EFFECTIVENESS OF NEW INSECTICIDES AS RESIDUAL SPRAYS FOR THE CONTROL OF *ANOPHELES QUADRIMACULATUS* IN BUILDINGS

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Since the discovery that residual sprays of DDT applied to the interiors of buildings remained effective against *Anopheles* mosquitoes for many months (Gahan and Lindquist, 1945; Gahan *et al.*, 1945a and 1945b), this method of control has been used extensively in malaria eradication campaigns. Because resistance to DDT and to dieldrin has now become prevalent in various parts of the world, substitute insecticides are needed that can reestablish control in such places. The Entomology Research Division is one of a group of organizations engaged in a continuing program for the evaluation of new insecticides that might be used as residual applications against disease-carrying species of mosquitoes.

We have had no great difficulty finding materials that kill adult mosquitoes, but

only a small proportion of these compounds remain toxic longer than 1 or 2 months after application to surfaces on which *Anopheles* mosquitoes rest. Most durable insecticides have been unsatisfactory for incorporation in malaria eradication programs primarily because they were highly toxic to warm-blooded animals, were too costly to manufacture, or had undesirable physical characteristics.

MATERIALS AND METHODS. In our laboratory studies, we have recently found five chemicals with low toxicities to rats that appeared to be highly effective against adults of *Anopheles quadrimaculatus* Say for at least 6 months when they were applied to plywood boards at the rate of 1 g/m^2. Another that has a moderate toxicity (135–163 mg./kg. of body weight) to rats also has been effective in this type of test. The designation, chemical name, and acute LD_{50} to rats (based on information received from the manufacturer) for the six compounds are:

Designation	Chemical name	Acute oral LD_{50} in rats (mg/kg)
Bay 38799	*o*–cyclopentylphenyl methylcarbamate	1000
Bay 77488	*O,O*–diethyl phosphorothioate *O*–ester with phenylglyoxylonitrile oxime	>2500
Bay 78182	*O,O*–diethyl phosphorothioate *O*–ester with (*o*–chlorophenyl)glyoxylonitrile oxime	>2500
Dursban ®	*O,O*–diethyl *O*–3,5,6–trichloro–2–pyridyl phosphorothioate	135–163
Neopynamin ®	2,2–dimethyl–3–(2–methylpropenyl)cyclopropanecarboxylic acid ester with N–(hydroxymethyl)–1–cyclohexene–1,2–dicarboximide	5200
Union Carbide UC–8454	5,6,7,8–tetrahydro–1–naphthyl methylcarbamate	325–470

The results obtained in laboratory tests with four of these compounds have been published previously (Gahan *et al.*, 1967); in these tests, Bay 38799 and UC-8454 killed 100 percent of the mosquitoes exposed to them throughout a 24-week test. Dursban killed 100 percent the first 20 weeks and 75 percent the 24th week; Neopynamin killed 98 percent the 4th and 16th weeks and 100 percent during the other test periods. Bay 77488 and Bay 78182 were tested after the report by Gahan *et al.* (1967); both materials consistently produced 100 percent mortality throughout the 24-week test.

During the summer of 1967, the same six compounds were compared with Bay 39007 (*o*-isopropoxyphenyl methylcarbamate) and carbaryl (1-naphthyl methylcarbamate) as residual sprays in buildings naturally infested with adult *A. quadrimaculatus*. Bay 39007 had been highly effective, and the carbaryl had been only moderately effective in tests of this type conducted previously (Gahan *et al.*, 1964, 1965). The compounds for this test were all obtained from commercial sources. The study was made in the vicinity of Stuttgart, Arkansas, one of the principal rice-growing sections of the United States, where many fields are flooded from May until September, and adult *A. quadrimaculatus* are numerous in the daytime in open buildings—particularly in those that house animals.

Only small wooden buildings on farms with livestock were used. Most tests were made in chicken houses or in barns that housed animals at night, but one carport close to an area where animals were maintained regularly was also used. Since spider webs provide resting places

for adult *Anopheles* and sprays do not adhere to them as well as to the wood, those webs that could be reached easily were removed before spraying.

Applications were made between June 12 and June 30, 1967 with a compressed-air sprayer with a nozzle that delivered a flat, fan-shaped spray pattern. Just enough material was placed in the tank to treat a single building at 2 g. of insecticide per square meter. Bay 38799 was used as a wettable powder, and all other materials were applied as water emulsions. Each spray contained 5 percent insecticide. Three buildings were treated with each insecticide by coating all potential resting places on the interior walls, ceilings, and pieces of equipment. The exterior surfaces and areas under the eaves were left untreated.

Effectiveness was evaluated by making pre- and posttreatment counts of the adult *A. quadrimaculatus* in the sprayed buildings and in four untreated buildings that were used as controls to determine variations in the normal densities in the area. No building was treated unless the infestation exceeded 150 *A. quadrimaculatus* in pretreatment observations made on each of two afternoons. In buildings with a density exceeding 500, an accurate count was impossible because mosquitoes moving from one spot to another disturbed those in the vicinity; instead, 50 or 100 were counted, and the total number was estimated.

