susceptibility levels. The use of an LT-50 also reduces the number of concentrations and mosquitoes required as compared to those necessary in making LC-50 determinations.

TABLE 33
Percent Mortality of *Aedes aegypti* Adults Exposed to Papers Impregnated with Fenitrothion

% Conc.	Percent Mortality 1/2*	1	2
0.1	0**	0	68(64-72)
0.2	5(0-4)***	29(16-48)***	99(96-100)
0.4	55(32-88)	99(96-100)	100
0.8	100	100	100
1.6	100	100	100
Check	0	0	0

* Hours of exposure.

** Four replicates of 25 females each - Total = 100 females/concentration.

*** Range in mortality.

TABLE 34
Percent Mortality of *Aedes aegypti* Adults Exposed to Papers Impregnated with Propoxur

% Conc.	Percent Mortality 1/2*	1	2
0.01	0**	0	0
0.02	0	0	7(0-16)***
0.04	0	1	50(42-58)
0.08	1(0-4)***	26(17-32)***	96(92-100)
0.16	39(12-52)	81(76-88)	100
Check	0	0	0

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TABLE 35
Percent Mortality of *Aedes aegypti* Adults Exposed to Papers Impregnated with Fenthion

% Conc.	Percent Mortality 1/2*	1	2
0.05	0**	0	1(0-4)***
0.1	0	0	8(0-16)
0.2	0	43(32-52)***	94(84-100)
0.4	8(0-16)***	95(88-100)	100
0.8	96(88-100)	100	100
1.6	100	100	100
3.2	100	100	100
6.4	100	100	100
Check	0	0	0

* Hours of exposure.

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*** Range in mortality.

TABLE 36
Percent Mortality of *Aedes aegypti* Adults Exposed to Papers Impregnated with Malathion

% Conc.	Percent Mortality 1/2*	1	2
0.1	0**	0	0
0.2	0	0	3(0-8)***
0.4	0	2(0-8)***	93(84-100)
0.5	0	3(0-4)	100
0.8	0	87(84-92)	100
1.6	81(72-100)***	100	100
3.2	100	100	100
5.0	100	100	100
6.4	100	100	100
Check	0	0	0

* Hours of exposure.

** Four replicates of 25 females each - Total = 100 females/concentration.

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Specifications

Recommendations were made to WHO on specifications for a variety of pesticidal products including BHC and BHC formulations, technical grade DDT, technical grade methoxychlor, malathion water-dispersible powder, and Abate emulsion concentrate.

Reference Center of WHO on Evaluation of Insecticides

Research data for 22 experimental compounds were provided to WHO for inclusion in their computerized summary of data from the insecticide evaluation program involving TDL and six other laboratories. The data related to residual sprays, larvicides and space sprays, and involved one to four test species. In April, the principal investigator attended a conference in Geneva to present TDL findings on insecticides, hormones, and biological pathogens.

