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EVALUATION OF ABATE INSECTICIDE FORMULATIONS AS LARVICIDES AGAINST *ANOPHELES GAMBIAE* IN NORTHERN NIGERIA

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The effectiveness of new synthetic organic insecticides as larvicides for the control of *An. gambiae*, the principal malaria vector in Africa, has not been previously evaluated in Nigeria. In view of the interest in larval control as a supplementary measure for house residual spraying in malaria control, Abate (OMS–786) [3] was evaluated in field plot tests under dry and wet season conditions during 1970–1971 by the World Health Organization Anopheles Control Research Unit, Number 1 at Kaduna, Nigeria.

LABORATORY EVALUATION

Prior to the selection of Abate for the field trials the susceptibility of *An. gambiae* to nine other insecticides was tested for LC_{95} values using a standard insectary strain (Kankiya) third instar *An. gambiae* larvae.

The WHO test procedures were followed except that the range of susceptibility was determined from three concentrations of the technical material in ethanol instead of five and disposable, waxed monocups were substituted for glass beakers. Each insecticide was tested at least twice with four replications of each test. The test data are shown in Table 1.

TABLE 1.—Suscepibility of third instar *An. gambiae* larvae (Kankiya Strain) to ten insecticides, Kaduna, Nigeria, 1970.

Insecticide	LC 50	LC 95	Oral LD-50 mg/Kg (female white rats)
Carbaryl	0.30	0.760	500*
Mobam	0.33	0.480	178
Propoxur	0.14	0.270	116
Malathion	0.048	0.106	1000*
Iodofenphos	0.011	0.032	1600
Bromophos	0.016	0.022	2000
Abate	0.0074	0.019	13000*
Fenthion	0.0049	0.013	245*
Ciba C-14814	0.0020	0.008	1000
Dursban	0.0022	0.006	82*

* World Health Organization Technical Report Series No. 443. All other values taken from Vol. 11 WHO/VBC/71.11 August 1971.

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[3] OMS-786 American Cyanamid, Abate, CL-52160 0,0,',0'-tetramethyl 0,0'-thiodi-para-phenylene phosphorothicate.

Among the ten insecticides tested, those with the lowest LC_{95} levels were Dursban, Ciba 14814 (OMS-1290), fenthion and Abate.

Although the LC_{95} level of Abate was only fourth in the list of the ten insecticides tested, it was selected for field trials because of its low mammalian toxicity. This factor is an important consideration for larviciding in Nigeria where ponds, streams and other breeding places of *An. gambiae* are often sources of drinking water for people and domestic animals.

FIELD PLOT TRIALS

WET SEASON TRIALS. The wet season in the Kaduna area occurs from May to October inclusive and rainfall during this period may range from a minimum of 1,000 mm. to over 1,500 mm. It is characterized by torrential downpours of short duration lasting from one to several hours. This results in flooding of streams and rivers and the extensive formation of temporary pools and ponds ideally suited to *An. gambiae* breeding. Under such conditions Abate 1 percent sand granules were tested in a field plot trial in August during the peak of the 1970 wet season. In this month 387.6 mm. of rain was recorded in 27 wet days. A second trial to test Abate emulsion 40 percent concentrate was carried out in September when 224.9 mm. of rain was recorded in 24 wet days.

ABATE 1 PERCENT SAND GRANULES TRIAL. *Methods:* The site of the trial was an abandoned highway borrow pit area covering about 5 hectares alongside the Kaduna-Zaria road 7 miles north of Kaduna. The borrow pit contained a number of shallow ponds which were naturally infested with *An. gambiae, An. funestus* and *Culex* sp. larvae and pupae.

Twelve test plots measuring 2 x 1 metres by 15 cm deep were laid out in areas between the scattered ponds. When filled, the plots held 300 litres of water and within a few days became naturally infested with *An. gambiae* larvae. A system of dykes and drains was installed to protect the plots from being washed out by surface runoff. Plastic sheets were laid over the bottom and sides of the plots and covered with about 10 cm. of soil to reduce water loss from percolation between rains.

The Abate granules were applied on 21st August, 1970, after first mixing with 5 kilograms of sand to facilitate distribution in three plot replicates of three concentrations of Abate at 0.2, 0.4 and 1.0 ppm. Three plots were left untreated as checks.

Only first instar *Anopheles gambiae* larvae were present in the plots at the time of treatment, but larvae of all instars and pupae collected from neighbouring breeding habitats were added to approximate an average density of 2 larvae per dip in the treated and check plots.

