national uncertainty are developing programs concerned with human welfare. I would particularly like to acknowledge the personnel of the Region 4 malaria program for the contributions they have made. This program will eventually serve as a model for malaria control for the rest of the country. Mr. H. J. Jankowski, USAID malaria advisor, has been instrumental in the development of this endeavor. The technical assistance of Mr. C. H. Nam, Sanitarian, Region 4 Korean Preventive Medicine Team and the administrative support of Dr. J. Ely and Mr. J. Stivers are gratefully acknowledged. Dr. G. Carner and Dr. R. Noblet of the Department of Entomology and Economic Zoology, Clemson University, Clemson, S.C. and members of the parasitology group at the University of Alberta, Edmonton, Alberta, Canada reviewed the manuscript.

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INSECTICIDE BAITS FOR ANOPHELINE LARVAE[1]

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The use of stomach poisons, applied as sprays or dust to natural food materials or incorporated into artificial baits, is an insect control method which antedates by a century today's contact insecticides. Because of their mode of entry via the alimentary canal, stomach poisons, notably arsenic and fluorine compounds, have been employed mainly against chewing insects important as pests of agricultural crops and stored food products. Their application in the control of disease vectors has been limited to the use of paris green against mosquito larvae. The feasibility of killing surface-feeding anopheline larvae with this arsenical, applied as a floating dust, was first demonstrated by Barber and Hayne in 1921. Use of paris green against anophelines became widespread during the 1920's and 1930's (Pal and Gratz, 1968) and it has been credited with eradication of *Anopheles gambiae* from Brazil and Egypt in the 1940's (Soper, 1966). Formulated as a non-floating preparation, paris green has

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also been used operationally against *Aedes, Psorophora,* and *Culex* larvae (Fehn *et al.*, 1959).

The demonstrated worth in mosquito control of a stomach poison in particulate form has suggested that floating poison baits compounded with modern organic insecticides would provide an effective new larviciding technique for use in malaria control. In theory, this method of insecticide application should be highly efficient. The primary site of entry into the larval body would be the midgut and, provided the treated food was not repellent, very low dosage/application rates should be effective because the normal feeding activities of the larvae could be expected to accumulate the insecticide until toxic levels were reached. The feasibility of using a number of the newer insecticides as stomach poisons for *Anopheles* larvae is being investigated in the laboratory and this paper is a progress report on those tests.

MATERIALS AND PROCEDURES. Baits consist of whole wheat flour treated with water-insoluble insecticides. The flour is presieved to a particle size range of 88 to 125 microns with Tyler[3] standard sieves. Twenty-five grams are placed in a ribbed, 300 ml, round-bottom flask with a predetermined amount of technical insecticide dissolved in approximately 50 ml of acetone. The acetone is removed from the resulting slurry with a rotary vacuum evaporator. Loose agitators in the flask promote uniform mixing of the flour with the insecticide/acetone solution during the evaporation process. Following this treatment, the flour is resieved to a final size range of 88 to 105 microns, which was shown to be most efficiently ingested by 3rd stage insectary-reared *Anopheles albimanus* larvae (Wilton *et al.*, 1972). Whole wheat flour is used for two reasons: it floats and disperses well on the water surface, and, as a component of our standard insectary diet for anophelines, its acceptability to the larvae is well proven.

Except as noted, larvae for these tests have come from a malathion, DDT, dieldrin-resistant colony of *A. albimanus* established with material collected in coastal El Salvador (Breeland *et al.*, 1970). Tests are run in duplicate in glass crystallizing dishes with 50, uniform size, 3rd stage larvae in 300 ml of tap water. Water depth is 2.0 cm with 3.4 sq cm of surface area per larva.

