FIELD AND LABORATORY ASSESSMENT OF TEMEPHOS FOR LARVAL CONTROL OF *ANOPHELES ALBIMANUS* IN EL SALVADOR AND EVIDENCE FOR RESISTANCE[1]

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ABSTRACT. Temephos [*0,0'*–(thiodi-4,1-phenylene) bis (*0,0*-dimethyl phosphorothioate); AI3-27165] applied by ground equipment at 9.2 g/ha provided up to 98% control of mosquito species in El Salvador, Central America, in 1976. Aerial applications of the same compound were made at a rate of 8.3 g/ha in 1976 and 1977 to anopheline breeding areas in a 150-km² area along the Pacific Coast. In 1978, laboratory bioassays confirmed that *An. albimanus* had acquired a 164-fold cross resistance to temephos. This resistance might have resulted from yearly exposures of these mosquitoes to chemicals for cotton insect control in the area. Such resistance might be lost within a few generations if the population is no longer under pressure from temephos or related compounds.

INTRODUCTION

A pilot test was initiated in El Salvador, Central America, in 1975 to evaluate the feasibility of using the sterile insect technique (SIT) in an integrated control program to control *Anopheles albimanus* Wiedemann. Since the SIT is most effective and practical when the target insect population is at a low density, initial supplementary control methods are usually necessary to lower the insect population. If this is done in combination with natural population fluctuations caused by environmental factors, it should be possible to reduce the density in the test area to a manageable level. In the El Salvador test, we decided to use insecticidal applications as the supplementary control method. However, because of the large size of the test site and the cost, application of an adulticide over the entire area was impractical.

Another critical factor was the high degree of resistance of the mosquitoes to many of the commonly used insecticides including malathion (*0,0*-dimethyl phosphorodithioate of diethyl mercaptosuccinate; AI3-17034) and propoxur (*o*-isopropoxyphenyl methylcarbamate; AI3-25671) (Georgiou 1972). This resistance was caused by the indirect selection of mosquitoes by pesticides employed on the large cotton plantations in the test area. However, Georghiou (1972) did not report mosquito resistance to the larvicide temephos (*0,0'*-(thiodi-4,1-phenylene) bis (*0,0*-dimethyl phosphorothioate); AI3-27165); thus we thought that this compound could be used effectively. We report here the results of field trials with temephos conducted over a 2-year period and include laboratory evidence that mosquito resistance to temephos had developed by 1978.

DESCRIPTION OF TEST AREA

The test site is a triangular-shaped area of about 150 km² on the Pacific coastal plain of El Salvador, bounded on the north by rapidly rising bluffs at the base of a mountain range and on the south by the Pacific Ocean. These two barriers join at the west end of the triangle at the city of La Libertad. The eastern end opens into a broad plain about 12.5 km wide, which is devoted mostly to the production of cotton, sugarcane, and dairy cattle.

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[1] This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the USDA nor does it imply registration under FIFRA as amended. Mention of a commercial or proprietary product does not constitute an endorsement of this product by the USDA.

Five major rivers flow south to the ocean across the test area; in addition, there are numerous smaller rivers, streams, and irrigation canals. The water flows into coastal estuaries that form low, swampy marshlands. During the wet season these estuaries are open to the ocean, and their salinity becomes high enough to prevent anopheline breeding. However, in the dry season (December to April), the outlets to the ocean become blocked with sand, and the salinity decreases due to the influx of fresh runoff water, thereby allowing anopheline breeding to occur. Runoff from the irrigation canals throughout the agricultural area creates additional mosquito breeding sites in low-lying fields and roadside ditches. Other breeding areas consist of small ponds, canals, and lowlands that become flooded during periods of excessive rainfall. Breeland (1972) described the various mosquito habitats throughout the diversified types of land use in the area.

Since we were attempting to reduce the *An. albimanus* population to the lowest level possible, the larvicide applications were made in March and April in 1976. During these months at the end of the dry season, the potential breeding area is at a minimum (ca. 404 ha). In 1977, however, the applications were delayed until July because of problems in rearing and processing the sterile males.