Twenty-four hours after treatment and daily thereafter the plots were inspected for larvae. In addition to a visual examination a minimum of 10 sample dips was made in each plot using a half-litre ladle. Concurrently a 500 ml. water sample was taken from each plot and bioassayed against third instar insectary-bred *An. gambiae* larvae.

Results. The plot inspection data are shown in Table 2. There were no larvae seen or collected in the treated plots in the 24 hour post-treatment inspection but check plots averaged 2.3 larvae per dip.

In the plots treated at 0.2 ppm, larvae first appeared on post-treatment day 4 averaging 0.5 first and second instars per dip. First and second instars were recovered again on days 6 and 9. On days 5, 7 and 8 the plots overflowed and inspections were negative. In the bioassays of water samples from these plots the mortality of third instar insectary-bred *An. gambiae* larvae was 100 percent through day 3 but fell to 36.6 percent on day 4. On days 5 and 6 all larvae survived and tests were discontinued.

TABLE 2.—Recovery of *An. gambiae* larvae from field test plots tested with Abate 1% sand granules in the wet season at Kaduna, Nigeria. August 1970.

Applied conc. ppm	Pre-treatment average no. larvae dip	Post treatment—Average number larvae per dip collected on indicated days[a]								
		1	2	3	4	5	6	7	8	9
0.2	2.0(4)	0	0	0	0.5(2)	0[b]	0.9(2)	..[b]	..[b]	0.8(2)
0.4	2.0(4)	0	0	0	0	0.03(1)	0.13(1)	0[b]	..[b]	1.5(2)
1.0	2.0(4)	0	0	0	0	0	0	0	0.23(1)	0.30(2)
Check	2.3(4)	0.8(4)	0.3(4)	1.0(4)	2.0(2)	1.3(2)	0.6(2)	—[b]	0.3(1)	0.9(2)

Figures in parentheses represent the highest larval instars present.
[a] 10 sample dips per replicate.
[b] Plots overflowed.

In the plots treated at 0.4 ppm, first instar larvae were first detected on day 5 and again on day 6 but on days 7 and 8 the plots were negative possibly due to flooding by heavy rains. On day 9 when water levels were normal, first and second instar larvae averaged 0.8 per dip. In the bioassay tests the mortality of third instar larvae was 100 percent for 4 days, but on the fifth and sixth day all larvae survived.

In the plots treated at 1.0 ppm. no larvae were recovered until day 8 when first and second instar larvae were collected, averaging 0.23 and 0.30 per dip. The mortality of test larvae in the bioassays was 100 percent on day 4 but fell to only 13 percent on day 5. On day 6 survival was 100 percent.

In the untreated check plots *An. gambiae* larvae in all instars were taken in 30 sample dips.

Discussion. In this trial it was assumed that there was no further insecticidal effect when second instar larvae appeared in the treated plots. Based on this criterion, the Abate gave control for only 3 days at 0.2 ppm, for 4 days at 0.4 ppm and for 7 days at 1.0 ppm. This brief period of insecticidal effect is attributed in part to dilution of the applied concentration in the plots by the torrential rains which fell during the observation period. It is, therefore, concluded that the effects of larviciding with 1 percent sand granules in the peak period of the wet season in the Kaduna area would be very limited.

ABATE EMULSIFIABLE CONCENTRATE 40 PERCENT PLOT TRIAL. In September 1970 Abate emulsion was tested in plot trials in the same borrow pit site as described for the sand granule trial. A total of 14 plots were laid out to provide for three replicates of four concentrations of Abate emulsion at 0.2, 0.4, 1.0 and 2.0 ppm and two untreated check plots.

The plots were treated on 2nd September, 1970 and 25 third instar insectary bred *An. gambiae* were introduced to each of the replicates and check plots to supplement the naturally occurring

larvae. The evaluation procedure was the same as described for the granular trial consisting of daily plot inspections and bioassays of water sample. Plots were considered negative for larvae if none were seen in visual examination and none taken in 30 dips. Bioassays were made with first instar larvae in addition to thirds to ascertain if an insecticidal effect could still be demonstrated with the earliest instars when third instars survived a 24-hour exposure period in the water samples from the treated plots.

As with the sand granule trial in August the water levels in the plots fluctuated from heavy rainfall, percolation and evaporation but to lesser extent because precipitation was appreciably less in September and terminated altogether near the end of the month. Due to the virtual disappearance of surface water, the trial was forced to a premature conclusion on post-treatment day 24.

