# The Effectiveness of Ultra-Low-Volume Applications of Malathion at a Rate of 6 US Fluid Ounces per Acre in Controlling *Aedes aegypti* in a Large-scale Test at Nakhon Sawan, Thailand \*

CLIFFORD S. LOFGREN, HUGH R. FORD, ROBERT J. TONN & SUJARTI JATANASEN 4

The effectiveness of ultra-low-volume (ULV) applications of technical malathion to control Aedes aegypti was studied in Nakhon Sawan, Thailand, in November 1968. Two treatments of 6 US fl oz/acre (438 ml/ha) were applied 4 days apart by a C-47 aircraft equipped with a fuselage-mounted spraying boom. An area of 7 square miles (18 km²), which included the entire city area, was treated.

The landing rate of adult A. aegypti adults was reduced significantly following each malathion application (95% and 99%, respectively), the reduction ranging from 88% to 99% during the 10-day post-treatment observation period. No eggs were deposited in ovitraps 4 days after the first application. Dissections of female mosquitos collected during the post-treatment period showed that only 8% of the females were parous compared with 30% before treatment and 40% in the check area. Night landing rates of other mosquitos were reduced by 82%–97%; housefly populations also decreased markedly. The test results indicate that UVL treatments with malathion applied by aircraft can be used to control A. aegypti during outbreaks of dengue haemorrhagic fever. The fuselage-mounted spraying system used in the tests performed very well. The equipment can be mounted on, and removed from, a C-47 aircraft very quickly, thus making it possible to convert such aircraft used for other purposes into spraying planes at quite short notice.

Dengue haemorrhagic fever is a serious arbovirus disease in South-East Asia that is transmitted by a mosquito, Aedes aegypti L. It primarily affects young persons under 16 years of age. During the past decade several epidemics of the disease have occurred in Thailand (Bangkok) and the Philippines (Manila) and, as a result, the World Health Organization established a research unit in Bangkok, Thailand, in 1966 to study the vector and develop methods for

its control. As part of this research programme, an evaluation of the ultra-low-volume (ULV) technique of insecticide application from aircraft was undertaken. The goal of this trial was the establishment of an inexpensive, readily usable means of vector control with which health authorities in south-east Asia might prevent or halt epidemics of haemorrhagic fever.

The first series of ULV tests in the programme were conducted early in 1968 and have been reported by Kilpatrick, Tonn & Jatanasen (1970) who obtained good control of A. aegypti in a Thai village on the outskirts of Bangkok with an application of ULV malathion applied at a rate of 6 US fl oz/acre (438 ml/ha) during a 7-day period. The applications were made with a Cessna-180 aircraft equipped with a Sorenson spraying unit. Tests with a lower rate of application, namely, 3 US fl oz/acre (219 ml/ha) were unsuccessful.

The Cessna-180 is a single-engined aircraft and

<sup>\*</sup>This study was supported jointly by US Public Health Service Grant No. CC 00174 from the National Communicable Disease Center, Atlanta, Ga., USA, and by the World Health Organization.

<sup>&</sup>lt;sup>1</sup> Entomologist, Entomology Research Division, US Department of Agriculture, Gainsville, Fla., USA.

<sup>&</sup>lt;sup>2</sup> Agricultural research technician, Entomology Research Division, US Department of Agriculture, Gainsville, Fla., USA.

<sup>&</sup>lt;sup>a</sup> Project Leader, WHO Aedes Research Unit, Bangkok, Thailand.

<sup>&</sup>lt;sup>4</sup> Communicable Diseases (Control) Division, Ministry of Health, Bangkok, Thailand.

therefore not entirely suitable for operation at low altitudes over metropolitan areas. As a result, it was decided to conduct additional studies with multiengined aircraft and arrangements were made with the Royal Thai Air Force for the use of a C-47 aircraft. In addition, the Insects Affecting Man and Animals Laboratory of the US Department of Agriculture at Gainesville, Florida, was asked to provide technical assistance and to install an insecticide-spraying apparatus in the C-47 aircraft.

For these trials, it was decided to make 2 applications of ULV malathion at the rate of 6 US fl oz/acre (438 ml/ha) to a city of about 50 000 inhabitants to determine if *A. aegypti* could be controlled effectively by this method and to determine how long the control would last.

Since the aircraft obtained for the tests was not already equipped for spraying, and for various reasons—such as cost, the need for modifications to the aircraft and the disruption of normal usage of the aircraft—it was impracticable to attempt to install a conventional spraying unit with wing booms. As a result, it was decided to evaluate a system used by the Gainesville Laboratory that utilizes a short boom mounted on the aircraft or just underneath it. A description of the spraying unit and its installation is given in another article (Lofgren et al., 1970). Additional information on the development of the spraying system and the results of the biological evaluation made during field trials conducted in late 1968 will be presented in a later paper.