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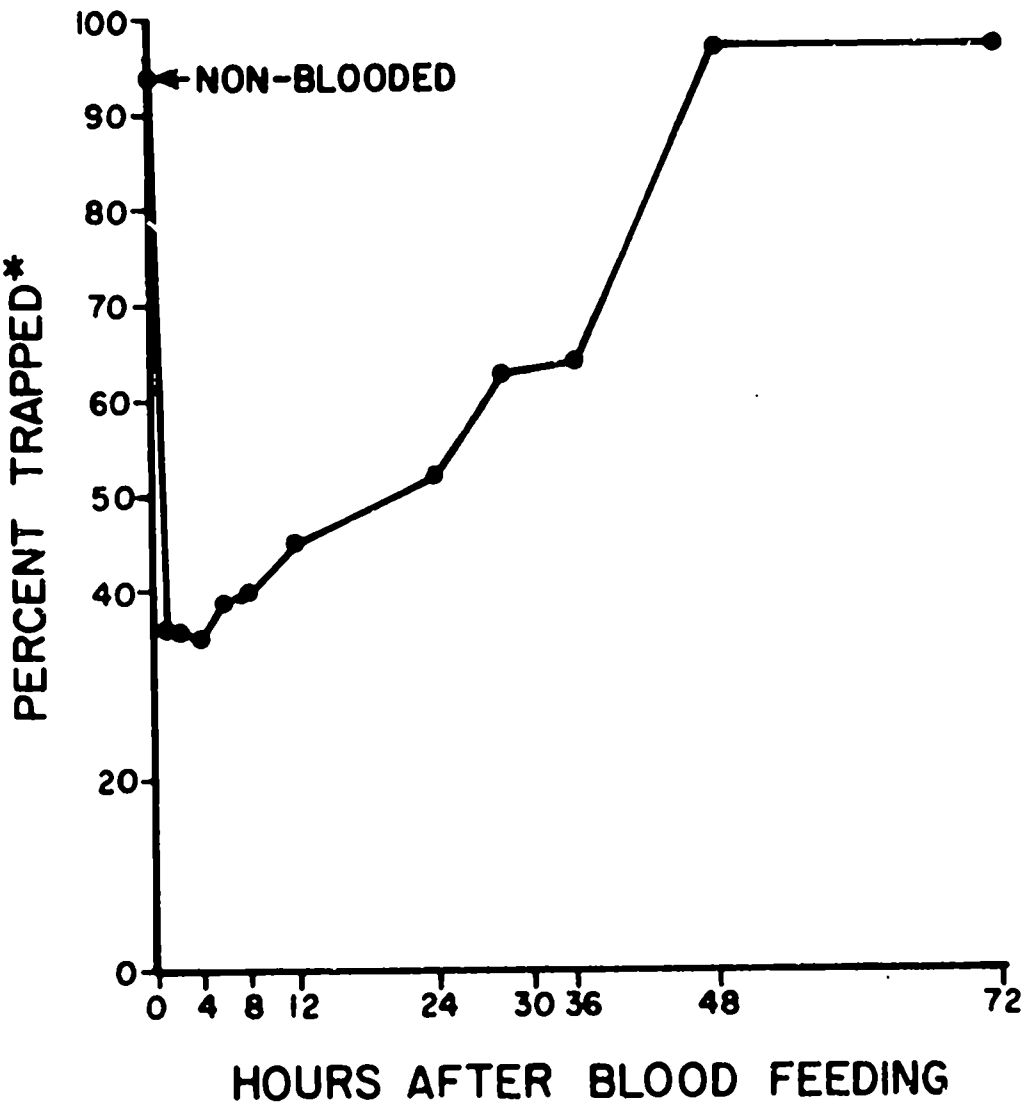

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0.5	0	3(0-4)	100
0.8	0	87(84-92)	100
1.6	81(72-100)***	100	100
3.2	100	100	100
5.0	100	100	100
6.4	100	100	100
Check	0	0	0

* Hours of exposure.

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*** Range in mortality.

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Specifications

Recommendations were made to WHO on specifications for a variety of pesticidal products including BHC and BHC formulations, technical grade DDT, technical grade methoxychlor, malathion water-dispersible powder, and Abate emulsion concentrate.

Reference Center of WHO on Evaluation of Insecticides

Research data for 22 experimental compounds were provided to WHO for inclusion in their computerized summary of data from the insecticide evaluation program involving TDL and six other laboratories. The data related to residual sprays, larvicides and space sprays, and involved one to four test species. In April, the principal investigator attended a conference in Geneva to present TDL findings on insecticides, hormones, and biological pathogens.

APPENDIX 3

Publications and Presentations

PUBLICATIONS

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Taylor, R.T. and Schoof, H.F., 1971. The relative effectiveness of malathion thermal aerosols and ground-applied ULV against three species of mosquitoes. *Mosquito News*, *31*:346-349.

Jakob, W.L. and Schoof, H.F., 1971. Studies with juvenile hormone-type compounds against mosquito larvae. *Mosquito News*, *31*:540-543.

Weathers, D.B., Taylor, J.W., and Jensen, J.A., 1971. A re-examination of the disk flow regulator. *Bulletin of WHO*, *44*:847-854.