Candidate baits are sprinkled on the water surface; controls receive untreated flour of the same size range in amounts equal to the lowest application rate being tested. After 24 hours, the larvae are classified as either normal or dead and moribund. The criterion used in the absence of evident toxic symptons for assigning larvae to the dead and moribund category is inability to regain the water surface and remain there after forced submergence. Baits treated to contain 1.0 percent, 0.5 percent, 0.1 percent, 0.05 percent, and 0.025 percent of several water-insoluble, technical grade insecticides have been evaluated in this manner at application rates of 0.046, 0.023, and 0.012 mg per sq cm (= 8.0, 4.0, and 2.0 mg per dish, respectively).

During the 24-hour test period the larvae have ample opportunity for physical contact with the insecticide-treated food and a question arises concerning the contact toxicity of the bait preparations. Studies to develop a method for distinguishing contact-caused mortality from that due to ingestion of insecticide baits have been initiated. In a preliminary test, ten 3rd stage *A. albimanus* larvae were individually observed for 4 to 5 minutes each under a binocular microscope to estimate what portion of their time larvae of this species spend feeding on untreated 88–105-micron flour particles. Since feeding (here defined as actions of the labral brushes effective in bringing food to the mouth) is a discontinuous activity, a cumulative type of stop watch was used to obtain totals for the observation periods.

[3] Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

Similar observations were made on 10 additional 3rd stage larvae from the same rearing pan exposed for ½ to 1 hour to a 1:2000 solution of tricaine methane sulfonate. Guttman (1967) reported this chemical (as MS-222 Sandoz) to be an effective anesthetic for blackfly larvae, but its ability to slow or stop the feeding activity of *Anopheles* larvae had not been determined.

Both anesthetized and normal 3rd stage *A. albimanus* larvae were then given short-term access to flour bait treated to contain 0.1 percent and 0.5 percent DDT. The test procedure was like that described above with two exceptions; known DDT-susceptible larvae were used, and they were confined in 2 x 2 cm stainless steel wire baskets. At the end of the feeding period the 50 larvae in each basket were vigorously tumbled in a stream of tap water to remove clinging food particles and immediately transferred to tap water in clean dishes for 24-hour mortality counts.

Results and Discussions. At one or more of the concentration/application combinations which were tested, each of the 12 baits included in Table 1 produced complete, or nearly complete, larval mortality. Those containing chlorpyrifos, phoxim, or Abate caused 99–100 percent mortalities with 0.025 percent preparations at an application rate of 0.023 mg/sq cm. Although none of the other baits approached this level of effectiveness, applications of 0.05 percent methyl chlorpyrifos, methoxychlor, or fenthion at the 0.023 application rate caused mortalities of 97–98 percent. The toxicity of baits containing coumaphos was somewhat lower. Results comparable to those above were obtained with this material at 0.1 percent concentration. DDT baits gave mortalities above 90 percent at both 0.05 percent and 0.1 percent concentration, but only when the application rate was increased to 0.046 mg/sq cm. Baits made with fenitrothion or resmethrin produced complete kills only at a toxicant concentration of 0.5 percent and similar results with methiocarb and carbaryl required 1.0 percent preparations. Allethrin and propoxur baits have also been tested but

Table 1.—Mortalities of third-stage *Anopheles albimanus* larvae after 24 hours access to 88–105 microns whole wheat flour treated with indicated target concentrations of technical grade insecticides. Except where noted all larvae were from a strain that was resistant to malathion, DDT and dieldrin.

Insecticide	% Conc.	Bait application (mg/cm²)	No. larvae	Mean % mortality	Equivalent amounts tech. insecticide/acre: Ounces	Grams
Chlorpyrifos	0.05	0.023	200	100	0.02	0.5
		0.012	200	100	<0.01	0.2
	0.025	0.023	300	100	<0.01	0.2
		0.012	300	100	<0.01	0.1
Phoxim	0.05	0.023	100	100	0.02	0.5
		0.012	300	100	<0.01	0.2
	0.025	0.023	300	100	<0.01	0.2
		0.012	300	94	<0.01	0.1
Abate	0.1	0.023	200	100	0.03	0.9
	0.05	0.023	300	100	0.02	0.5
		0.012	300	96	<0.01	0.2
	0.025	0.023	300	99	<0.01	0.2
		0.012	300	92	<0.01	0.1
Methyl chlorpyrifos	0.1	0.023	300	100	0.03	0.9
		0.012	300	94	0.02	0.5
	0.05	0.023	300	98	0.02	0.5
		0.012	300	86	<0.01	0.2

