

ARTICLES

RESIDUAL TOXICITY OF SOME NEW INSECTICIDES TO ADULTS OF *ANOPHELES QUADRIMACULATUS* SAY

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This paper is one in a series presenting the results obtained in a program to develop new insecticides as residual sprays for the control of adult mosquitoes. In previous papers LaBrecque *et al.* (1958, 1960), Gahan and Hoffman (1959), and Gahan *et al.* (1961) showed malathion and *m*-isopropylphenyl methylcarbamate (Hercules AC-5727) were sufficiently effective to justify extensive testing in infested areas where the chlorinated hydrocarbon insecticides have failed to give satisfactory control. The present report includes data obtained in the laboratory with five new compounds and in naturally infested buildings with four of the compounds mentioned in previous papers cited above.

LABORATORY TESTS. In the laboratory evaluations, acetone solutions of the compounds were sprayed on plywood surfaces at one or more dosages. At intervals after treatment approximately 50 adults of *Anopheles quadrimaculatus* Say (mixed sexes) were exposed on the treated surfaces under half sections of Petri dishes for periods of 1, 5, 15, 30, or 60 minutes. All the exposures were not used each time but an effort was made to achieve a range of mortalities that reached 100 percent. After exposure, the insects were transferred to untreated screen holding cages, furnished cotton that had been saturated with 10 percent honey solution, and held for 24-hour mortality counts.

The strain of *A. quadrimaculatus* used in these tests has developed a high level of resistance to dieldrin in the course of many years of laboratory maintenance. At the time of these tests the larvae had about 100-fold resistance and adult mortality did

not exceed 16 percent with the W. H. O. standard test kit following a 1-hour exposure on papers treated with 0.8-percent dieldrin.

One of the compounds, *o*-isopropoxyphenyl methylcarbamate (Bayer 39007), was closely related to the *m*-isopropylphenyl methylcarbamate (Hercules AC-5727) that was highly effective in previous studies. It is a white crystalline material that forms an almost clear solution in acetone. When applied to plywood, it did not stain and had only a slight odor. The other compounds are esters of phosphorothioic or phosphinothioic acids. *O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate (Bayer 41831) is a brown liquid with a mild unpleasant odor. It gave a yellow solution in acetone and stained the plywood boards very slightly. *O,O*-diethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37341) and *O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37342) are both colorless liquids that do not stain. *O*-(4-methylthio-*m*-tolyl) dimethylphosphinothioate (Bayer 34098) is a white powder with a mild unpleasant odor. It forms a colorless solution in acetone and does not stain. DDT, malathion, and Hercules AC-5727 were included in these tests as standards.

The minimum exposure times required to produce between 90 percent and 100 percent mortality within 24 hours after the residues had aged various periods of time are presented in Table 1.

Bayer 39007 and Bayer 41831 were the compounds of greatest interest. When they were used at dosages of 100 mg. per square foot, exposures as short as 1 to 5

TABLE 1.—Minimum exposure times required to produce at least 90-percent mortality within 24 hours among *Anopheles quadrimaculatus* exposed on plywood panels treated with various insecticides and aged for various periods

Insecticide	Dosage (mg./sq. ft.)	Minutes needed after aging residues—								
		1 wk.	2 wk.	4 wk.	8 wk.	12 wk.	16 wk.	20 wk.	24 wk.	32 wk.
Bayer 39007	100	1	5	1	5	15	15	1	30	5
	10	5	15	15	60	30	>60
	1	60	30	30	>60	60	>60
Bayer 41831	100	5	5	5	5	15	30	30	30	15
	10	60	30	60	15	>60
Bayer 37341	100	60	60	60	>60
Bayer 37342	100	30	30	30	60	60	60	60	>60	..
Bayer 34098	100	60	30	>60
Hercules AC-5727	100	1	1	1	1	5	5	15	15	15
	10	5	15	15	15	30	30	30	30	60
Malathion	1	15	60	30	30	60	30	60	30	60
	100	30	60	30	15	60	30	15	30	15
DDT	1	15	15	30	15	30	15	30	30	..
	100	30	30	15	15	15	60	30	60	..

minutes killed above 90 percent of the insects during the first 2 months of aging. Throughout this same period DDT and malathion needed exposures of 15 to 30 minutes to produce similar results. By the time the residues were 12 to 32 weeks old Bayer 39007 was still better than malathion or DDT, and Bayer 41831 was about as effective as malathion, but superior to DDT. Bayer 39007 was somewhat less effective than Hercules 5727, especially at dosages of 1 and 10 mg. per square foot. At 10 mg. per square foot both these compounds were better than Bayer 41831.

