the remaining half of the road, but only one side was treated because the aerosol could be used only during inversion, and air movements toward the other side occurred only during lapse conditions.

In a series of 10 tests with fronts up to a half-mile, the effective penetration was not sufficient to prevent reinvasion of mosquitoes and blackflies within 24 hours.

There was some evidence that blackflies were not readily killed by the aerosols used.

STUDIES USING DDT APPLIED IN AIRPLANE THERMAL EXHAUST AEROSOLS FOR THE CONTROL OF ANOPHELINE LARVAE IN RICE FIELDS IN CALIFORNIA [1]

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I. INTRODUCTION

The control of *Anopheles freeborni* (Aitken) larvae in California rice fields has always been a difficult task. Mosquito control ground crews are limited in the extent to which they can treat rice fields with spray equipment. Dr. Basil G. Markos (1949) has shown that in California anopheline larvae breed throughout a rice field, in the center as well as along the edges. Spray crews can treat only the edges of rice field checks, and are unable to penetrate the center of the checks with larvicides. Therefore airplane treatment would be a very desirable method of applying larvicides to cover the extensive area of individual rice fields.

Krusé and Metcalf (1946) successfully controlled anopheline larvae in impounded water by the use of DDT applied in airplane thermal exhaust aerosols. The Sutter-Yuba Mosquito Abatement District during 1946 conducted airplane thermal exhaust aerosol operations using similar equipment and techniques with apparent control of *Anopheles* larvae in rice fields.[2] The studies reported herein were conducted in the Sutter and Yuba Counties during routine control operations in 1947.

In cooperation with the Sutter-Yuba Mosquito Abatement District, the State Department of Public Health, Bureau of Vector Control, conducted fourteen studies during September, 1947, which were part of a routine program to treat 18,000 acres of rice in the vicinity of Yuba City and Marysville.

II. OBJECTIVES

1. To determine if the thermal aerosol dispersal method was applicable for the control of anopheline larvae in rice fields in the Sutter-Yuba Mosquito Abatement District.
2. To determine the minimum dosage of DDT required to give 90% control of *A. freeborni* larvae in rice fields.
3. To determine the rapidity of recurrence of *A. freeborni* larvae after treatment in rice fields.

[1] This report is one of a series on continuing mosquito control operational and biological studies begun in 1946 by the Bureau of Vector Control, Division of Environmental Sanitation of the California State Department of Public Health in cooperation with various Mosquito Abatement Agencies.

[2] Unpublished records are in the permanent file of the Sutter-Yuba Mosquito Abatement District, Yuba, California.

4. To determine the economics of airplane applications of DDT larvicides for anopheline larval control in rice fields.

III. EQUIPMENT AND TECHNIQUES

The equipment and techniques used in these studies were largely those developed by the Tennessee Valley Authority for the control of anopheline larvae with DDT aerosols produced by airplane exhaust aerosol generators as reported by Krusé and Metcalf (1946).

Airplane Equipment

A converted Navy Primary trainer with a 300 horsepower motor was used in these studies. It was equipped with a 6″ diameter exhaust stack that extended the length of the fuselage. A 2″ venturi was fitted onto the end of the exhaust stack. Two flat spray nozzles obtained from an Essick ground sprayer, projected into the venturi. Essick WS-45-3 discs were used in each nozzle. The rate of discharge was between 1.8 to 2.5 gallons per minute. This was dependent upon the viscosity of the material, the pump pressure, the cleanliness of each spray disc, and the operating efficiency of the airplane engine.

A pressure of approximately 80 pounds was maintained by a wind-driven, fan-type pump, which was mounted on the upper surface of the lower wing.

Flight Data

For the majority of the studies the airplane was flown at a height of 25 feet with 100-foot swaths paced off on the ground by one man. This swath gave an adequate distribution of the aerosol. Several studies were flown at 75-foot swaths in order to compare the effectiveness of this width with 100-foot swaths.