## DESCRIPTION OF TEST AREA

Nakhon Sawan was selected as the test site for a number of reasons. The *A. aegypti* population was comparable with that in Bangkok and several cases of dengue haemorrhagic fever were reported in children during 1968. The Provincial Health Officer, whose approval was needed for the tests, had observed some of the preliminary tests conducted by Kilpatrick, Tonn & Jatanasen (1970) and was convinced of the safety of the treatment and the need for the trial. Another important factor in the selection of this city was the presence of an airfield on which the C-47 aircraft could be landed for preliminary briefings before the spraying treatment began.

Nakhon Sawan has been a river port for many years but it is also served by the northern railroad, Highway 1, and a small airport. It is located about 341 km north of Bangkok where 2 forks of the Chao

Phaya River join. The terrain around the city is mostly plains but some hills, 200–300 feet high (60 m–90 m) are found on the northern and southwestern sides. The climate is similar to that of Bangkok and during the trial it was warm and dry. There was some ground fog in the morning but it disappeared before spraying operations began.

The total area of the city was about 3 square miles (7.75 km²); however, to ensure complete insecticide coverage, an area 2.2 miles by 3.3 miles (3.5 km by 5.3 km) or 7 square miles (18 km²) was treated. The city area contained 8804 houses and shophouses. The total urban population was approximately 46 000, of whom 18 277 were school-children. Nakhon Sawan is also the site of one of the regional government plague laboratories which was used as headquarters and laboratory for the trials.

One week before the first application of malathion 4 study areas in which the effectiveness of the treatments was to be assayed were selected. These areas were chosen on the basis of location and type of housing. A brief description of each site follows.

Site 1 was a shophouse—market area in the business district of the city. The majority of the shophouses were cement 2-storey buildings but a few were single-storey wooden-framed buildings. The market area was mostly under cover but some goods were sold in open-air stalls. As in most shophouse areas, there were very few open spaces and the majority of the water containers were stored inside the buildings. Many of the windows were screened.

Site 2 was a middle-income to low-income housing area; all the structures were wooden. Some shophouses lined the streets but the majority of the buildings were single-family homes. Many of these houses were built on stilts with a sleeping or cooking area underneath. The streets were gravel or clay. The area had a piped water system with a tap on nearly every premise. Water jars were located both indoors and outdoors, many being beneath the houses. Few of the houses were screened and most of the people slept under mosquito netting.

Site 3 was a low-income to slum area near Wat Nakhon Sawan. All the structures in this area were wooden and the majority were single-family premises. There were a few shophouses and a market in the centre of the area. Here, as in site 2, the majority of the houses were on stilts. There was standing water under many of the houses and guppies were used to control *Culex*. The area had a piped water supply with a tap on nearly all premises. Water jars were located both indoors and outdoors.

Site 4 was a middle- to high-income housing area; all the buildings were wooden. Facing the road were several shophouses but the majority of the structures were single-family houses. Some of the houses were on stilts with a bathing-toilet area and a cooking or sleeping area under the house. Other houses were constructed on cement slabs. No standing water was observed. Each house had a piped water supply. Water jars were found indoors and outdoors.

A check area was established in the market area of the village of Nongben, located about 18 km north of Nakhon Sawan on Highway 1. The area consisted of 2-storey wooden shophouses lining both sides of the road and some single-storey wooden houses behind the shophouses. There were a number of open sewers near the houses but no standing water. Water was drawn from wells and more containers were used for storage than in Nakhon Sawan.

## **EVALUATION TECHNIQUES**

Prior to treatment, a 1-larva-per-container survey (Sheppard, Macdonald & Tonn, 1969) was made to establish the extent of the Aedes population at each study site. The effects of the malathion treatments were assayed by several techniques: landing-rate counts were made of A. aegypti in the morning and of all mosquitos in the evening, ovitraps were used to check the incidence of gravid females, and dissections of female mosquitos were made to determine the age structure of the population. The housefly population was also assayed with fly-grid counts in the markets. Bioassay tests with caged adults and larvae of A. aegypti and Culex pipiens fatigans were made in each study area. Finally, distribution of malathion droplets was determined with the aid of oil-sensitive, red dye cards. Detailed descriptions of most of these techniques are given in Kilpatrick et al. (1970) and only brief descriptions are presented here.

## Landing-rate counts

Collections of male and female mosquitos attracted to, and landing on, mosquito scouts were made from 0730 hours to 1200 hours and from 1900 hours to 2100 hours. For the morning, or *Aedes*, landing collections, 2 scouts were assigned to each of the first 3 evaluation sites and to the check site. Each scout was assigned 9 houses and collections were made for 20 minutes in each house. Evening collections of all mosquito species were

made including A. aegypti, C. p. fatigans, 4 species of Anopheles, species of Mansonia and various other species of Culex. Each scout was assigned 1 house in which to make the collections. The houses were permanent sites and the same scouts visited the same houses during each collection period.