Bayer 39007 is another fast-acting compound like Hercules AC-5727. On 7-day-old residues of 100 mg. per square foot, exposures for only 5 minutes caused 100 percent knockdown before the mosquitoes could be removed from the boards. Exposures as short as 1 minute produced 100 percent knockdown within 30 minutes. Bayer 41831 was not as rapid in action, for the knockdown in 30 minutes was 0 percent after a 5-minute exposure and only 37 percent after a 30-minute exposure. Fast action is not a necessary characteristic of compounds for use in malaria eradication programs, but it is highly desirable so that mosquito annoyance can be reduced as rapidly as possible.

Bayer 37341, Bayer 37342, and Bayer 34098 at dosages of 100 mg. per square foot required exposures of 30 to 60 minutes to cause mortality above 90 percent the first week after treatment, and the residues were less durable than those of the previously mentioned compounds. None caused any 30-minute knockdown after 30 minutes of continuous exposure, nor were they highly toxic at dosages of 10 mg. per square foot.

Toxicological information received from the manufacturers indicated that at least three of the compounds probably would be safe to use in actual control work. The oral toxicity to warm-blooded animals based on an LD₅₀ in mgs./kg. of body weight was 95–200 mg. to rats for Bayer 39007¹ and 1180 mg. to mice for Bayer 37342. The latter compound was not toxic to mice at 500 mg. The status of Bayer 41831 is not clear but it appears to be no more toxic than Bayer 39007. No information was available on the toxicity of Bayer 37341 or Bayer 34098.

¹ Information on this compound received from Chemagro Corporation, based on data collected by K. P. DuBois, Department of Pharmacology, University of Chicago, as well as the Farbenfabriker Bayer Company, Elberfeld, West Germany.

FIELD TESTS. Four compounds that were previously reported to be effective in laboratory tests by LaBrecque *et al.* (1960) or Gahan *et al.* (1961) were tested in buildings naturally infested with *Anopheles quadrimaculatus*. A dog house treated with a malathion standard was built of concrete blocks, but the other buildings were made of wood. All buildings were located in a swampy area around Bushnell, Sumter County, Florida, where cattle, pigs, or chickens were plentiful. Two series of tests were made; in the first series the applications were made on February 10, 1960, and in the second on July 13, 1960. Hercules AC-5727 was applied at 100 mg. per square foot in a calf barn in series 1 and in a small tool shed in series 2, and at 10 mg. in a small pump house in series 1. Dimethoate, Methyl Trithion (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate), and Sevin (1-naphthyl *N*-methyl carbamate) were applied to two buildings each at 100 mg. per square foot in series 2. Animal barns were used for the dimethoate and one of the Methyl Trithion treatments. The other Methyl Trithion application was made in an old deserted building with broken windows and doors through which the mosquitoes could enter. Two privies at a rural church were sprayed with Sevin. In each series DDT and malathion were used at 100 mg. per square foot as standards. One of the buildings treated with malathion was a tractor shed on a farm that had cattle, pigs, and chickens; the other buildings regularly housed animals. All residues were applied as water emulsions. Hercules AC-5727, Methyl Trithion, Sevin, and DDT sprays were prepared from emulsion concentrates containing 25 percent insecticide, 5 percent xylene, and 10 percent Triton X-100. The malathion and dimethoate concentrates were formulated by their manufacturers.

Pre- and post-treatment counts were made of the *Anopheles* mosquitoes resting on the treated buildings as well as in eight untreated buildings, four of which were used as checks in series 1 and four in series

2. Pre-treatment counts were made on at least two occasions within 1 month of the spray applications and all counting was done as near the middle of the day as practicable. Averages of the counts made before treatment and the percentage reduction in the mosquito population at intervals after treatment are presented in Table 2. A test was completed as soon as the percentage reduction became less than 90 percent.