MATERIALS AND METHODS

Although earlier studies (Georghiou 1972) showed no resistance of *An. albimanus* larvae to temephos (LC_{50} = .005 ppm) and because this was the material of choice for selective mosquito larval control by local officials, we decided to run a series of bioassays to assure the effectiveness of the compound under actual field conditions.

Numerous aquatic locations throughout the experimental area were examined for the presence of anopheline larvae with 350-ml dippers, which collected water from about 750 cm^2 of surface area. Collection records were tabulated on printed cards that indicated the numbers collected in each dip for: (1) 1st and 2nd-stage anopheline larvae; (2) 3rd and 4th-stage anopheline larvae; (3) pupae; and (4) all other species. The areas that were positive for anopheline larvae were evaluated for the relative numbers present and for size and accessibility for bioassay treatment. Thirteen acceptable sites were selected that were representative of the different breeding habitats that would be encountered in a large-scale treatment; these sites ranged in size from 5 to 130 m^2. A single application of temephos was made at each site, and all sites were treated during a 4-wk. period in February, 1976.

Temephos was applied to the bioassay plots at a rate of 9.2 g/ha with 9.5-liter stainless steel hand pump sprayers. The applicators sprayed a standardized swath width of 2.4 m (1.2 m on each side of the applicator) at a walking pace of 0.5 m/sec. Each sprayer was fitted with a TeeJet® nozzle and calibrated for a known rate of delivery at 276 kN/m^2 (40 psi). The delivery rate and appropriate amount of chemical to be added per liter of water was labeled on each sprayer. The chemical for each sprayer was weighed in the laboratory and taken to the field in labeled, glass screwtop vials to permit rapid and accurate refill of the sprayers.

At each site, pretreatment counts of mosquitoes were made immediately prior to application and subsequent collections were made 2 and 24 hr posttreatment. For 5 of the treatments, additional collections were made at 48, 72, and 96 hr posttreatment.

During March 1976, two aerial applications of temephos were made to all major breeding areas. A Rockwell Thrush Commander® agricultural aircraft fitted with Micronair® nozzles was used to make the applications, which were spaced 6 days apart. The delivery was calibrated to achieve a rate of 8.3 g of temephos in 1.5 liters of water/hectare (0.045 lb/acre/gal).

Because of the diverse types and irregular shapes of the breeding sites, it was impossible for the pilot to fly straight-line patterns; therefore, it was left to his discretion how best to obtain optimum application after considering the meandering curves of rivers, tree line vegetation, heights of bridges, and locations of utility lines. Treatments began immediately after daybreak when the influence of winds was at a minimum. Approximately 3,406 liters of the temephos formulation were needed to cover the area for the 1st application; the aircraft carried 1,135 liters per sortie. Prior to the treatment, members of the field crew were stationed in predetermined locations to make pretreatment counts of larvae and pupae, and to subsequently check the efficiency of application. After treatment, daily counts were made at 12 separate locations for a period of 5 days. The 2nd application was completed 6 days after the first; however, 4 sorties were made (4542 liters) to compensate for an inadequate treatment in 2 locations and additionally discovered breeding sites. Also, 2-man crews with spray pumps were sent to a few locations where river gorges were too deep or too winding to allow adequate aircraft application or where trees along the streams were too dense to allow penetration of the spray droplets.

The 2 aerial applications made in July 1977 were similar to those in 1976 except that adjustments were made for changes in breeding sites and the number of posttreatment assays was decreased. Larval assays were made at 8 locations before and 24 hr after the 1st treatment. Larval counts made immediately prior to the 2nd application served the dual purpose of pretreatment (0-hr) assays for the 2nd application and a 7-day (168-hr) posttreatment sample for the 1st application. Counts were made at 6, 24, and 168 hr after the 2nd treatment.

In 1978 after reviewing the data from previous temephos applications, and with the knowledge that large quantities of diverse chemicals were being used for control of agricultural pests throughout the area, we conducted laboratory bioassays to determine the susceptibility of mosquitoes to temephos.