Prior to the first treatment, and to establish the pretreatment population level of mosquitos, 3 collections were made. After treatment, landing-rate counts were made.

# **Ovitraps**

Altogether, 20 traps were set out in each of the 4 evaluation sites and the check area and placed near existing larval habitats. Those traps placed outdoors were located near water jars and those indoors near either water jars or ant traps. The paddles were collected after being exposed for 48 hours and examined under  $\times 10$  magnification for the presence or absence of eggs; the total number of eggs was also recorded.

The traps were employed for a period equal to 3 life-cycles to establish a pretreatment level of egg-laying. On the day of treatment, new paddles were placed in the traps which were then run continuously until the end of the evaluation.

#### Dissections

A. aegypti females collected during the landing collections were dissected to determine the state of feeding and ovarian development (Christophers' stages) and whether or not they were parous or nulliparous. All these factors provide evidence on the age of the females.

# Fly counts

During the ULV insecticide trials using a singleengined aircraft (Kilpatrick et al., 1970), it was noted that the spray apparently killed large numbers of flies in the markets. It was decided to test the validity of this observation in the present study. A wooden grid, 61 cm² with 10 strips each about 3.5 cm wide, was used. The grid was placed in a market area and the mosquito scouts counted the number of flies which landed on the strips from 1300 hours to 1400 hours. Counts were made in the first 3 sites at Nakhon Sawan and at the check area.

# Bioassay

Caged A. aegypti and C. p. fatigans adults were used for the bioassays. The larvae were caged in 8-oz (about 220-ml) paper cups containing 100 ml

of water. The adults in the first test were exposed in wire-screen cages measuring 1 in by 4 in by 6 in (2.5 cm×10 cm×15 cm). These cages were very cumbersome to use and many mosquitos escaped through holes. In addition, no facilities were available for anaesthetizing the mosquitos so that they could be transferred to clean holding-cages. For the second test, a new type of cage was constructed by cutting away all but 2 narrow strips of the sides of 8-oz paper cups and covering the cups with nylon tulle. The nylon tulle was of a finer mesh than the screen originally employed and the nylon strands were much finer than the wire. As a result, more insecticide could probably penetrate these cages.

The mosquitos were exposed at 10 locations within each study area and during the second treatment also along a road that bisected the city. At sites 1, 3 and 4, half the locations were inside houses and half underneath the same houses. In other words, all the bioassay material was under at least partial cover. At site 1, the shophouse area, half the material was placed outside the houses and half inside. In all cases, the material inside the houses was placed on the floor near the centre of a room. Two sets of 5 cages of *Culex* and *Aedes* adults and larvae were taken to the check area prior to the treatment and recovered about 4 hours after the spraying was completed.

The cages in the test area were exposed to the spray for 45 minutes after which they were returned to the laboratory. In the first test, the adults were transferred to clean cages and the mortality was determined after 12 hours; in the second test the mosquitos were left in the cages and mortality was determined after 6 hours. All larval mortalities were read after 24 hours.

# Droplet collections

As an indication of the distribution of the spray, malathion droplets were collected on oil-sensitive red dye cards. One card was placed at each of the bioassay stations in the treatment area and at intervals of 300 feet (about 90 m) along a road that bisected the city. The cards were collected about ½-1 hour after the end of the insecticide spraying. Examinations for spots left by droplets of insecticide were made with the aid of a dissecting microscope. Droplets were also collected on glass slides treated with silicone and were measured with a compound microscope and an ocular micrometer. Mass median diameters were calculated by the method of Yeomans (1949).

### INSECTICIDE APPLICATION TECHNIQUES

The insecticide spray system consisted of an Oberdorfer gear pump driven by a 12-volt motor that pumped the insecticide through a small boom mounted 30 inches (75 cm) below the fuselage near the cargo doors of the aircraft. The system depends on fine atomization of the spray and on cross-winds to drift the insecticide across the treatment area to obtain the desired swath width, namely, 500 feet (152 m). The boom was equipped with 10 Tee-Jet nozzles and No. 8008 flat-fan tips. The nozzles were positioned at an angle of 45° forwards into the wind in order to obtain good atomization. Smaller tips would have produced smaller droplets but under the operating conditions the tips used were the smallest that would deliver the amount of insecticide required—namely, 7 US gallons per minute (26.4 1/min) at a pump pressure of 46 lbf/in<sup>2</sup> (1.1 kgf/cm<sup>2</sup>). During the application of insecticide, the aircraft was flown at a height of 150 feet (46 m) and a speed of 150 miles/h (241 km/h) with a swath width of 500 feet (152 m). It was necessary to rely primarily on the pilot's ability to fly a consistent flight pattern at 500-feet (152-m) intervals. A pilot untrained in low-level insecticide spraying flew the aircraft during the first application. He had difficulty in maintaining uniform spacing which resulted in non-uniform application. This problem was overcome during the second application when an experienced pilot was available.