Table 1.—Continued

Insecticide	% Conc.	Bait application (mg/cm²)	No. larvae	Mean % mortality	Equivalent amounts tech. insecticide/acre	
					Ounces	Grams
Methoxychlor *	0.05	0.046	300	98	0.04	1.0
		0.023	300	97	0.02	0.5
		0.012	300	86	<0.01	0.2
Fenthion	0.5	0.023	100	100	0.16	4.7
		0.012	100	100	0.08	2.4
	0.1	0.023	300	99	0.03	0.9
		0.012	300	89	0.02	0.5
	0.05	0.023	300	97	0.02	0.5
		0.012	300	74	<0.01	0.2
Coumaphos	0.5	0.023	200	100	0.16	4.7
		0.012	200	100	0.08	2.4
	0.1	0.023	300	99	0.03	0.9
		0.012	300	98	0.02	0.5
	0.05	0.023	300	85	0.02	0.5
		0.012	300	70	<0.01	0.2
DDT *	0.1	0.046	200	97	0.06	1.8
		0.023	100	81	0.03	0.9
		0.012	100	80	0.02	0.5
	0.05	0.046	300	91	0.04	1.0
		0.023	300	82	0.02	0.5
		0.012	300	54	<0.01	0.2
Fenitrothion	0.5	0.023	300	100	0.16	4.7
		0.012	300	99	0.08	2.4
	0.1	0.023	300	13	0.03	0.9
Resmethrin	0.5	0.023	200	100	0.16	4.7
		0.012	300	91	0.08	2.4
	0.1	0.023	300	54	0.03	0.9
Methiocarb	1.0	0.046	100	100	0.66	18.8
		0.023	100	99	0.33	9.4
	0.5	0.023	300	77	0.16	4.7
Carbaryl	1.0	0.046	200	100	0.66	18.8
		0.012	200	97	0.16	4.7
	0.5	0.023	300	1	0.16	4.7

* Larvae used in these tests were susceptible to DDT and malathion but were resistant to dieldrin.

were omitted from the table because mortalities with these materials did not exceed 48 percent.

Results to date allow no conclusions regarding the possible influence of the concentration/application ratio on the effectiveness of a given insecticide. With fenthion, for example, 4 mg of 0.05 percent bait appeared somewhat more effective than 2 mg of 0.1 percent material (97 percent vs. 89 percent mortality) though both applications delivered the same amount of insecticide to the test dish. Coumaphos, however, was just the reverse; 2 mg of 0.1 percent bait caused 98 percent mortality whereas 4 mg of 0.05 percent material gave only 85 percent kill. With the remaining compounds, mortality differences associated with application-concentration differences either were not detected or amounted to less than 5 percent.

The effect on larval feeding produced by a 1:2000 aqueous solution of tricaine methane sulfonate is shown in Table 2. Whereas the normal larvae averaged nearly 57 percent of their time in feeding, those exposed to the anesthetic fed only

Table 2.—Feeding by third-stage susceptible *A. albimanus* larvae held for 30 to 60 minutes in water (normal) and in 1:2000 tricaine methane sulfonate * (anesthetized).