Measurement of Acreage

Acreage was measured by determining the treatment time with a stop watch that was held by the pilot. Since the plane speed was practically constant at 80 miles per hour and the swath width of 100 feet was measured from the ground, it was easy to calculate the acreage treated by the plane in any given time by multiplying the swath width by the length of the field.

DDT Recovery and Droplet Studies

Six floats were set out in the vegetation with four clear 1 x 3″ glass slides on each float. In addition, one magnesium oxide-coated slide was placed on each of five of the floats. After the airplane treatment of a rice field, the slides were collected and the clear slides were chemically tested for DDT deposit. The magnesium oxide-coated slides were examined for droplet size distribution, mean diameter of the droplets and number of droplets in one square centimeter.

Meteorological Data

(1) *Relative humidity* was determined with the use of a sling psychrometer.
(2) *Wind velocity* was determined with the aid of an anemometer. The wind velocity reading was taken three times during each flight, and an average of the three was recorded.
(3) *Conversion and Inversion Temperature* conditions were determined by placing a thermometer at one foot above the ground and another thermometer at six feet above the ground. A difference in temperature would indicate whether the air was rising or falling. If the 1-ft. reading was higher than the 6-ft. reading, this would give rising or conversion air currents. If the 1-ft. reading was lower than the 6-ft. reading, this would result in falling or inversion air currents.

For airplane aerosol application, inversion conditions are desirable, because of the slow settling rate of the smaller aerosol droplets. A majority of the flights were made early in the morning in order to take advantage of the inversion climatic conditions which were usually present at that time of day.

Water Factors

The water in the rice fields originated from wells or irrigation canals. The water tended to be slightly acid with a pH of 6.0 to 7.0, the pH being determined with pH Hydrion paper. Other water data included temperature, depth, and presence or absence of algae and flotage. The growth of the rice in the fields was considered to be of heavy vertical density and of medium height.

Method of Selecting and Sampling Study Areas

A single check of a rice field was selected for each study. Pre-treatment dips, 24 hour post-treatment, and other post-treatment dips were made at later dates. Three man-hours were devoted to each check. If two men were dipping, each dipped for one and a half hours; if three men were dipping, each dipped for one hour. Each man took two dips every four steps along the edge of the rice check. The dips were taken in multiples of ten. The surface of the water was skimmed for a distance of about 1½ feet with a white dipper attached to a five foot pole. The checks selected for the studies had their levees more or less at right angles to one another. Thus, each walked in a perpendicular direction to the other, so that during the airplane treatment, one man's series of checks would parallel the line of flight of the plane.

If the rice grew adjacent to the levee the men walked on the levee and dipped into the rice, but where "borrow" furrows were present and the rice was sparse in the furrows, they walked into the borrow furrows and dipped into the main stand of rice.

Entomological

Anopheles freeborni (Aitken) and *Culex tarsalis* (Coquillett) were the dominant species of mosquitoes found. *Anopheles franciscanus* (McCracken) and *Culex erythrothorax* (Dyar) were present in lesser numbers. Anopheline larvae were counted according to separate instars and pupae. *Culex* larvae were separated into three groups: First and second (1–2) instars, third and fourth (3–4) instars, and pupae.

Larval and pupal counts were taken 6–24 hours before treatment, and 24 hours after treatment. Post-treatment larval and pupal counts were made for the recurrence of anopheline larvae and pupae up to at least 14 days after treatment. The degree of control was based upon the per cent of larval reduction.

A large number of dips (130–230) were necessary in order to have sufficient larvae to give an analysis of the percentage of larval reduction. There was an average of at least one larva per dip, frequently two and three. There was considerable variation among the rice fields in the number and density of larvae.

Materials Used

A stock solution of a technical DDT concentrate in General Petroleum Distillate 544C (PD-544C), the basic material used, contained 1.71 pounds of DDT per gallon. This concentrate was diluted with diesel oil in various ratios. One study was made with straight diesel oil and DDT.