Results: The data on larval recovery from the treated plots is given in table 3. Table 4 shows the mortalities of first and third instar larvae bioassayed in water samples from the plots.

At the applied concentration of 0.2 ppm one first instar larva was detected on post-treatment day 7, but none were found on days 8 and 9 and only one first instar was recovered on day 10. On day 11 first instar larvae averaged 0.3 per dip. The plots were not inspected on day 12 and 13 but on day 14 and each day thereafter to the end of the observations on day 24, some larvae were present, although none beyond second instar were ever recovered. The mortality of third instar *An. gambiae* larvae in bioassays was 100 percent for 3 days before falling to a negligible 6.6 percent on day 4. But when first instar larvae were bioassayed on day 14 a low level mortality was noted in the range of 6 percent to 13 percent.

At the applied concentration of 0.4 ppm. larvae did not reappear until post-treatment day 9 when first instars were recovered. On day 12, second instar larvae were collected but thereafter only first instars were found. In bioassays the mortality of third instar larvae was 100 percent for 3 days after treatment, dropping to 50 percent, 40 percent and 10 percent on days 4, 5 and 6, respectively. Mortality continued at the 10 percent level until nil on day 13. However, when first instars were used in the bioassay on day 14 a mortality of 46 percent was recorded and continued above 30 percent until day 21.

At the applied concentration of 1.0 ppm, first instar larvae first appeared on day 10. On day 13, first and second instars were present but thereafter, only first instar larvae were observed. The bioassay mortality of third instar larvae through day 13 was only slightly higher than at 0.4 ppm. concentration. When first instar larvae were used on day 14, the mortality increased appreciably but was nil on day 25.

At the applied concentration of 2.0 ppm one first instar larva was recovered on day 10 in the three replicates. Thereafter, some live first instars were detected each day from day 14 to the end of the observation period.

To check on the effect of Abate against older instars, 250 field-collected anopheline larvae of all instars consisting mainly of *An. gambiae,* were added to each of two of the three plots on day 18, and 125 larvae to two of the three check plots. On day 19 and 20 only first instar larvae were recovered in the three plot replicates. Concurrently the check plots yielded all instars including pupae. The check plot without larvae added contained first and second instar larvae on day 19 and third instar on day 20. The bioassay mortality of third instar larvae was 100 percent for 5 days after treatment and remained in the 60 percent to 100 percent range until day 13 when it fell to 53 percent. But when first instar larvae were bioassayed on day 14 the mortality was 100 percent and continued at this level until day 20. On day 21 the mortality fell to 70 percent and declined to 43 percent on day 25

TABLE 3.—Recovery of *An. gambiae* larvae from field test plots treated with Abate 40% EC in the wet season, Kaduna, Nigeria, September, 1970.

Pre-treatment		Post-treatment									
Applied conc. ppm	Avg. no. larvae/dip	Avg. no. larvae collected in 30 sample dips on indicated days (3 plot replicates)									
		1[a]	7	8	9	10	11	12	13	14	15
0.2	2.0(4)[b]	0	.03(1)	0	0	.03	0.3(1)	..[c]	..[c]	0.6(2)	1.0(2)
0.4	2.0(4)	0	0	0	.3(1)	0.5(1)	0.5(1)	2.2(2)	2.2(1)	0.4(1)	0.2(1)
1.0	2.0(4)	0	0	0	0	.16(1)	0	..[c]	2.0(2)	0	.07(1)
2.0	2.4(4)	0	0	0	0	.07(1)	0	..[c]	0.4(1)	.06(1)	.03(1)
Check	1.3(4)	0.3(3)	0.5(2)	0.3(2)	0.3(2)	0.3(2)	2.2(2)	3.9(2)	2.4(3)	2.0(1)	2.3(3)
		16	17	18	19	20	21	22	23	24	
0.2		1.7(1)	1.3(1)	0.7(1)	2.2(1)	2.3(1)	2.8(1)	2.3(1)	0.6(1)	1.1(1)	
0.4		1.0(1)	0.0(1)	0.02(1)	2.0(1)	1.4(1)	0.7(1)	1.6(1)	0.3(1)	0.7(1)	
1.0		0.5(1)	0.03(1)	0.3(1)	2.0(1)	0.8(1)	0.7(1)	0.5(1)	0.01(1)	0.4(1)	
2.0		0.03(1)	0.02(1)	0.01(1)[d]	0.7(1)	0.36(1)	0.02(1)	0.02(1)	0.01(1)	0.02(1)	
Check		1.6(1)	1.5(1)	1.9(1)[e]	4.3(4)	2.3(4)	3.2(4)	4.7(3)	9.6(1)	3.9(1)	

[a] All plots negative day 1–6 inclusive but check plots contained III and IV larval instars during 5 of the 6 days.
[b] The number in parentheses represents the highest larval instar present.
[c] Plots overflowed day 12 and 13 from torrential rains.
[d] 250 field collected *An. gambiae* and other anopheline larvae added to two of three replicates.
[e] 125 field collected *An. gambiae* and other anopheline larvae added to two of three plot replicates.