Climatic conditions at the time of both applications were favourable. During the first flight (18 November 1968) the wind was from the south-east at approximately 30° to the flight path during most of the treatment period (0700 hours to 0830 hours). The wind speed varied considerably but was about 2 miles/h (3.2 km/h) at the start and 3–6 miles/h (4.8 km/h–9.6 km/h) by the end of the test. The air temperature was from 71°F to 75°F (21.7°C–23.9°C). During the second application (22 November 1968) the wind was very light (less than 2 miles/h (3.2 km/h) at ground level) and from a northerly direction. The air temperature was from 65°F to 70°F (18.3°C–21°C) during the application period (0700 hours to 0800 hours).

A total of 460 US gallons (about 1740 litres) of malathion (95%) was applied during the 2 trials. A little more than half (256 US gallons; 969 litres) of this amount was applied during the first treatment Shorter swaths accounted for the lesser amount applied during the second treatment.

## RESULTS

The results of the 1-larva-per-container survey (see Table 1) show that the *Aedes* premises index, or the percentage of houses with at least 1 container with *Aedes* larvae, varied from 58% in the shophouse area (site 1) to 94% in the check area. The average number of receptacles per household varied from 4.9 at site 2 to 8.1 at the check area, while the average number of positive receptacles per premises ranged from 1.6 at sites 1 and 3 to 3.8 at site 2. These data indicate a substantial *A. aegypti* population in all the study areas.

Excellent control of A. aegypti was obtained with the 2 malathion applications (see Table 2). The average control for the 3 study sites ranged from 92% to 95% following the first treatment and reached a high level of 99% the day after the second treatment. Thereafter, control declined and ranged from 88% to 92% during the remainder of the 10-day observation period. This degree of control represented an actual reduction in mosquitos collected from 8.6 per man per hour prior to treatment to less than 1, except for one collection period when it was 1.1 per man per hour. The slight increase in

numbers of mosquitos caught per hour at the end of the test was expected because of the large number of breeding sites in the area and since malathion does not have long residual control effects.

Dissections of female mosquitos collected during the post-treatment period (see Table 3) showed that most of the females were young (92% being nulliparous compared with 70% before treatment and 60% in the check area). In addition, almost 75% were unfed compared with 38.3% before treatment and egg development in 89.7% of the females was in the first of Christophers' stages.

Ovitrap data (Table 4) indicated a sharp drop in oviposition from the second to the fourth day after the first treatment (21.5%-2.5% positive paddles) and after the fourth day no more eggs were deposited. All these data also indicate that the mosquito population after the first treatment was young and, conversely, that the old mosquitos that would be the virus carriers in an epidemic situation were eliminated.

The control area showed a steady decline in mosquitos caught throughout the test period. This was also reflected in a decrease in the number of positive ovitraps. Despite this decrease, the collection rate

TABLE 1
SUMMARY OF THE RESULTS OF PRETREATMENT 1-LARVA-PER-CONTAINER SURVEYS
IN NAKHON SAWAN AND NONGBEN MARKET, THAILAND

	Site 1	Site 2	Site 3	Site 4	Nongben market
Premises index (%)	58.0	88.0	67.0	70.0	94.0
Average no. of containers per house	5.4	6.9	4.9	7.8	8.1
Average no. of positive containers per house	1.6	3.5	1.6	2.8	3.1
Fotal containers	540	689	490	784	807
Containers positive for A. aegypti (%)	28.9	50.9	33.3	35.7	38.0
Fotal outdoor containers	55	195	111	152	206
Outdoor containers positive for A. aegypti (%)	· 43.6	52.3	30.6	25.0	48.5
Fotal outdoor water jars	47	182	106	116	168
Outdoor water jars positive for A. aegypti (%)	46.8	51.6	29.2	27.6	50.6
Total indoor containers	485	494	379	632	601
ndoor containers positive for A. aegypti (%)	27.2	50.4	34.0	38.2	51.1
Total indoor water jars	199	259	215	258	334
Outdoor water jars positive for A. aegypti (%)	20.6	37.1	26.0	20.9	48.2
Total ant traps	193	214	144	244	180
Ant traps positive for A. aegypti (%)	38.3	69.1	46.5	63.9	55.0

TABLE 2
CONTROL OF AEDES AEGYPTI IN NAKHON SAWAN, THAILAND,
WITH 2 APPLICATIONS OF ULV MALATHION a APPLIED AT 6 US FL OZ/ACRE b

Site	Pretreatment count (mosquitos/	Percentage reduction after no. of days indicated following first treatment with malathion						
	man/hour)	1	2	3	5	6	8	10
1	3.9	87	96	92	100	100	92	96
2	15.7	99	94	89	100	82	90	83
3	6.3	89	100	100	97	95	97	100
Average	8.6	95	95	92	99	88	92	89
Check area	17.1	5	0	22	29	26	35	47

<sup>&</sup>lt;sup>a</sup> Malathion applied on days 0 and 4. All collections made between 0730 hours and 1200 hours.