IV. DISCUSSION OF RESULTS

Toxicity Data

The first eleven studies are summarized in Table 1, where they are listed according to the amount of DDT applied per acre. One study in which DDT and diesel oil alone was used and two of the studies in which 75-foot swaths were used are listed separately. It is evident that an average of 96.5% control of anopheline larvae was obtained in the studies with the exception of Nos. 1, 2, & 3 in which the disc from one of the nozzles was missing. The insecticidal solution was dispersed as a dense spray rather than as an aerosol.

Of significance was the fact that 98% anopheline larval control was obtained with as little as one pint of DDT solution per acre, which gave a dosage of 0.025 pounds of DDT per acre. These studies have not indicated an approach to the minimum lethal dosage of DDT per acre.

There were six studies with sufficient *Culex* larvae present to give significant data of larval reduction. A minimum of 100 *Culex* larvae per study in the pretreatment count was considered a significant number to be tabulated. Four of the six studies gave better than 98% larval control. Studies Nos. 2 and 5 resulted in a 64% and 63% larval reduction. The low larval reduction rate of study No. 2 may have been due to the fact that the disc of the larvicide injection nozzle was missing.

Measured Factors

Inspection of the data on the measured factors as listed in the following paragraph did not seem to indicate that the results were controlled by any single factor or combination of factors, since the results demonstrated above 85% control with the exception of one study.

The rate of application varied from 0.116 to 0.331 gallons per acre; pounds of DDT from 0.025 to 0.228 per acre; wind velocity from 0 to 10 miles per hour; relative humidity from 18 to 76%; inversion and conversion temperature differences from +1 to −4° F.; air temperature from 56° F. to 96° F.; water temperature from 59° F. to 73° F.; pH from 6.0 to 7.0; and water depth from 6″ to 24″. The mean droplet diameter for each study varied

TABLE I.—Reduction of Mosquito Larvae in DDT Airplane Thermal Exhaust Aerosol-Treated Rice Fields in California, September, 1947

Study No.	Material Used [2]	Swath	Lbs. DDT per Acre	Gallons Per Acre	*Anopheles* percentage reduction	*Culex* percentage reduction
2 [1]	2 gals. PD 544C—DDT Con. 3 gals. pure PD 544C	100′	0.228	0.331	96.5	64.0
3 [1]	1 gal. PD 544C—DDT Con. 4 gals. diesel oil	100′	0.100	0.292	96.5	–
4	1 gal. PD 544C—DDT Con. 4 gals. diesel oil	100′	0.062	0.176	99.5	–
5	1 gal. PD 544C—DDT Con. 4 gals. diesel oil	100′	0.054	0.157	89.0	63.0
6	1 gal. PD 544C—DDT Con. 4 gals. diesel oil	100′	0.054	0.157	100	100
12	1 gal. PD 544C—DDT Con. 4 gals. diesel oil	100′	0.040	0.117	99.5	98.0
13	1 gal. PD 544C—DDT Con. 4 gals. diesel oil	100′	0.040	0.117	85.0	100
7	1 gal. PD 544C—DDT Con. 8 gals. diesel oil	100′	0.031	0.165	100	100
9	1 gal. PD 544C—DDT Con. 9 gals. diesel oil	100′	0.026	0.132	98.5	–
14	1 gal. PD 544C—DDT Con. 9 gals. diesel oil	100′	0.025	0.117	98.0	–
15	1 gal. PD 544C—DDT Con. 9 gals. diesel oil	100′	0.025	0.117	100	–
10	0.4 lbs. DDT. per gal. diesel oil	100′	0.046	0.116	97.0	–
1 [1]	1 gal. PD 544C—DDT Con. 1 gal. pure PD 544C 8 gals. diesel oil	75′	0.069	0.403	55.0	–
8	9 gals. PD 544C—DDT 51 gals. diesel oil	75′	0.055	0.214	99.5	–

[1] Disk from one nozzle missing; insecticide emitted as coarse drops.
[2] One gallon of PD 544C concentrate contains 1.71 lbs. DDT.

from 12.5 to 54.0 microns; the mean diameter for all studies was 29.5 microns. The number of droplets per square inch varied from 7 to 73 drops per study with the exception of study No. 9 which had 145 drops. Of these droplets, 75% were less than 10 microns in diameter.