TABLE 4.—Bioassay mortalities of *An. gambiae* larvae held 24 hours in water samples from field plots treated with Abate 40% EC at applied concentrations 0.2, 0.4, 1.0 and 2.0 ppm, September 1970.

Applied conc. ppm	Percent mortality 3rd instar insectary larvae after indicated days[a, b]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
0.2	100	100	100	6.6	6.6	13	10	3	3	0	0	0	0
0.4	100	100	100	50.6	40	10.3	10.6	10.3	10.6	10	10.3	10.6	0
1.0	100	100	100	90	43	43	10.0	13	6.0	20	26.0	10	20
2.0	100	100	100	100	100	80	70	66	76	100	90	60	53
Check	0	0	0	0	0	3	3	0	0	0	0	0	0

	Percent mortality 1st instar insectary larvae after indicated days											
	14	15	16	17	18	19	20	21	22	23	24	25
0.2	6.0	13	10	0	6	0	0	3	0	3	0	0
0.4	46	46	33	47	33	80	36	13	0	0	0	0
1.0	46	50	66	90	83	73	56	40	23	20	10	0
2.0	100	100	100	100	100	100	100	70	63	60	53	43
Check	3	0	3	6	0	0	0	0	0	0	0	0

[a] Number exposed: 30 larvae, 10 per replicate of 3 replicates.
[b] Plots flooded by torrential rains day 12, 13 and plots replenished with water day 1, 7, 9, 16, 18, 21, 23.

when the observations were terminated.

The untreated check plots during the trial period yielded varying numbers of larvae of different instars and the mortality never exceeded 3 percent in bioassays.

Conclusions: Under the condition of this trial the Abate treatment prevented reinfestation of the plots by *An. gambiae* and other mosquito larvae for a period of 7 days at an applied concentration of 0.2 ppm and for 10 days at 2.0 ppm. After the appearance of first instars on day 10 at the latter concentration the insecticidal effect was apparently sufficient to curb larval development beyond first instars as later instars never appeared in the plots during the 24-day observation period. This was corroborated by continuing 100 percent mortality of first instar larvae in bioassays of water samples taken from the 2.0 ppm plots, after the mortality of third instar had fallen to only 53 percent on day 13.

There was also evidence of higher insecticidal activity in the plots themselves than in the water samples taken from the plots for bioassays. For example, when the 2.0 ppm plots were challenged on post-treatment 18 with third and fourth instar larvae, 100 percent mortality was recorded on day 19, but bioassays of third instar larvae in water samples taken from the same plots 5 days earlier on day 13 registered 53 percent mortality.

The higher mortality in the plots is attributed to Abate being deposited on the mud and particulate matter, from which it was slowly released into the water. Retention of the Abate in the water itself is ruled out because the plots were replenished with fresh water several times during the course of the trial. This would suggest that larval mortality in the plots may have been due to a sorption-release phenomenon such as was reported to take place in clay jars used for water storage in Thailand by Y. H. Bang and R. S. Tonn;[4] or possibly to larvae in the plots contacting or ingesting Abate deposited on mud or on suspended or floating particulate matter. Evidence of Abate 'plating out' on glass, plastic and paper vessels was seen in the laboratory in bioassays of water samples from treated plots.

Although the Abate emulsion was clearly more effective than the sand granules, the much heavier rainfall during the sand granule trial in August may have contributed to the reduced insecticidal effect.

DRY SEASON TRIALS

The dry season extends from about October to mid-April in the Kaduna area of Nigeria and *An. gambiae* adults and larvae are generally very scarce between November and February during the cooler period of the season even though there are some potential breeding habitats present. However, in March *An. gambiae* reappears in large numbers coinciding with a pronounced increase in temperature and relative humidity which annually precedes the onset of the wet season beginning about late April and early May. Although surface water available for mosquito breeding is very limited in the March–April period, hut densities of *An. gambiae* rise 50 to 100 times the level in February. Anti-larval measures under such limited conditions of breeding would appear feasible as a method of counteracting this resurgence. To be effective, however, the measures would need to be initiated in February before the onset of the resurgence, and continued until the beginning of the wet season in May while breeding places are still readily accessible and limited. It was with this consideration in view, that the Abate plot trials described below were undertaken during the late dry season.