Hercules AC-5727 at 100 mg. per square foot eliminated all the mosquitoes from the treated buildings during the first 9 to 11 weeks and the control remained above 90 percent for at least 25 to 28 weeks. The test in the building under observation for 25 weeks was still highly effective when this manuscript was prepared and the final answer on its durability was not available at that time. Effective control deteriorated more rapidly with this compound at the 50-mg. dosage for although it also eliminated all mosquitoes for 9 weeks it was not highly effective after 11 weeks.

Incomplete information on its safety obscures the future of this compound in mosquito control campaigns at the present time.

Some mosquitoes were present on almost every occasion in the buildings sprayed with dimethoate, Methyl Trithion, and Sevin, and control deteriorated rapidly. In addition to those counted on the walls and ceilings in these buildings, mosquitoes that could not be counted were flying in the air. These compounds appeared to be too slow acting to be of much value.

The malathion standard applied in the dog house constructed of cement blocks caused less than 90 percent reduction as early as 6 to 7 weeks after treatment, but this insecticide remained highly effective for 9 weeks in the wooden tractor shed. The DDT standard was almost completely effective, with control above 99 percent, for at least 25 to 36 weeks.

The *Anopheles* mosquitoes in this part of Central Florida obviously were not highly resistant to DDT. In studies conducted during 1958 in Arkansas by Gahan

TABLE 2.—Continued
Percent reduction ^a at indicated number of weeks following treatment

Type of building	Pre-treatment count	Percent reduction ^a at indicated number of weeks following treatment												
		1-2	3-4	6-7	9	11	14	18	25	28	32	36	40	
Animal barn	410	99.7	99.9	99.8	..	99.9	100	100	100	
						DDT								
						Untreated								
Storage shed	72	46	74	45	..	49	46	56	
Tractor shed	375	+60	+60	+33	
Garage	172	40	71	71	..	79	55	80	
Roof shelter	47	+28	26	+527	..	+538	+332	+318	

^a Plus signs indicate percent increase above the pretreatment count.

and Hoffman (1959), DDT caused above 90 percent reduction for only 2 to 11 weeks in infested buildings treated at 200 mg. per square foot. In the present series of tests treatments applied at half that dosage remained effective more than twice as long as the most effective application made in Arkansas. No malaria eradication program has been conducted recently in this part of Florida and the farmers do not apply insecticides to their crops as frequently as in Arkansas so the mosquitoes probably contact surfaces treated with DDT less frequently.

SUMMARY. Residual spray tests conducted in the laboratory showed *o*-isopropoxyphenyl methylcarbamate (Bayer 39007) and *O,O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothioate (Bayer 41831) to be highly effective against adults of *Anopheles quadrimaculatus*. At dosages of 100 mg. per square foot they were superior to the DDT and malathion standards during the first 2 months of aging and exposures as short as 1 to 5 minutes were sufficient to produce above 90 percent mortality. After 16 to 32 weeks Bayer 39007 was the best of these four materials and Bayer 41831 was about as effective as malathion, but superior to DDT. Bayer 39007 was somewhat less effective than a closely-related compound, *m*-isopropylphenyl methylcarbamate, (Hercules AC-5727) which was used as a standard. Both these compounds were fast acting; 5-minute exposures on 7-day-old applications produced 100-percent knockdown before the mosquitoes could be removed from the treated surfaces. *O,O*-Diethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate

(Bayer 37341), *O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate (Bayer 37342), and *O*-(4-methylthio-*m*-tolyl) dimethylphosphinothioate (Bayer 34098) also were effective, but were less toxic and durable than the other compounds.

Four compounds were evaluated as 100-mg.-per-square-foot residues in buildings naturally infested with adults of *Anopheles quadrimaculatus*. Hercules AC-5727 at 100 mg. per square foot eliminated all the mosquitoes from treated buildings during the first 9 to 11 weeks and produced control above 90 percent for at least 25 to 28 weeks. Dimethoate, Methyl Trithion (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate), and Sevin (1-naphthyl *N*-methyl carbamate) failed to eliminate all *Anopheles* mosquitoes from treated buildings as fresh applications and control deteriorated rapidly.

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