Studies Nos. 6, 12, and 9 had 122%, 76% and 181% DDT recovery, respectively. Aside from these three studies, the percentage of DDT recovery varied between 9% to 52% with an average recovery of 23%. Excessively large recoveries may have been due to unusual microclimatic conditions which resulted in extra large amounts of the material being deposited on the slides. One excessively large drop on a slide could throw off the results. One droplet of 100 microns in diameter is equal by weight to 1000 droplets of 10 microns in diameter. Perhaps the main source of variation was that six floats with four slides each did not afford large enough sampling for the recovery test. Perhaps 10 or 12 floats with four slides on each would have given better samples and thus would have eliminated the source of sampling error.

Recurrence of Anopheline Larvae in Rice Fields

A study of the recurrence of anopheline larvae in treated rice fields was made. Six treated fields were observed at varying intervals of days up to at least 14 days. In two fields first instar larvae recurred within three days; in two fields the larvae recurred within five days; and in two fields the larvae recurred within six days. This indicated that there was no lasting effect from the airplane treatments.

The recurrence of anopheline larvae in one selected rice field treated on September 10 is graphically illustrated in Figure I. It brings out the fact that DDT aerosol treatments did not have any lasting effect. The number of first instar larvae continued to build up. The rate of development of the larvae seems to follow the mean daily temperature. From September 18 to September 22 there is a drop in the number of first instar larvae, and an increase of second and third instar larvae. Third and fourth instar larvae were more numerous as a result of normal larval development. The drop of first instar larvae on September 22 may be due to an earlier decline of the mean daily temperature on September 19. This drop in temperature may have retarded the incubation period of the eggs that were deposited, or else have limited the activities of ovipositing female mosquitoes.

On September 24th there was a very large increase in the number of first instar larvae and a corresponding increase in the fourth instar larvae. The increase in the first instar larvae appears to have followed a hot spell that occurred a few days earlier. There were a few early fourth instars at this time and if much more time were allowed to elapse, pupation would have occurred and adults would have emerged.

Under the existing temperature conditions prevalent in this area it seems advisable to treat the rice fields on the 10th day after the first treatment, to prevent an increase of pupae and subsequent adult emergence.

V. COST OF PROGRAM

On the basis of the preceding results a total of 18,000 acres of rice fields were treated by the Sutter-Yuba Mosquito Abatement District at approximately one pint per acre of an oil solution containing 0.4 of a pound DDT per gallon.

Four solvents were used: A mixture of PD 554C and diesel oil, straight diesel oil, Standard Base Oil W.T. and Union Solvent. The latter two were free samples from the respective oil companies. However, in computing the cost of the treatment, the prices of these two solvents were included in order to determine what the total cost of operations would have been if the district had had to pay for these materials.

A breakdown of the total costs of operations is listed in Table 2 under three headings: Cost of materials, ground crew (wages of one man for six weeks), and hiring a pilot and renting a plane. The cost was thirty cents per acre, which is