Technical Development Laboratories, 1972. 1972 Center for Disease Control Report on public health pesticides for mosquitoes, flies, fleas, bedbugs, ticks, chiggers, lice, cockroaches, venomous arthropods, and rodents. *Pest Control*, *40*(4):35-65 passim.

Jakob, W.L. and Schoof, H.F., 1972. Mosquito larvicide studies with MON 585, a juvenile hormone mimic. *Mosquito News*, *32*:6-10.

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ABSTRACTS, FY 1972

Taylor, R.T., and Schoof, H.F., 1971. Experimental field treatments with larvicides for control of *Anopheles*, *Aedes*, and *Culex* mosquitoes. *Journal of Economic Entomology*, *64*:1173-1176.

Grassy plots were treated with granular, wettable powder, and emulsifiable concentrate formulations of 22 different insecticides to determine their residual life as larvicides. Larvae of *Anopheles albimanus*, *Aedes taeniorhynchus*, and *Culex pipiens quinquefasciatus* were used as test insects. Thirteen of the compounds gave satisfactory results for six or more weeks against at least one of the test species. Dursban at 1 lb./acre was the most effective compound; fenthion at 2 lb./acre, and Abate and Mobam at 4 lb./acre gave comparable results. At 0.5 lb./acre Dursban gave 6 and >12 weeks of 90% kills of *Ae. taeniorhynchus* and *A. albimanus*. Carbamates, such as propoxur, carbaryl, and Mobam were effective against one or more of the three species for >12 weeks when applied at rates of 2 to 4 lb./acre. Residual activity varied markedly with the dosage rate and species tested.

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Taylor, R.T., and Schoof, H.F., 1971. The relative effectiveness of malathion thermal aerosols and ground-applied ULV against three species of mosquitoes. *Mosquito News*, *31*:346-349.

Malathion ULV and thermal fogs applied with ground equipment were evaluated against caged *Anopheles albimanus*, *Culex pipiens quinquefasciatus* and *Aedes taeniorhynchus* at distances of 150, 300, 450, and 600 feet from the discharge nozzle. Delivery rates used were 1.7 to 10.0 fl. oz./min. with the ULV equipment and 85 and 170 fl. oz./min. with the thermal fogs. Open and wooded areas were included in the test sites. At 5 mph, flow rates of 3.9 and 4.1 fl. oz./min. were required to give kills of the three species approximating those achieved with thermal fogs (85 fl. oz./min.). At 10 mph, flow rates of 4.3 fl. oz./min. or above produced kills equivalent to the thermal fog (170 fl. oz./min.) up to the 300-foot stations. Thermal fogs were superior to ULV applications in producing kills in cages held at wooded sites. ULV adulticiding, however, offers the advantages of lower cost, reduced traffic hazard, and less environmental contamination.

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Jakob, W.L., and Schoof, H.F., 1971. Studies with juvenile hormone-type compounds against mosquito larvae. *Mosquito News*, *31*:540-543.

Techniques have been developed to evaluate compounds with juvenile hormone-like activity against mosquito larvae. All tests were conducted in the laboratory, starting with third instar larvae, and mortality was based on emergence of "normal" adults. The LC-95 values against each test species are given. ZR-0342 was highly effective against *Culex pipiens quinquefasciatus* at a dosage of 0.0025 ppm. Most materials were effective against this species and the others tested at 0.1 to 0.25 ppm. Activity of the compounds was usually exhibited by the inhibition of adult emergence.

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Weathers, D.B., Taylor, J.W., and Jensen, J.A., 1971. A re-examination of the disk flow regulator. *Bulletin of WHO, 44*:847-854.

It is important in residual spraying programmes for malaria eradication that a uniform deposit of insecticide should be applied to the walls of dwellings. The uniformity of application with hand compression sprayers is greatly enhanced if a disk flow regulator is used, but this device has not been popular for field use as its properties alter after a few days' use. This study explains the mode of action of the device and discloses that swelling of the disk caused by the DDT formulation is the primary cause of the gradual reduction of output. The need for proper mating of the disk with the nozzle tip is explained, and the 9504E-type tip is described as best suited for the desired spray output. Use of the disk flow regulator is recommended because it permits uniform spray application and reduces nozzle-tip erosion.