TABLE 3

RESULTS OF MICROSCOPIC DISSECTIONS OF FEMALE

AEDES AEGYPTI FOR CONDITION, CHRISTOPHERS'

STAGES AND PAROUS RATES BEFORE AND AFTER

ULV MALATHION APPLICATION

	Spray area, before spraying	Spray area, after spraying	Check area
Condition:	-		
Unfed	38.3	74.3	32.5
Partly fed	25.5	23.1	48.8
Semi-gravid	23.4	2.6	11.3
Gravid	12.8	0	7.4
Christophers' stages:			
1	44.7	89.7	30.0
2	21.3	7.7	33.8
3	21.3	2.6	28.8
4	12.7	0	7.4
Parous rate:			
Parous	30.0	8.0	40.0
Nulliparous	70.0	92.0	60.0

in the check area was still above the pretreatment average for the test area at the end of the test (9 compared with 8.6 per man per hour).

Results from the night-time landing collections also indicated very good mosquito control (Table 5). Reductions ranged from 91% to 97% from the first

TABLE 4

EFFECT ON OVIPOSITION OF AEDES AEGYPTI

OF 2 TREATMENTS WITH 6 US FL OZ/ACRE <sup>a</sup>

OF ULV MALATHION IN THE CITY OF

NAKHON SAWAN, THAILAND

Site	Percentage of positive paddles before and at indicated days after first application of malathion								
	Pretreat- ment	2	4	6	8	10	11		
1	10.2	5.0	5.0	0	0	0	0		
2	53.3	40.0	0	0	0	0	0		
3	40.0	25.0	5.0	0	0	0	0		
4	30.0	15.9	0	0	0	0	0		
Average	33.4	21.5	2.5	0	0	0	0		
Check area	52.0	55.0	50.0	55.0	30.0	40.0	30.0		

a 438 ml/ha.

to the seventh days of the post-treatment observation period. On the last collection day the control dropped to 82%. This was due to a large increase in one of the study areas; control in the other two study areas was still good.

The housefly population was also decreased significantly by the malathion treatments (Table 6). Control ranged from 87% to 95% from the second to the seventh days, then decreased to 71% but rose to 91% on the last observation day. The final increase in control was probably attributable to

<sup>&</sup>lt;sup>b</sup> 438 ml/ha.

TABLE 5 CONTROL OF NIGHT-BITING MOSQUITOS IN NAKHON SAWAN, THAILAND, WITH 2 TREATMENTS WITH ULV MALATHION  $^a$  APPLIED AT THE RATE OF 6 US FL OZ/ACRE  $^b$ 

Site	Pretreatment count (mosquitos/	Per	Percentage reduction after days indicated following first treatment with malathion							
	man/hour)	0.5	1.5	2.5	4.5	5.5	7.5	9.5		
1	17.6	76	99	89	93	99	99	91		
2	4.6	89	95	95	100	100	100	100		
3	7.6	77	97	93	100	93	80	47		
Average	9.9	78	97	91	96	97	94	82		
Check area	5.2	3	18	47	0	0	0	0		

<sup>&</sup>lt;sup>a</sup> Malathion applied on days 0 and 4. All collections made between 1900 hours and 2100 hours. Mosquitos collected included A. aegypti, C. p. fatigans, 4 species of Anopheles, Mansonia sp. and Culex spp.

TABLE 6
CONTROL OF HOUSEFLIES IN NAKHON SAWAN, THAILAND, WITH 2 TREATMENTS
OF ULV MALATHION <sup>a</sup> APPLIED AT THE RATE OF 6 US FL OZ/ACRE <sup>b</sup>

Site	Pretreatment count (mosquitos/	Percentage reduction on days indicated following first treatment with malathion							
	man/hour)	0.5	1.5	2.5	4.5	5.5	7.5	9.5	11.5
1	561	94	98	97	99.9	99	97	93	91
2	1 940	81	81	94	92	90	73	65	94
3	1 038	55	92	96	94	93	92	88	86
Average	1 180	77	87	95	94	92	88	76	91
Check area	2 582	13	0	0	0	14	13	4	1

 $<sup>^</sup>a$  Malathion applied on days 0 and 4. All grid counts were made between 1300 hours and 1400 hours.

factors other than the insecticide treatment since malathion would not be expected to give residual control for more than about 2 days.