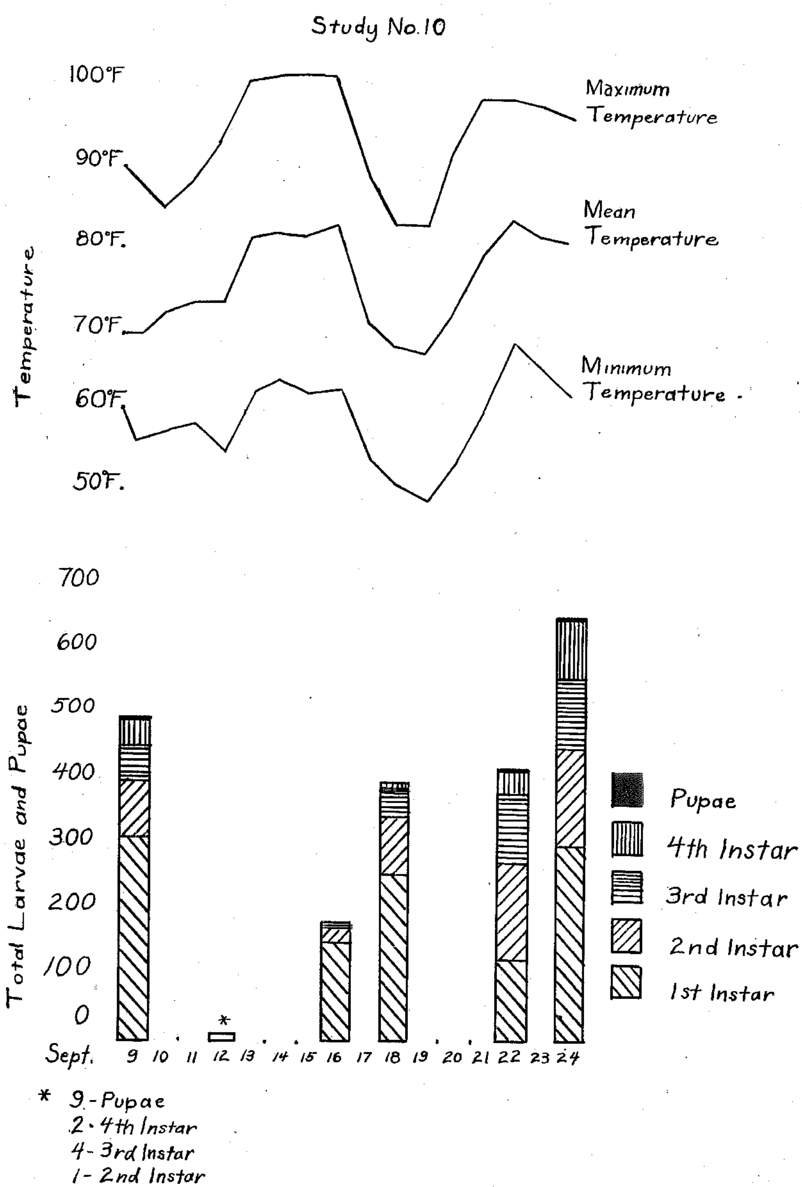

Figure 1. Recurrence of *Anopheles freeborni* Larvae in a Rice Field Following DDT-Airplane Treatment, Sutter-Yuba Mosquito Abatement District, 1947

considered to be economical for accomplishing mosquito control.

under temperature conditions prevailing in the Sutter-Yuba Mosquito Abatement District. Under the temperature conditions prevalent in the fall in this area, rice fields should be treated again by the 10th day after the first treatment.

TABLE 2.—Cost of Treating 18,000 Acres of Rice Fields by Airplane in the Sutter-Yuba Mosquito Abatement District.

September, 1947

1. *Material*						
PD 544C	—	1000 gal. at 29¢	—	$ 290.00		
Diesel Oil	—	3525 " " 8¢	—	280.00		
Standard Base Oil W.T.	—	100 " " 19¢	—	19.00		
Union Solvent	—	200 " " 17¢	—	34.00		
Total	—	4825 " "	—	623.00		
Pounds DDT Tech. Grade	—	2260 lb. " 50¢	—	1,130.00		
Total Cost of Material			—			$1,753.00
2. *Ground Crew* at $200/mo.			—			350.00
3. *Total Flight Time* (74 hours at $45/hr.)			—			3,330.00
GRAND TOTAL COST						$5,433.00

Cost per Acre — 30 Cents.

VI. SUMMARY AND CONCLUSIONS

(1) The techniques and equipment developed by Krusé and Metcalf (1946) under the auspices of the Tennessee Valley Authority for the control of anopheline larvae using DDT as airplane thermal exhaust aerosols were applicable to the control of anopheline larvae in rice fields in the Sutter-Yuba Mosquito Abatement District.

(2) As little as 0.025 pounds of DDT per acre applied by this means gave 98% mortality of anopheline larvae.

(3) By flying at 25-foot heights resulting in at least a 100-foot effective swath, 98% control was obtained with a rate of one pint of insecticide which gave 0.025 pounds of DDT per acre. Flying less than 100-foot swaths was not economical.