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Technical Development Laboratories, 1972. Public Health Pesticides. *Pest Control, 40*(4):35-65 passim.

"Public Health Pesticides" is the annual release of the Center for Disease Control (CDC) that presents current information on the use of pesticides in the control of arthropods and rodents of public health significance. The data included are from CDC investigations supplemented by published findings and unpublished experimental results furnished by certain research organizations. The purpose of the article is to keep organizations and personnel engaged in the control of disease vectors and rodents currently informed on compounds that are effective and can be used safely. Emphasis is placed on new developments in the chemical control field and on those pesticides in current use by agencies engaged in control or eradication programs.

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Jakob, W.L., and Schoof, H.F., 1972. Mosquito larvicide studies with MON 585. a juvenile hormone mimic. *Mosquito News, 32*:6-10.

Laboratory and simulated field studies show that MON 585, a synthetic juvenile hormone mimic, was effective against larvae of *Aedes aegypti, Culex pipiens quinquefasciatus, Culex tarsalis, Aedes taeniorhynchus, Anopheles albimanus,* and *Anopheles stephensi*. Activity of the compound is expressed in death of newly formed pupae. Exposure of third instars to relatively high concentrations for intervals of six hours or less similarly resulted in mortality at the larval-pupal moult. The compound did not cause any larval mortality.

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Wilton, D.P., Fetzer, L.E. Jr., and Fay, R.W., 1972. Quantitative determination of feeding rates of *Anopheles albimanus* larvae. *Mosquito News, 32*: 23-27.

A colorimetric technique using dyed food particles floated on the water has been developed for investigating the relation of particle size to food consumption by anopheline larvae. Simultaneous study of large numbers of larvae is possible with results expressed as µg. of food/larva/unit time. Tested with larvae of *Anopheles albimanus*, the method has demonstrated the relation of food particle size to feeding efficiency.

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PAPERS IN PRESS

McKinney, R.M., Spillane, J.T., and Holden, P. Identification of mosquito blood meals by fluorescent antibody methods. In press, *American Journal of Tropical Medicine and Hygiene.*

Miles, J.W., Goette, M.B., Guerrant, G.O., and Churchill, F.C. Argentimetric method for determination of malathion in formulations. In press, *Journal of the Association of Official Analytical Chemists.*

Schoof, H.F., and Taylor, R.T. Recent advances in insecticides for malaria programs. In press, *American Journal of Tropical Medicine and Hygiene.*

Wilton, D.P., and Fay, R.W. Air flow direction and velocity in light trap design. In press, *Entomologia Experimentalis et Applicata.*

Wilton, D.P., and Fay, R.W. Responses of adult *Anopheles stephensi* to light of various wavelengths. In press, *Journal of Medical Entomology.*

PRESENTATIONS AT SCIENTIFIC MEETINGS

Guerrant, G.O., Goette, M.B., and Miles, J.W. Determination of malathion in formulations by the argentimetric method. Association of Official Analytical Chemists Meeting, October 11-14, 1971.

Jakob, W.L. Studies on selection of *C. p. quinquefasciatus* with MON 585, a synthetic with JH-like activity. Entomological Society of America Meeting, November 29-December 2, 1971.

Jakob, W.L. Field trials with MON 585 against mosquito larvae. American Mosquito Control Association Meeting, April 23-26, 1972.

McCray, E.M. Jr. and Fay, R.W. The biological control of *Culex* in outdoor tests with *Lagenidium culicidum*. American Mosquito Control Association Meeting, April 23-26, 1972.

Miles J.W., Goette M.B., and Guerrant, G.O. Argentimetric method for determination of malathion. Association of Official Analytical Chemists Meeting, October 11-14, 1971.

Taylor, R.T. Observations on the effect of a juvenile hormone on *Triatoma infestans*. Entomological Society of America Meeting, November 29-December 2, 1971.