Standardized assays were conducted in 600-ml glass beakers containing 250 ml of distilled water. Serial dilutions were prepared from technical grade temephos, and amounts were added to each beaker to provide 6 concentrations ranging from 0.005 to 1.0 ppm. Tests were conducted with 25 early 4th-stage larvae that had been reared from eggs oviposited by fieldcollected adult females. Two controls were used; the 1st consisted of a beaker containing 1 ml of ethanol (the original solvent) added to 249 ml of distilled water, and the 2nd was distilled water only. For these tests, larval food (ground hog supplement, 40% protein, Purina Food Corp.) was added immediately after the larvae were introduced and the water temperature in the beakers was recorded. Mortality in each beaker was recorded 24 hr posttreatment. A 2nd test was conducted 2 wks later with larval progeny from a separate field collection of adult females. Three replicates were completed in each test.

RESULTS AND DISCUSSION

The results obtained in the small area bioassay test with hand spray applications appear in Table 1. A high level of control of all stages was achieved for 48 hr. After this time the control of 1st- and 2nd-stage larvae began to decrease, but good reduction of 3rd- and 4th-stage larvae and pupae was achieved for 96 hr. These results were consistent with the hypothesis that the temephos effectively killed all larval stages and that reinfestation began as larvae hatched from eggs that were unaffected by the chemical. Data from individual treatments indicated that no one site was sufficiently different from the others to drastically alter the results, and the averages shown are for all treatments. In these tests, mosquito species other than anophelines, although not specifically identified, were also controlled for 48 hr before they began to increase in numbers.

Since good control of mosquito larval populations had been achieved in single

Table 1. Numbers of immature mosquitoes collected from 13 breeding sites after one application of temephos at 9.2 g/ha. with hand sprayers in El Salvador in 1976.

Time post-treatment (hr)[a]	Avg. no. of dips per site	Average number collected per dip[b]			
		Anopheles larvae (stages)			
		1–2	3–4	Pupae	Other mosquito species
0	100	2.06	1.86	0.25	3.40
2	94	.40	.61	.27	.41
24	114	.06	.13	.11	.20
48	58	.05	.03	.04	.18
72	60	.39	.02	.04	.85
96	53	.89	.04	.01	1.52

[a] Zero indicates pretreatment counts made immediately before treatment.

[b] Numbers shown are averages for all thirteen applications at 0–24 hr and 5 of the sites at 48–96 hr.

treatments with ground equipment, we decided that for large-scale application 2 treatments applied 6 days apart would kill developing larvae hatched after the 1st treatment and provide control of a complete generation of the natural population. The degree of control achieved with aerial application (see Table 2) was much lower than with the ground applications, but the pilot verified that during the 1st application complete coverage of some locations was not possible because of topographical obstacles. By 120 hr after the second application, all collections of immature stages of mosquitoes, except third- and fourth-stage anopheline larvae, were equal to or higher than those recorded before the first treatment.

Because it was thought that the mediocre results of the 1976 applications may have been a result of inadequate insecticide distribution, detailed instructions concerning the breeding sites were given to the pilot prior to the 1977 aerial applications. Areas difficult to delineate from the air were marked by flagmen, and 2-way radio communication was established from ground vehicles to the airstrip used for reloading the aircraft to report areas receiving inadequate treatment. Also, extensive treatments were applied by ground crews in inaccessible areas. The results shown in Table 2 indicate that the level of control observed 24 hr posttreatment exceeded 85% and was similar to that observed with ground treatment the year before, and that collections of anopheline larvae made 7 days posttreatment were still below the level observed in pretreatment counts.

Several factors could account for the differences in results of the 1976 and 1977 aerial applications. First, a more efficient use of ground application crews during 1977 reduced the amount of chemical used in aerial treatment of difficult areas. Second, the pilot was more familiar in 1977 with the terrain and the need to cover specific locations. However, the most important factor may have been that during the treatments in 1976 the natural population was in a very high rate of natural increase. The applications during 1977 coincided with a delay in the onset of the rainy season, at which time the natural population was at a low level. Therefore, the reduction observed was achieved at a time when the population was at its lowest rate of increase.

Prior to requesting permission from the local government authorities to apply temephos into the SIT area in 1978 for the 3rd consecutive year, the previously mentioned laboratory bioassays were conducted to determine whether resistance to temephos (or a cross-resistance derived from pressure of related compounds) had developed. The preliminary bioassay to determine the range of dosages to be used gave the 1st evidence that

Table 2. Numbers of immature mosquitoes collected from 20 breeding sites after aerial applications of temephos at 8.3 g/ha in El Salvador in 1976 and 1977.