Most posttreatment observations were also made in the afternoons to allow time for the insecticides to affect the mosquitoes. If a visit was made before noon and mosquitoes were present, a return trip was made later in the day, and the afternoon count was the one used. All interior surfaces were examined with the aid of a flashlight, and particular attention was paid to the corners of the room, the insides and undersides of chicken nests, the insides of empty boxes, barrels, or feeding troughs, and other places where the intensity of light was low. Then the percentage reduction was determined by comparing the number of *A. quadrimaculatus* observed before treatment and at intervals after treatment. By the middle of September, most of the rice fields had been drained, and the mosquito population in the untreated buildings was gradually decreasing. The experiment was therefore discontinued after the first week in September when it became obvious that further observations would not give accurate information on the effectiveness of the treatments. Each building was thus observed for 10 to 12 weeks after treatment unless it became obvious prior to that time that the treatments were no longer effective. The results are shown in Table 1.

The efficacy of the residual deposits was also verified by collecting adult *A. quadrimaculatus* from untreated buildings and confining 13–38 of them under half sections of petri dishes on each of the four walls of a building for 60 minutes. The dishes were held in place by rubber bands stretched tightly between small staples driven into the wall on opposite sides of the containers. The times required for 50 and 100 percent knockdown were recorded if this degree of effectiveness was achieved within 1 hour. After 1 hour, percentage knockdown was recorded, pieces of thin cardboard were inserted between the insects and the treated wall, and the dishes were transferred in a cooled box to an air-conditioned room maintained at a temperature of about 80° F. There a small piece of cotton that had been dipped in 10 percent sucrose in water was added to each dish. The percentage mortality was recorded 24 hours after the exposures were made. Such tests were made as time permitted on residues that were at least 4–12 weeks old. The results are presented in Table 2. Exposures were made in two or three buildings treated with each compound except carbaryl. Carbaryl was omitted because the afternoon counts demonstrated almost at once that it was one of the least effective of the test compounds.

RESULTS. Bay 39007 and Dursban were

TABLE 1.—Effect of insecticidal residues on densities of mosquitoes in buildings during the afternoon.

Type of building	Pretreatment count	% Reduction at indicated week after treatment											
		1	2	3	4	5	6	7	8	9	10	11	12
							Bay 38799						
Cow barn	1000+	100	100	99.7	99.9	99.6	99.8	99.3	100	100	99	99.4	100
Dog house	248	100	100	100	100	100	99	100	100	100	96	100	...
Chicken house	714	100	100	100	100	100	100	100	100	100	100	...	...
							Bay 77488						
Pig barn	742	100	100	100	99.9	100	100	100	99.9	92	99.6	99	91
Horse barn	568	100	100	100	100	100	100	100	100	100	100	100	100
Horse barn	635	100	100	100	100	100	100	100	100	100	100	...	...
							Bay 78182						
Horse barn	813	100	100	100	100	99.8	99	100	100	92	100	100	95
Cow barn	445	100	100	100	100	100	100	100	100	100	88	100	100
Horse barn	688	100	100	100	100	100	100	100	100	100	100	...	...
							Bay 39007						
Pig barn	1000+	100	100	100	99.1	100	100	100	100	99.5	100	99	99.8
Carport	344	100	100	100	100	100	100	100	100	100	100	100	...
Chicken house	398	100	100	100	100	100	100	100	100	100	100	...	...
							Carbaryl						
Cow barn	840	100	90	50	99.4	93	93	44	30	...	...	...	...
Cow shed	459	99.1	84	99.1	87	98	93	97	98	86	55	99	...
Chicken house	797	99.5	92	0	75	87	93	98	40	74	0	0	...
							Dursban						
Pig shed	1000+	100	100	100	100	100	100	100	100	100	100	100	100
Pig barn	1000+	100	100	100	100	100	100	100	99.5	100	99.8	100	...
Cow barn	783	100	100	100	100	100	100	100	100	99.5	100	...	...
							Neopynamin						
Animal barn	1000+	100	99	94	92	48	4	13	...	...	...	...	...
Dog house	170	100	99.4	100	100	99.4	100	97	21	57	0	61	...
Dog house	238	100	100	99.2	100	84	91	95	75	75	75	...	...
							UC-8454						
Horse shed	444	100	100	99.8	100	100	99.3	100	100	94	100	98	99.3
Goat shed	297	100	100	100	100	99	99.3	100	100	100	99.3	99.7	...
Cow barn	375	100	99	98	100	100	100	99.2	99.2	99.5	99.5	...	...

highly effective throughout the entire 10–12 week period. They consistently reduced the mosquito infestations in the buildings 99 to 100 percent during the afternoon. Bay 38799, Bay 77488, Bay 78182, and UC-8454 also produced at least 99 percent control on most occasions; however, some temporary increase in the mosquito density occurred in one or more buildings treated with each of these materials. For example, the infestation in the dog house sprayed with Bay 38799 increased slightly during the 10th week but decreased again the 11th week. The pig barn treated with Bay 77488 showed substantial increases the 9th and 12th weeks, but many dead and dying mosquitoes were seen there. Also, at least two of the three buildings treated with Bay 78182 or UC-8454 had less than 99 percent reduction during one or two observations, and in the cow barn treated with Bay 78182, the control was as low as 88 percent the 10th week though it increased to 100 percent the next week.