Taylor, R.T., Weathers, D.B., and Taylor, J.W. Aerial ULV studies with malathion against caged mosquitoes in open and protected sites. American Mosquito Control Association Meeting, April 23-26, 1972.

Wilton, D.P. and Fay, R.W. Some innovations for surveillance of mosquitoes. American Mosquito Control Association Meeting, April 23-26, 1972.

APPENDIX 4

Working Papers Prepared by TDL Staff for WHO Meetings

WORKING PAPERS PREPARED BY TDL STAFF FOR WHO MEETINGS

7th Meeting of the Expert Committee on Insecticides, 1957

Development of a test kit for detecting insecticide resistance in adult mosquitoes.

Evaluation of methods for detecting insecticide resistance in mosquito larvae.

8th Meeting of Expert Committee on Insecticides, 1958

Interpretation of results of resistance tests.

Recommended methods for vector control.

9th Meeting of Expert Committee on Insecticides, 1958

Factors affecting the suspensibility of DDT water-dispersible powders.

Factors affecting the suspensibility of dieldrin water-dispersible powders.

Summary of inspection analysis of 75% DDT water-dispersible powders purchased by ICA in 1955-58.

Studies on the adsorption of DDT by mud in the presence of solvents.

Storage stability of DDT water-dispersible powders.

Specifications for water-dispersible DDT powders for use in malaria control programs.

10th Meeting of Expert Committee on Insecticides, 1960

Recommended methods for vector control.

Informal Meeting on Chemistry and Specifications of Pesticides, 1961

The effect of compaction on the storage stability of DDT water-dispersible powders.

A new high temperature test for predicting the storage stability of DDT water-dispersible powders.

A review of specifications for malathion and its formulations.

Analytical method for pyrethrins.

ICA Specification for 75% DDT water-dispersible powders.

Specifications for 75% dieldrin water-dispersible powders.

Informal Meeting on Chemistry and Specifications of Pesticides, 1963

Studies on dieldrin water-dispersible powders.

Review of specifications for malathion water-dispersible powders.

Determination of melting point and mixed melting point.

Method for determination of malathion.

Sieving test after tropical storage.

Emulsion stability test.

Accelerated storage test for DDT water-dispersible powders.

Dustability test after tropical storage.

Packaging of insecticides.

13th Meeting of Expert Committee on Insecticides, 1963

Variations in testing for resistance to insecticides.

Laboratory Directors' Meeting, WHO Program on Evaluation of Insecticides for Vector Control, 1964-1968.

Summary of research by TDL on chemical control of arthropods.

15th Meeting of Expert Committee on Insecticides, 1965

Wet sieve analysis of DDT water dispersible powders.

Specifications for DDT water-dispersible powders.

Determination of suspensibility in distilled water.

A high temperature accelerated storage treatment for dieldrin water-dispersible powders.

Recommended changes in the specification for dieldrin water-dispersible powder.

Water content of dieldrin water-dispersible powder.

Concentration, graduate size, and aliquoting for suspensibility determinations on water-dispersible powders.

Methods of analysis for malathion.

Recommended specifications for malathion water-dispersible powders.

Standardization of glassware for suspensibility tests.

Packaging requirements in AID specifications for DDT, 75% water-dispersible powder.

16th Meeting of Expert Committee on Insecticides, 1967

Chemical control methods for vectors and pests of public health importance.

Laboratory Directors' Meeting, WHO Program on Evaluation of Insecticides for Vector Control, 1968

Test procedures and criteria of acceptance.

Informal Consultation with Chemists on Chemistry and Specification of Pesticides, 1968

Laboratory analytical trials with DDT.

Factors influencing storage stability of DDT powders.

New analytical method for malathion.

Analytical methods for Abate.

Specification for insecticide, 43% Abate emulsifiable concentrate.

Specification for insecticide, 1% Abate granules.

Flash point requirements for pesticide emulsion concentrates.

17th Meeting of Expert Committee on Insecticides, 1968

Chemical control methods for vectors and pests of public health importance.

18th Meeting of Expert Committee on Insecticides, 1970

Revision of WHO specifications for the hand compression sprayer.