Results of the bioassays (Table 7) indicated only fair mosquito kills from the first malathion application, but good over-all kills of both larvae and adults during the second treatment. This is probably a reflection of the more uniform spacing of the swaths by the more experienced pilot. In almost every instance, better kills of mosquitos placed under houses than of those placed inside the houses were obtained but the kills of both larvae and adults in

the second test indicate that the insecticide sprays did penetrate most of the houses.

The data obtained from collecting droplets of insecticide on oil-sensitive red dye cards (Table 8) shows that generally good distribution was obtained. Droplet deposition varied greatly, depending upon the location of the dye card. Penetration of droplets into the houses did occur and generally more drops were found on cards inside and under houses in the second test than in the first. This supports the bioassay data which indicated much better penetration of droplets into the houses as evidenced by a greater

<sup>&</sup>lt;sup>b</sup> 438 ml/ha.

<sup>&</sup>lt;sup>b</sup> 438 ml/ha.

TABLE 7

MORTALITY OF CAGED ADULTS AND LARVAE
OF AEDES AEGYPTI AND CULEX PIPIENS FATIGANS

EXPOSED TO 6 US FL OZ/ACRE <sup>a</sup>

SPRAYS OF ULV MALATHION <sup>b</sup>

		Percentage mortality						
Site	Location of insects	Aedes a	aegypti	Culex pipiens fatigans				
		Larvae	Adults	Larvae	Adults			
	First application	(18 <b>N</b> ov	ember 19	68)				
1	Inside shophouse	1	79	39	23			
	Behind shophouse	53	89	98	68			
2	Inside house	11	63	73	69			
	Under house	85	98	100	79			
3	Inside house	18	62	30	18			
	Under house	61	71	64	28			
4	Inside house	20	90	46	55			
	Under house	60	75	63	71			
Check	_	0	0	5	14			

## Second application (22 November 1968)

1	Inside shophouse	41	100	91	100
	Behind shophouse	93	100	100	100
2	Inside house	19	100	98	100
	Under house	99	100	99	99
3	Inside house	29	100	84	100
	Under house	74	100	98	100
4	Inside house	82	100	83	100
	Under house	85	100	98	100
5	Road ditch	84	100	97	100
Check	_	0	3	2	7

a 438 ml/ha.

mosquito kill. The dye-card evaluation system has definite limitations for detecting droplets inside houses since only small droplets drift inside and these droplets do not readily fall on to the cards. In addition, droplets smaller than 20  $\mu$  in diameter may not make a spot on the red dye cards. As a result, the bioassays are more useful for determining the penetration of insecticide droplets into, or under, houses.

The average number of droplets on 10 red dye cards placed 300 feet (90 m) apart on the ground along a road ditch was 48.4/in<sup>2</sup> (7.5/cm<sup>2</sup>) in the first applica-

tion and 32.5/in<sup>2</sup> (5.0/cm<sup>2</sup>) in the second. The lower number and greater range in the second test is thought to be due to an early cut-off of the spray system before one of the swaths over the area where the cards were located was completed.

It had been hoped to make a determination of the droplet-size spectrum; however, satisfactory droplet collections that gave a completely representative sample of the spray could not be made in the test area. Droplets as small as 3  $\mu$  and as large as 200  $\mu$  in diameter were collected and measured on silicone-treated glass slides. On the basis of droplets collected, it is estimated that the mass median diameter of the spray was about 60  $\mu$ . Glass slides were also placed at the bioassay sites inside the houses. Again, the number of droplets collected was insufficient for a good evaluation; however, droplets ranging from 3  $\mu$  to 42  $\mu$  in diameter were observed and the mass median diameter of 70 droplets that were measured was 20  $\mu$ . This diameter should not be regarded as an optimum size for mosquito kills in houses because our collection methods were not efficient and very probably many of the smaller droplets were not collected. The smaller droplets could be collected with a cascade impactor but even with this device it is difficult to discriminate between malathion droplets and other airborne droplets or particles that are only a few microns in size.

## DISCUSSION AND CONCLUSIONS

It has been established that A. aegypti is a primary vector of dengue in areas of South-East Asia where haemorrhagic fever occurs. Other mosquitos such as A. albopictus are probably also important vectors. A. aegypti is highly anthropophilic and is collected primarily indoors. However, it does breed in containers found both indoors and outdoors. In epidemics of haemorrhagic fever, control of this mosquito would be a major point of attack in arresting or limiting the epidemic. Mosquito control should be rapid and of sufficient magnitude to curb the population until hosts in viraemia are no longer capable of transmitting the virus.