(4) Variations of operational, meteorological, and biological factors did not appear to affect the results, as 96.5% control was obtained in all but the first three studies.

(5) There were not enough studies to warrant recommending the application of DDT for the control of *Culex* larvae, at the same dosage that was used for the successful control of anopheline larvae.

(6) Anopheline larvae recurred in rice fields within three days after treatment under temperature conditions prevailing in the Sutter-Yuba Mosquito Abatement District. Under the temperature conditions prevalent in the fall in this area, rice fields should be treated again by the 10th day after the first treatment.

(7) The control of anopheline larvae which were breeding in rice fields was economically accomplished by the airplane application of DDT applied in an aerosol produced from a thermal exhaust aerosol generator.

(8) More studies should be made in order: To determine the minimum lethal dosage of DDT applied as an aerosol with an airplane; to determine the effect of variations of operational, meteorological, and biological factors on anopheline larvae control when this method of DDT dispersal is used; to determine the number of thermal exhaust aerosol treatments that would be required in order to control recurring anopheline larvae following treatment.

ACKNOWLEDGMENT

Acknowledgment and appreciation is extended to the following men who assisted in the project: Grafton Campbell, Thor A. Holm, and Edmond C. Loomis, Bureau of Vector Control; Thomas M. Sperbeck, Manager, and William Miller of the Sutter-Yuba Mosquito Abatement District; Bud Marquis, pilot; Dr. R. L. Metcalf, University of

California, who visited the project and offered suggestions pertaining to the technical aspects of the project; and T. E. Hillis, Chemist, Division of Laboratories, California State Department of Public Health.

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LARVICIDE TESTS ON BLACKFLIES IN NEW HAMPSHIRE [1]

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In the summer of 1947 tests were made to evaluate several compounds as larvicides for blackflies breeding in streams in the White Mountains of New Hampshire. A material was sought which would control these insects but would be harmless to the fish population.

Little published information on this subject has been noted. O'Kane (1926) used oils of a soluble or miscible type, and these were effective under some conditions but were injurious to trout at dosages necessary to control larvae. Fairchild and Barreda (1945) found DDT effective against larvae in streams of Guatemala when applied as an emulsion at 0.1 p.p.m. Garnham and McMahon (1947) reported the eradication of simuliid larvae from an area in Kenya Colony, East Africa, with DDT dosages of 1.3 to 35.6 p.p.m.

In New Hampshire pyrethrum, DDT, benzene hexachloride, TDE, chlordan, caustic soda, and chlorinated lime were first tested in flumes at different dosages. The three most effective of these materials —TDE, DDT, and benzene hexachloride —were then tested under natural conditions in streams.

Procedure.—Flumes, or troughs, 6 feet long, lined with metal and water-proofed with shellac, were used for the preliminary tests with measured dosages of larvicides. The ends of the flumes were covered with fine-mesh wire. The flumes were placed in stream rapids so that a constant flow of water was maintained through them. Blackfly larvae attached to blades of grass were placed in the current. The larvicides were introduced into the flumes from a drip funnel gaged to deliver 1,000 cc. over a 10-minute interval. The desired dosages of toxic agent were obtained by varying the concentration of the solution or emulsion. At intervals of 10, 30 and 60 minutes counts were made of the detached larvae and of the normal larvae that remained attached to the blades of grass. Control counts were made of larvae subjected to the same handling in the flumes except that water alone, or acetone solvent alone, was added through the drip funnel.

In the stream tests pretreatment counts of the larval population on marked rocks and blades of grass were made in measured sections of a stream. The larvicides were introduced at the upper end of the test area during a 10-minute period, and posttreatment counts were made 1 or 1½

[1] This work was conducted under a transfer of funds, from the Office of the Surgeon General, Department of the Army, and, in part, from the Bureau of Medicine and Surgery, Department of the Navy, to the Bureau of Entomology and Plant Quarantine.

[2] The work was carried out under the supervision of C. C. Deonier.