Neopynamin was the least effective of the new chemicals and appeared to have insufficient residual effect to be considered promising. In all three buildings, it was highly effective less than 5–8 weeks and on one or more occasions, it appeared to have little or no effect in two buildings 6–10 weeks after application.

As in previous tests, results with carbaryl were erratic. Control was as low as 0–50 percent after 2–3 weeks but on later occasions was as high as 93–98 percent in the same buildings.

Counts of *A. quadrimaculatus* in the four untreated buildings used to house animals demonstrated that adults were active in the vicinity of Stuttgart throughout the period of this test. They averaged between 564 and 683 mosquitoes per building the last half of June, 401 and 603 throughout July, 214 and 496 throughout August, and 238 and 309 in the first week in September.

The age of the deposits at the time of the wall exposure tests (Table 2) varied so much that close comparisons of the results are not justified. However, most of the treatments caused 100 percent mortality in 24 hours after a 1-hour exposure. Again, Neopynamin was the least durable of the new chemicals; 8-week-old residues of this compound produced only 6 and 54 percent kill in 24 hours in two buildings. All other insecticides produced 100 percent kill in at least one or two buildings when the treatments were 7–12 weeks old. Bay 78182 and UC-8454 were considerably less effective in one building than the Bay 39007 standard during the 9th week; they caused only 72 and 16 percent mortality, respectively, in 24 hours compared with 100 percent for Bay 39007. However, the other applications of these two insecticides were still highly effective after 4–8 weeks of aging. An application of Dursban in a pig shed that was evaluated the 6th and 12th weeks showed little if any loss in toxicity. Bay 38799, Bay 77488, Dursban, and the Bay 39007 appeared to be the most effective compounds.

An outstanding characteristic of Bay 39007 is its fast action. In the wall exposure tests, it caused 100 percent knockdown within 13 and 25 minutes after the mosquitoes were confined to the treated walls. Bay 38799 was the only one of the new compounds that compared favorably with Bay 39007 in speed of action. It caused 50 percent knockdown in 13 and 16 minutes and 100 percent knockdown in 23 and 28 minutes. Only two other treatments caused 100 percent knockdown in less than 60 minutes: UC-8454 applied in a goat shed caused 50 percent knockdown in 23 minutes and 100 percent knockdown in 35 minutes; Dursban applied in a cow barn produced 50 percent knockdown in 20 minutes and 100 percent knockdown in 50 minutes.

SUMMARY. Five chemicals that have low and one that has moderate toxicity to rats were evaluated as residual sprays in buildings naturally infested with adult *Anopheles quadrimaculatus* Say. All

TABLE 2.—Toxicity of residues in wall exposure tests (mosquitoes exposed to treated surfaces under half sections of petri dishes).

Type of building	Age of residues (weeks)	Avg. no. mosquitoes per dish	Avg. knockdown and kill: Minutes to knockdown of— 50%	100%	% knockdown after 1 hour	% kill after 24 hours
			Bay 38799			
Cow barn	10	29	16	28	100	100
Chicken house	4	24	13	23	100	100
			Bay 77488			
Pig barn	8	29	>60	>60	43	100
Horse barn	4	20	40	>60	88	100
Horse barn	7	30	35	>60	92	100
			Bay 78182			
Horse barn	9	28	>60	>60	9	72
Cow barn	4	21	>60	>60	38	100
Horse barn	7	23	36	>60	97	100
			Bay 39007			
Pig barn	9	23	14	25	100	100
Chicken house	6	27	9	13	100	100
			Dursban			
Pig shed	6	21	>60	>60	64	100
	12	28	>60	>60	48	100
Pig shed	9	27	>60	>60	45	100
Cow barn	6	27	20	50	100	100
			Neopynamin			
Dog house	8	29	>60	>60	28	54
Dog house	8	22	>60	>60	7	6
			UC-8454			
Horse shed	9	23	>60	>60	7	16
Goat shed	7	23	23	35	100	100
Cow barn	8	28	>60	>60	63	100

treatments were applied at the rate of 2 g of insecticide per square meter. Dursban was highly effective throughout the entire test period of 10–12 weeks; it consistently reduced the infestations in the buildings 99–100 percent during the daytime. Bay 38799, Bay 77488, Bay 78182, and UC-8454 also produced at least 99 percent control on most occasions. However, some temporary increase in the density of mosquitoes occurred in one or more of the buildings treated with each of these materials. Neopynamin had insufficient residual effect to be considered promising. The Bay 39007 standard was at least equal to Dursban, but the carbaryl standard gave erratic results. When adult *A. quadrimaculatus* were exposed to treated walls for 1 hour under half sections of petri dishes, Bay 38799, Bay 77488, Dursban, and Bay 39007 proved to be the most toxic compounds, and Bay 39007 and Bay 38799 were the fastest acting materials.

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