ABATE FLOATING GRANULES PLOT TRIAL. Three Abate floating granule formulations, 1 percent in perlite, krobite and vermiculite diluent, were tested in field plots against *An. gambiae* larvae during

[4] Residual life of OMS-786 Abate Emulsion Concentrate in water jars in Thailand. Y. H. Bang and R. S. Tonn, WHO/VBC/69.156.

the period February 15 and March 17, corresponding with the pre-wet season resurgence of *An. gambiae* previously noted.

Methods: The plots were designed to simulate natural dry season habitats of *An. gambiae* larvae. Shallow pits with sloping sides varying from 40–60 cm. in diameter and from 20–30 cm. deep were dug at the edge of a marsh where the high water table provided subsurface seepage which maintained a uniform depth of water varying from 10–20 cm. during the trial period.

Treatment was postponed until the suitability of the plots as anopheline habitats was confirmed by the appearance of natural infestations of larvae and introduced *An. gambiae* insectary-bred, first instar larvae had developed to older instars and pupae. Anopheline larvae naturally infesting the plots in February included *An. rufipes* and *An. maculipalpis.*

There were 30 plots in the trial, 9 for each of the three formulations to be tested in three replicates at an applied concentration of 0.2, 0.5 and 1.0 ppm. with an additional 3 plots as checks. On 17 February, 25 first and 25 third instar insectary-bred *An. gambiae* larvae were introduced into each of the 30 plots and the insecticide applied at the dosage indicated. Thereafter, inspections were made on a daily basis. This included a visual examination and dipping with a one-half litre ladle at ten dips per plot. The numbers of larvae recovered were recorded, classified by instar and identified. If none were seen nor taken in ten dips the plot was considered negative.

In addition to the inspections the three formulations were bioassayed until post-treatment day 14 by testing water samples from the plots against first and third instar *An. gambiae* insectary-bred larvae in the laboratory for 24 hour mortalities. Thereafter, the bioassays were made in the plots themselves. The method consisted of fastening waxed paper cups containing the test larvae to the side of a stake inserted in the plots until the cups were immersed to nearly rim level. The sides of the cups were pierced with numerous pin holes to allow circulation of the plot water in the cups. After a 24 hour exposure period larval mortality was recorded in the test and check plots.

Results: The data of larval recovery from the plots for each of the three formulations, perlite, Krobite and vermiculite are given in tables 5, 6 and 7 respectively. Table 5 also gives the check plot data.

There were no larvae in the treated plots 24 hours after application, but the three check plots were infested with anopheline larvae of all instars and pupae including the introduced *An. gambiae.*

All plots treated with the three formulations were negative for larvae until day 12 when natural infestations of first instar anophelines (species unknown) appeared in the 0.2 ppm concentrations of the three formulations. To determine if *An. gambiae* would develop in the plots third instar larvae were introduced (10 per plot) on day 13. On day 14 only first instars were present. This suggested that oviposition and hatching was occurring in the plots but the first instars were not surviving to later instars. This assumption was supported by the fact that third instars did not appear until day 21 in the 0.2 and 0.5 ppm concentrations in the case of krobite (table 6) and with perlite (table 5) not until day 21 at 0.2 ppm. and day 23 at 0.5 ppm. Third and fourth instars were first detected in the vermiculite (table 7) treated plots at 0.5 ppm on day 22.

In the plots treated at the applied concentration of 1.0 ppm, first instar larvae first appeared in the krobite plots as early as 13 days after treatment but not until day 19 in the perlite and vermiculite treated plots.

Since there was no evidence of larval development beyond first and second instars from natural infestations in the 1.0 ppm plots, these plots were challenged with introduced insectary-bred, third instar

TABLE 5.—Recovery of *An. gambiae* larvae from field test plots treated with Abate 1% floating granules (perlite) at concentrations of 0.2, 0.5, 1.0 ppm in 3 plot replicates in the late dry season, February–March, 1971, Kaduna, Nigeria.