	Normal larvae			Anesthetized larvae		
	Observation time (sec.)			Observation time (sec.)		
Larva	Total	Feeding	% Time feeding	Total	Feeding	% Time feeding
1	335	259	77.3	271	3	1.1
2	240	92	38.3	275	141	51.3
3	270	118	43.7	270	0	0
4	240	80	33.3	270	7	2.6
5	262	233	88.9	270	63	23.3
6	307	60	19.5	270	63	23.3
7	270	216	80.0	270	0	0
8	276	179	64.9	270	63	23.3
9	255	38	14.9	270	52	19.3
10	270	266	98.5	301	58	19.3
Mean	273	154	56.4	274	45	16.4

* "Finquel" supplied by Ayerst Laboratories, New York, N.Y. 10017. "Finquel" is equivalent to "MS-222 Sandoz."

16 percent of the time they were under observation. The feeding rate of the normal larvae thus averaged about 3.5 times that of the anesthetized larvae. The mortalities sustained by normal and anesthetized larvae following short-term access to DDT bait (Table 3) are consistent with the differences in their respective feeding rates. It is apparent that the anesthetic afforded the larvae a marked degree of protection from otherwise toxic bait applications and it seems evident that the protection resulted from decreased feeding activity. These results agree with those of White and Jones (1968) who found that a number of anesthetics increased the survival of 4th stage *Anopheles quadrimaculatus* larvae exposed for 10 minutes to an acetone suspension of DDT. In the present test, the data, although limited, indicate that larval mortality attributable to body contact with the DDT-treated flour was of minor significance and that feeding activity provided the toxicant with its principal route of entry into the larval body.

The per acre equivalent amounts of technical insecticide represented by each of the bait applications in the test dishes are included in Table 1. Although extrapolations of such magnitude are not reliable, it is nevertheless evident that unusually small amounts of insecticide are highly effective when eaten by the larvae. If application rates similar to those found effective in the laboratory should prove

Table 3.—Mortalities of normal and anesthetized * third-stage susceptible *A. albimanus* larvae fed DDT-treated 88–105 microns whole wheat flour.

	Larvae			
Flour	No.	Condition	Min. access to food	% 24-hour mortality
With 0.1% DDT	150	Normal	40	95
	150	Anes.	40	13
With 0.5% DDT	100	Normal	10	97
	100	Anes.	10	28
Without DDT	50	Normal	40	
	50	Anes.	40	2

* Exposed to 1:2000 aqueous tricaine methane sulfonate 30 minutes prior to, and during, access to food.

effective in the field also, the use of floating baits compounded with water-insoluble, organic insecticides would provide a new technique for anopheline control which would be highly advantageous in terms of low insecticide cost and reduced environmental contamination.

ACKNOWLEDGMENT. The authors wish to thank Dr. Stafford A. Lindsay of Ayerst Laboratories, New York, New York 10017, for making available a sample of Finquel (tricaine methane sulfonate) for use in this study.

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EFFECTIVENESS OF GROUND ULV AEROSOLS AGAINST LARVAE OF *PSOROPHORA CONFINNIS* (LYNCH-ARRIBÁLZAGA)

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The ULV ground aerosol technique of applying insecticides has recently become widely accepted for mosquito control. Considerable research has been performed with this method of application, primarily against caged adult mosquitoes. However, little has been reported on the effectiveness against mosquito larvae. Lofgren (1970) stated that ULV spraying works well against adult mosquitoes, but is limited for larval breeding areas because vegetation reduces its effectiveness. Also, larval breeding is limited to relatively small, distinct areas, and the main objective is to deposit insecticide at these sites.

There is much concern regarding mosquito resistance to insecticides, and it would appear useful to understand the effect of ULV ground application upon larvae as well as adults since larvae are often present in areas where adulticides are used. The objectives of this research were to ascertain the effectiveness of ULV application of insecticides against dark ricefield mosquito larvae, *Psorophora confinnis* (Lynch-Arribálzaga). No tests have been reported against larvae of this species using ground ULV aerosols.

Most ULV larviciding has been accomplished by aerial spraying. Mount *et al.* (1970) applied aerial sprays of malathion and fenthion against larvae contained in

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