The tests reported in this paper illustrate that ULV applications of malathion by aircraft will effectively control A. aegypti and can, therefore, be considered for use when epidemics of haemorrhagic fever occur. All methods of evaluation of the test complemented each other, but the most convincing data were those obtained with the ovitraps and the dissections of

<sup>&</sup>lt;sup>b</sup> Mortality of adults determined after 12 hours in first test and after 6 hours in the second; all larval mortalities recorded after 24 hours.

TABLE 8 DATA ON THE NUMBER OF DROPLETS COLLECTED ON OIL-SENSITIVE RED. DYE CARDS PLACED AT VARIOUS LOCATIONS IN THE AREA TREATED WITH MALATHION AT THE RATE OF 6 US FL OZ/ACRE  $^{\alpha}$ 

-		Number of droplets collected on dye cards						
Site	Location	First ap	plication	Second a	pplication			
		Average/in² <sup>b</sup>	Range	Average/in² b	Range			
1	Inside shophouse	1.6 <sup>c</sup>	0-4.5	2.7	0–10.0			
		(0.25)	(0-0.70)	(0.41)	(0-1.55)			
	Behind shophouse	64.1	13.8-158.5	36.4	7.5-83.0			
		(9.94)	(2.12 <b>–24.57)</b>	(5.64)	(1.16–12.86)			
2	Inside house	41.9 <sup>c</sup>	5.3-112.8	3.6 <sup>c</sup>	0.8-6.8			
1		(6.49)	(0.82-17.48)	(0.55)	(0.12-1.05)			
ļ	Under house	41.1	10.8-61.3	61.7	36.0-129.5			
		(6.37)	(1.67 <b>–9.50)</b>	(9.56)	(5.58–20.07)			
3	Inside house	1.0	0-2.3	8.0	2.0-14.8			
		(0.15)	(0-0.35)	(1.24)	(0.31-2.28)			
	Under house	4.5	0.3-15.5	9.66	2.0-18.0			
		(0.70)	(0.04–2.40)	(1.48)	(0.31–2.79)			
4	Inside house	7.0 <sup>d</sup>	1.0–14.8	8.3 <sup>c</sup>	2.3–21.5			
		(1.85)	(0.15-2.29)	(1.28)	(0.35-3.33)			
	Under house	5.5	0.3-13.0	14.6	8.8-20.5			
		(0.85)	(0.04–2.01)	(2.57)	(1.36–3.17)			
5	Road ditch	48.4	13.8–103.8	32.5	1.0-127.0			
		(7.50)	(2.02-16.09)	(5.04)	(0.15-19.69)			

a 438 mi/ha.

females. These data show conclusively that the posttreatment population was made up almost entirely of individuals that had emerged after the treatments. Conversely, this means that the older female mosquitos, those that would be carrying the virus during an epidemic, were eliminated. In addition to this, the mosquito population was kept at a very low level throughout the evaluation period, which lasted 11 days. It was unfortunate that the evaluation could not have been extended but other duties intervened.

Acceptance of the experiment by the residents of Nakhon Sawan was excellent. No complaints concerning the low-flying aircraft, odour of the insecticide, or adverse effects on organisms other than mosquitos were received. In fact many of the residents invited the team to spray again the following year.

The fuselage-mounted spray boom functioned well mechanically throughout the trial. Data from

red dye cards and the biological effectiveness of the spray indicate that the spray droplets were distributed fairly uniformly over the entire test area. This indicates that wing booms are not essential for obtaining the required swath width if the droplet size is kept small. The actual physical means by which the droplets became distributed over the swath is not completely clear. The insecticide is emitted in a narrow band and remains visible for several seconds after the plane has passed. The spray appears to be carried and distributed by cross-winds which, in these tests, were always stronger at an altitude of 150 feet (46 m) than at ground level. It may be that wind currents produced by the aircraft, particularly by the wing vortices, may help to distribute the spray; however, this effect was not visible from ground level. It is obvious that more studies are needed to determine the mechanics of droplet distribution so that the limits of effectiveness of the spraying system can be defined.

<sup>&</sup>lt;sup>b</sup> No. of droplets per cm<sup>2</sup> in parentheses.

 $<sup>^</sup>c$  Average of 4 cards; the fifth card in both tests contained an abnormally high number of droplets that were probably not malathion.

<sup>&</sup>lt;sup>d</sup> One card lost; average of 4 cards.

The possibility of using a fuselage-mounted boom has important implications for programmes involving mass treatment of large areas during epidemics of mosquito-borne diseases. Very few countries have a large number of aircraft available for spraying operations at short notice, such as was the case in the encephalitis epidemic in Dallas, Texas, in 1966 when C-123 aircraft of the special aerial spraying flight of the US Air Force were used. Even if conventional spraying equipment were stockpiled, the time required for the installation of the wing booms would preclude its use in emergencies. In addition, conventional equipment, once fitted, would prevent the use of the aircraft for other purposes. Most of these disadvantages can be eliminated by use of the fuselage-mounted boom. The equipment can be installed within a few hours once it is all assembled. Almost any airworthy C-47 aircraft can be used, although differences in models of the aircraft might require some modification of the manner in which the equipment is mounted. Possibly, other multiengined aircraft might also be used. It is not known . whether the system will function on slower, singleengined aircraft, but this possibility should be investigated.