Days* after treatment	Applied conc. 0.2 ppm — Avg. no. instars (10 dips per plot)					Applied conc. 0.5 ppm — Avg. no. instars (30 dips)					Applied conc. 1.0 ppm — Avg. no. instars (30 dips)					Check plots — Avg. no. instars (30 dips)				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
0[a,b]	75	..	75	..	..	75	..	75	..	..	75	..	75	..	..	75	..	75	..	..
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0.3	0.6	0.4	0.2
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0.4
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.3	0	0	0.2
12	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0.8	0.4	0	0
13[c]	0.2	0.1	0	0	0	0.03	0	0	0	0	0	0	0	0	0	0.3	1.3	0.3	0	0
14	0.3	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0	1.4	0.7	0	0
15	0.5	0.1	0	0	0	0.2	0	0	0	0	0	0	0	0	0	0.1	0.1	0.7	0.1	0.2
16	0.7	0	0	0	0	0.03	0.07	0	0	0	0	0	0	0	0	0	3.8	0.9	0.1	0
19	1.4	0.6	0	0	0	0.8	0.03	0	0	0	0.3	0	0	0	0	0.7	1.8	1.8	1.4	1.0
20	1.0	0.6	0	0	0	0.6	0.06	0	0	0	0.6	0	0	0	0	0.8	1.0	1.1	1.0	0.7
21	1.0	0.8	0.4	0.06	0	0.7	0.3	0	0	0	0.9	0.1	0	0	0	0	1.5	1.4	1.3	1.1
22	0.9	0.8	0.3	0.1	0	1.1	0.2	0	0	0	0.4	0	0	0	0	0.5	1.2	1.2	1.6	1.4
23	0.6	0.7	0.3	1.0	0	0.6	0.2	0.03	0	0	0.3	0	0	0	0	0.8	2.0	3.8	3.4	1.1
24	..	..	..	..	..	..	..	..	..	..	0.2	0	0	0	0	0	5.1	2.6	2.9	0.7
26	..	..	..	..	..	..	..	..	..	..	0.2	0	0	0	0	0	0	2.2	1.5	0.4
27[d]	..	..	..	..	..	..	..	..	..	..	0.07	0	0	0	0	..	..	..	..	..
28	..	..	..	..	..	..	..	..	..	..	0.7	0	0	0	0	1	2.3	2.1	0	0
29	..	..	..	..	..	..	..	..	..	..	0.5	0	0.03	0	0.3	1	1.9	2.3	0.7	0
30	..	..	..	..	..	..	..	..	..	..	0.1	0	0.1	0.1	0.4	0.8	0.7	0.6	0.4	0.2

* Date of treatment 17.2.71.
[a] 150 insectary bred *An. gambiae* third instar larvae added to plot replicates.
[b] At time of treatment plots naturally infested with anophelines (*An. rufipes* and *An. maculipalpis*).
[c] 10 third instar *An. gambiae* larvae introduced into each plot.
[d] 75 third instar larvae introduced into plot replicates 1 ppm only.

TABLE 6.—Recovery of *An. gambiae* larvae from field test plots treated with Abate 1% floating granules (krobite) at concentrations of 0.2, 0.5 and 1.0 ppm in three plot replicates in the late dry season, February–March, 1971, Kaduna, Nigeria.

Days* after treatment	Applied conc. 0.2 ppm					Applied conc. 0.5 ppm					Applied conc. 1.0 ppm				
	Avg. no. instars (10 dips per plot)					Avg. no. instars (30 dips)					Avg. no. instars (30 dips)				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
0[a, b]	75	..	75	..	..	75	..	75	..	..	75	..	75	..	..
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13[c]	0.27	0	0	0	0	0.07	0	0	0	0	0.07	0	0	0	0
14	0.40	0	0	0	0	0.2	0	0	0	0	0	0	0	0	0
15	0.43	0	0	0	0	0.3	0	0	0	0	0.1	0	0	0	0
19	2.0	0.3	0	0	0	0.7	0	0	0	0	0.5	0	0	0	0
20	1.7	0.9	0	0	0	1.6	0	0	0	0	0.8	0	0	0	0
21	2.2	0.6	0.5	0	0	1.7	0.8	0.1	0	0	0.4	0.1	0	0	0
22	3.3	1.3	0.5	0.2	0	1.7	0.6	0.1	0	0	0.5	0.2	0	0	0
23	2.2	1.2	0.5	0	0.3	1.6	0.6	0	0.1	0.3	0.6	0	0	0	0
24	..	..	..	..	..	..	..	..	..	..	1.7	0	0	0	0
26	..	..	..	..	..	..	..	..	..	..	0.23	0	0	0	0
27[d]	..	..	..	..	..	..	..	..	..	..	0.26	0	0	0	0
28	..	..	..	..	..	..	..	..	..	..	0.16	0	0	0	0
29	..	..	..	..	..	..	..	..	..	..	0.4	0	0	0	0
30	..	..	..	..	..	..	..	..	..	..	0.8	0	0.07	0.13	0.3

* Date of treatment 17.2.71. For check plot results see table 5.