Date of treatment	Time posttreatment (hr)[a]	Total no. of dips[b]	Average no. collected per dip			
			Anopheles larvae (stages) 1–2	3–4	Other species	Pupae
			1976			
March 4	0	490	1.00	0.74	0.88	0.08
	24	477	.84	.49	.51	.03
	48	160	.51	.23	.43	.01
	72	235	.48	.37	.66	.03
	96	280	1.09	.83	2.37	.15
	120	80	.76	.55	3.30	.05
March 10	24	480	.17	.16	.42	.05
	48	400	.49	.31	.37	.02
	72	120	.59	.07	.73	.02
	120	320	1.25	.35	.98	.08
			1977			
July 22	0	160	0.87	0.32	0.89	
	24	160	.08	.04	.38	
July 29	0	160	.30	.17	.14	
	6	160	.03	.00	.02	
	24	160	.06	.00	.00	
	168	160	.13	.17	.86	

[a] Zero indicates pretreatment counts; no pretreatment counts were made before the second application in 1976 since all areas had been previously treated.

[b] An average of 40 dips was taken from each of the 12 locations in 1976 but not all sites were sampled each day; 20 dips were sampled from each of 8 locations in 1977.

F_1 larvae might be resistant. When mortality was recorded 6 hr posttreatment, all laboratory colony larvae died at a dosage of 1.0 ppm, but only 6% of the field stock died at the same concentration; there was no mortality of the control larvae in untreated water. Averages for the 6 standardized bioassay treatments with dosages ranging from 0.005 to 1.0 ppm appear in Table 3. After we corrected for control mortality, probit analysis established that the LC_{50}, LC_{90}, and LC_{95} values were 0.125, 0.4554, and 0.6562 ppm, respectively. Georghiou (1972) established a dosage of 0.005 ppm for an LD_{50} in 1970 and then showed that this level did not change in 3 collections of adults made in 1971. When we used the recommended concentration of 0.004 ppm, previously shown to be the appropriate dosage for an LD_{95}, the field-collected mosquitoes in these tests exhibited a 164-fold resistance to temephos. At this juncture, the plans for the 1978 application of temephos were abandoned.

Table 3. Susceptibility of fourth-stage F_1 larvae obtained from field-collected adult female *An. albimanus* to temephos in El Salvador in 1978.[a]

Concentration (ppm)	Number tested	Number dead[b]	Percentage mortality
1.0	149	144	96.6
.5	149	141	94.6
.1	149	72	48.3
.05	150	20	13.3
.01	150	5	3.3
.005	150	7	4.7
Controls			
Ethanol and water	150	5	3.3
Water	150	6	4.0

[a] Results shown are for 6 bioassays with 25 larvae per replicate.

[b] Mortality was recorded 24 hr posttreatment.

We have not fully ascertained why adequate control was achieved by ground applications in 1976 and the aerial applications in 1977, and yet field-collected stock showed such a high level of resistance in 1978. One possibility could be associated with the schedule of application of chemicals for control of cotton pests in the coastal area of El Salvador. Large quantities of chemicals are used throughout the area during the cotton-growing season from August to December, which exerts selective pressure to insecticide resistance upon the natural population of mosquitoes. We first applied temephos from aircraft in March of 1976 and achieved only moderate control. However, in 1977 the treatments were not made until July, or approximately 6 months after cessation of the cotton spraying. In contrast, the laboratory bioassays in 1978 were conducted with progeny of adults collected in January or early February. It is interesting to speculate whether the natural population of *An. albimanus* could attain a high level of chemical resistance after only a few generations of selection and then rapidly lose that resistance. The data of Georghiou (1972) indicated that this did occur in *An. albimanus* collections made in February and June of 1971 and exposed to propoxur and carbaryl. However, another possibility is that over the duration of our investigations the natural population has actually acquired a resistance to temephos as a result of continued exposure to organophorous compounds applied for cotton insect control. Since we cannot be sure of the cause, we report here only that resistance to temephos has developed in a natural population of *An. albimanus* in El Salvador. This resistance should be considered in any future mosquito control programs.

References Cited

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