The ULV insecticide-dispensing system mounted in a C-47 or a similar aircraft appears to satisfy all the requirements needed to control the mosquito vectors during an epidemic of haemorrhagic fever. The method provides rapid coverage of extremely large areas. For example, the Nakhon Sawan test area was 7 square miles (18 km²) and was treated in about 45 minutes. The results indicated that a very high percentage of adult mosquitos were killed and the population remained small for a period of 10 days. The cost of the application might be higher than when ground spraying methods are employed, but the greater speed and ease with which large areas are covered would probably be more important than the actual cost. Since it could not be determined whether a double treatment was absolutely necessary, or whether the 6 US fl oz/acre treatment was the optimum rate, further investigations on the number of treatments and rate of application might lead to a reduction in the cost of ULV spraying.

## ACKNOWLEDGEMENTS

We wish to thank the Royal Thai Air Force and the Ministry of Public Health for their generous co-operation and assistance throughout the study. We are particularly grateful to Dr Soonthorn Thongkong, Nakhon Sawan Provincial Health Officer, for his help in making local arrangements with other government officials and the

press prior to our study and for his guidance during the work. We also wish to thank Mr Tongbai Suipout for allowing us the use of the Plague Center and a vehicle during the project. Finally, we wish to express our appreciation to the staff of the WHO Aedes Research Unit for their assistance.

# RÉSUMÉ

EFFICACITÉ DES APPLICATIONS DE MALATHION SOUS ULTRA-FAIBLE VOLUME À LA DOSE DE 438 ml/ha DANS LA LUTTE CONTRE AEDES AEGYPTI AU COURS D'UN ESSAI D'ENVERGURE EFFECTUÉ À NAKHON SAWAN, THAÏLANDE

Deux applications de malathion sous ultra-faible volume à la dose de 438 ml/ha ont été faites par épandage aérien à 4 jours d'intervalle sur un territoire englobant la totalité de la ville de Nakhon Sawan (46 000 habitants environ).

A la suite du 1<sup>er</sup> traitement, le taux de capture d'*Aedes aegypti* est passé de 8,6 par homme/heure en moyenne à moins de 1, soit une réduction de 92-95%. Le lendemain du 2<sup>e</sup> traitement, les captures ont diminué dans la proportion de 99% et sont restées à un niveau faible (environ 1 moustique par homme/heure, soit une réduction de 88-92%) pendant le reste de la période d'observation de

10 jours. Les taux de ponte ont baissé brutalement du 2e au 4e jour après le 1er traitement et aucune oviposition n'a plus été observée après le 4e jour. Les dissections d'Aedes femelles récoltés après le 1er traitement ont montré un taux de parité de 8% contrastant avec le taux de 30% environ noté avant le traitement et le taux de 40% relevé dans un secteur témoin. On en conclut que, après le traitement, la population d'Aedes était composée en majeure partie de moustiques jeunes éclos après le début des applications d'insecticide.

On a également enregistré une réduction satisfaisante des populations d'autres moustiques. Les captures de

nuit, qui atteignaient 9 moustiques par homme/heure avant le traitement, ont diminué dans la proportion de 91-97% du 1er au 7e jour et de 82% lors de la dernière séance de capture.

Au cours du 1<sup>er</sup> traitement, la mortalité des adultes et des larves exposés dans le secteur traité a été assez élevée; le 2<sup>e</sup> traitement a entraîné en revanche une destruction

quasi complète des adultes encagés et une forte mortalité parmi les larves. Cette différence d'efficacité entre les deux applications est probablement due à une précision accrue des opérations lors de la deuxième.

De l'examen des cartes colorées disposées dans le secteur traité, il ressort que l'on a pu obtenir une répartition uniforme de l'insecticide.

# **REFERENCES**

Kilpatrick, J. W., Tonn, R. J. & Jatanasen, S. (1970)
Bull. Wld Hlth Org., 42, 1-14
Lofgren, C. S., Ford, H. R., Tonn, R. J. & Jatanasen, S. (1970)
Bull. Wld Hlth Org., 42, 157-163

Sheppard, P. M., Macdonald, W. W. & Tonn, R. J. (1969) Bull. Wld Hlth Org., 40, 467-468
Yeomans, A. H. (1949) Bull. US Dept Agric., Bur. Ent. Plant Quarant., No. ET267