[a] Total of 150 third instar insectary bred *An. gambiae* larvae introduced into plot replicates.

[c] 10 third instar *An. gambiae* larvae introduced into each plot.

[b] At time of treatment plots naturally infested with anophelines (*An. maculipalpis* and *An. rufipes*).

[d] 75 third instar larvae introduced into plot replicates treated at applied conc. 1.0 ppm only.

TABLE 7.—Recovery of *An. gambiae* larvae from field test plots treated with Abate 1% floating granules (vermiculite) at concentrations of 0.2, 0.5, and 1.0 ppm in 3 plot replicates in the late dry season February–March, 1971, Kaduna, Nigeria.

Days after treatment	Applied conc. 0.2 ppm					Applied conc. 0.5 ppm					Applied conc. 1.0 ppm				
	Avg. no. instars (10 dips per plot)					Avg. no. instars (30 dips per plot)					Avg. no. instars (10 dips per plot)				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
0[a,b]	75	..	75	..	..	75	..	75	..	..	75	..	75	..	..
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0.37	0	0	0	0	0.1	0	0	0	0	0	0	0	0	0
13[c]	0.67	0	0	0	0	0.27	0	0	0	0	0	0	0	0	0
14	0.6	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0
15	0.67	0	0	0	0	0.23	0	0	0	0	0	0	0	0	0
19	1.5	0.9	0	0	0	0.57	0.2	0	0	0	0.97	0	0	0	0
20	0.84	0.67	0	0	0	0.67	0.14	0	0	0	0.64	0	0	0	0
21	0.87	0.54	0	0	0	0.84	0.3	0	0	0	0.67	0	0	0	0
22	0.9	0.27	0.10	0	0	0.27	0.4	0.34	0.1	0	0.50	0.67	0	0	0
23	0.84	0.54	0.17	0	0.03	0.97	0.34	0	0	0	0.37	0.03	0	0	0
24	..	..	..	..	..	..	..	..	..	..	0.30	0	0	0	0
26	..	..	..	..	..	..	..	..	..	..	0.34	0	0	0	0
27[d]	..	..	..	..	..	..	..	..	..	..	0.70	0	0	0	0
28	..	..	..	..	..	..	..	..	..	..	0.20	0.34	0.44	0	0
29	..	..	..	..	..	..	..	..	..	..	0.84	0	0	0.34	0.87
30	..	..	..	..	..	..	..	..	..	..	0.5	0	0.07	0.3	0

[a] Date of treatment: 17.2.71. For check plot results see Table 5.
[a] Total 75 third instar insectary bred *An. gambiae* introduced into plot replicates.
[b] At time of treatment plots naturally infested with other anophelines. (*An. maculipalpis* and *An. rufipes*).
[c] 10 third instar larvae introduced into plot replicates 1.0 ppm only.
[d] 75 third instar *An. gambiae* larvae introduced into plot treated at applied conc. 1.0 ppm only.

An. gambiae larvae numbering 24 per plot on day 27. From these introductions fourth instars and pupae were recovered from all the plots by the 29th and 30th day after treatment.

Bioassays: The results of bioassays of the three formulations are shown in table 8. The mortality of first and third instar *An. gambiae* larvae in water samples taken from the 0.2 ppm plots of the three formulations in the laboratory fell below 70 percent mortality on day 9 but remained in the 70–100 percent range at least through day 12 at 0.5 and 1.0 ppm concentration. On day 15 to the close of the trial on day 30 the bioassays were made in the plots themselves. Considerable variation was observed in the mortality of the test larvae in the several bioassays made, but in the 1.0 ppm concentration, a mortality of 90 percent and above was recorded for first and third instars on day 23 for perlite and 78 percent and 62 percent respectively for krobite. The vermiculite formulations registered only 30 percent and 12 percent for first and third instars respectively. On day 28 the bioassay mortalities for perlite and krobite had fallen below the 70 percent level and testing was terminated. Larval mortality in the check plot bioassays was nil in all tests.

Conclusions: The three Abate formulations, perlite, krobite and vermiculite appear to be equally effective against *An. gambiae* larvae for a period of about 27 days at 1.0 ppm and under the conditions of this trial, these formulations are considered suitable for *An. gambiae* control during the dry to wet season transition period when there is a resurgence of the *An. gambiae* population.