Field trials of modified X-Pert Sprayers.

Nozzle tip erosion evaluation - a standard method.

Nozzle tip replacement schedule - a proposal.

The disc flow regulator - a reassessment.

Compatibility of the disc flow regulator with OMS-33.

Specifications for nozzles.

19th Meeting of Expert Committee on Insecticides, 1971

Evaluation of methods for determination of the active ingredient in Abate formulations.

Use of the potassium salt of O,O'-Dimethyl Phosphorodithioc acid as a standard for colorimetric and titrimetric methods for analysis of malathion.

Studies on the chemistry, methods of analysis, and storage stability of malathion formulations.

Recommended changes in methods of analysis for malathion in malathion technical, malathion water-dispersible powders, malathion emulsion concentrates and malathion dusting powders.

Recommendations on the accelerated storage test for malathion water-dispersible powders.

Storage stability of 75% DDT water-dispersible powder as predicted from accelerated storage testing.

Comparative evaluation of four fenitrothion water-dispersible powder samples.

Studies on the chemistry, methods of analysis and storage stability of malathion formulations.

APPENDIX 5

AID Specifications for DDT Water-dispersible Powder, 1954-70

AID specifications for DDT water-dispersible powder, 1954-70

Specification			Suspensibility requirement, %			Accelerated storage treatment	Required volume of container
			As received	After accelerated storage	After long term storage		
FOA	43054	1954	1.5[a]	1.5[a]		55 ± 2°C- 20 hr	None
FOA	71554-2	1954	1.5[a]	1.2[a]		" "	"
ICA	101055	1955	1.5[a]	1.2[a]		" "	5.25 cu ft/100 lb
ICA	81056	1956	1.5[a]	1.2[a]		" "	26 gal/100 lb
ICA	9458	1958	1.5[b]	1.2[b]		" "	26 ± 3/4 gal/100 lb
ICA	1859	1959	1.5[c]	1.2[c]		" "	"
ICA	72059	1959	1.75[c]	1.5[c]		" "	"
ICA	2373-1	1961	1.75[c,d]	1.35[c]		70 ± 0.1°C - 2 hr	"
AID	2373-2	1964	1.75[c,d]	1.35[c]		" "	17-3/4 ± 1/2 gal/75 lb
AID	2373-3	1964	1.75[c,d]	1.35[c]	1.25[c,e]	" "	"
AID	2373-4	1966[g]	1.75[c,d]	1.35[c]	1.25[c,f]	" "	"
AID	2373-5	1966[g]	1.75[c,d]	1.625[c]	1.25[c,f]	" "	18.24 ± 1/4 gal/75 lb
PHS/NCDC-I-102		1967[g]	1.75[c,d]	1.625[c]	1.25[c,f]	" "	"
PHS/NCDC-I-102a		1970[h]	1.75[c,d]	1.625[c]	1.25[c,f]	" "	"

a 5% suspension prepared in a quart jar and diluted to 2.5% in 100 ml graduate.

b 2.5% suspension in a 250 ml graduate. Aliquot taken from center.

c 2.5% suspension in a 100 ml graduate. Aliquot taken from center.

d Tested in distilled water.

e After 6 months in ambient storage.

f After 12 months in ambient storage.

g Batch size increased to 11,000 lb. No composite samples.

h Batch size increased to 21,000 pounds. No composite samples.

APPENDIX 6

Specifications for Pesticides other than DDT

Specifications for Pesticides other than DDT

Specification	Year	Insecticide
ICA No. 1	1957	50% Dieldrin Water-Dispersible Powder.
ICA 5958	1958	75% Dieldrin Water-Dispersible Powder.
ICA 72259	1959	75% Dieldrin Water-Dispersible Powder.
AID 2361-1	1961	Malathion, 50% Water-Dispersible Powder.
PHS/NCDC-I-202	1968	Insecticide, 82% Malathion Emulsifiable Concentrate.
PHS/NCDC-E-100	1967	Surfactant, Nonionic Anionic Blend.
PHS/NCDC-E-100a	1970	" " " "