TABLE 8.—Bioassay mortalities of *An. gambiae* larvae held 24 hours in water samples from field plots treated with Abate 1% floating granules (perlite, krobite and vermiculite) at applied concentrations of 0.2, 0.4 and 1.0 ppm. February–March, 1971. Kaduna, Nigeria.

Days after[a] treatment	Perlite						Krobite						Vermiculite					
	0.2 ppm		0.5 ppm		1.0 ppm		0.2 ppm		0.5 ppm		1.0 ppm		0.2 ppm		0.5 ppm		1.0 ppm	
	I	III	I	III	I	III	I	III	I	III	I	III	I	III	I	III	I	III
0–7[b]	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
8	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
9	56	40	93	97	100	100	27	27	93	96	100	100	25	30	76	66	100	100
12	23	13	100	90	100	97	..	..	100	100	100	100	20	20	53	33	100	60
15[c]	..	..	95	93	..	..	..	..	..	75	100	100	..	..	..	..	..	80
19	..	..	..	..	..	..	..	..	70	76	..	..	..	..	..	..	..	..
23	..	..	..	..	93	90	..	..	..	..	78	62	..	..	..	..	30	12
28	..	..	..	..	53	63	..	..	..	..	10	38	..	..	..	..	..	..

[a] Day of treatment was 17th February, 1971. Larval mortality in the check plot was nil in all bioassays.
[b] Bioassays made in water samples taken from plots days 1–14.
[c] Bioassays made directly in plots days 15–18.

SUMMARY

Abate insecticide was evaluated as a larvicide against *An. gambiae* in field plots simulating natural habitats of the species under wet and dry season conditions by WHO ACRU I near Kaduna, Nigeria in 1970 and 1971. The LC95 of *An. gambiae* larvae to Abate was 0.019 ppm.

In the wet season trials 1st instar *An. gambiae* larvae were recovered 8 and 10 days after treatment respectively in plots treated with 1 percent Abate sand granules and with an emulsion formulation at applied concentrations of 1.0 ppm. The presence of only 1st instar larvae in the emulsion treated plots during a 24-day observation period suggested a sorption-release activity of Abate maintaining sufficient insecticidal effect to kill larvae before reaching 2nd or 3rd instar.

In the dry season trials 1st instar *An. gambiae* larvae were first recovered from field plots treated with Abate 1 percent floating granules at the applied concentration of 1.0 ppm, 19 days after treatment with vermiculite and perlite formulations and 13 days in the krobite treated plots.

Further evidence of a sorption-release activity was suggested by the lower mortality rates of third instar *An. gambiae* larvae bioassayed in plot water samples than the rates observed in bioassays made in the plots themselves.

ASSAYS FOR THE FRIEND MURINE LEUKEMIA VIRUS (FLV) COMPLEX IN THE STABLE FLY, *STOMOXYS CALCITRANS*

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INTRODUCTION. In 1956, Friend (1956) isolated a Type C virus pathogenic to mice which had been inoculated with cell-free extracts prepared from the Ehrlich ascites mouse carcinoma. The agent, originally considered as a single virus, caused a leukemia-like disease syndrome similar to erythroblastosis, with characteristic enlargement of spleen and liver; subsequently the Friend murine leukemia virus (FLV) was found to consist of a complex of viruses. The component which induces lymphatic leukemia *in vivo* is known as the Friend lymphatic leukemia virus (FLLV or LLV).

Axelrad and Steeves (1964) developed a rapid quantitative assay for Friend leukemia virus based on enumeration of macroscopic surface spleen foci following 30–60 seconds immersion of the extirpated spleen in a fixative composed of ethyl alcohol, glacial acetic acid, formaldehyde and water. The spleen focus-forming virus is "defective" making it incapable of undergoing its entire infectious cycle and of inducing erythroleukemia in the absence of co-infection with LLV (Steeves and Eckner, 1970). Thus LLV serves as a helper virus, supplying factors missing from the SFFV genome, which then permits the production of infectious SFFV virus. In the Mirand strain of the FLV complex, which induces spleen focus formation and polycythemia, there is also present lactic dehydrogenase–elevating virus (LDV) (Riley *et al.*, 1960).

Since 1965, experiments have been carried out at the University of North Dakota to determine the extent to which various murine, avian, and feline oncogenic viruses could be transmitted mechanically or biologically by a variety of blood-sucking arthropods. The complex nature of the

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This work was conducted under Contract No. PH43-66-8 within the Special Virus Cancer Program of the National Cancer Institute, NIH, USPHS.