the 1962 outbreak of encephalitis in Florida, even prior to its incrimination as a vector. This was because Dibrom had only been tested against *Aedes taeniorhynchus* at the 1 oz. and 1¾ oz. levels prior to 1962 (Rathburn and Rogers, 1961) and the only known effective dosage at the start of the 1962 encephalitis outbreak was 1¾ oz. per gallon. The recommended dosage rate of 1½ oz. of Dibrom per gallon for *Aedes taeniorhynchus* (Rathburn and Rogers, 1963) was not established until November of 1962.

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RECENT INVESTIGATIONS ON THE USE OF BHC AND EPN TO CONTROL CHIRONOMID MIDGES IN CENTRAL FLORIDA

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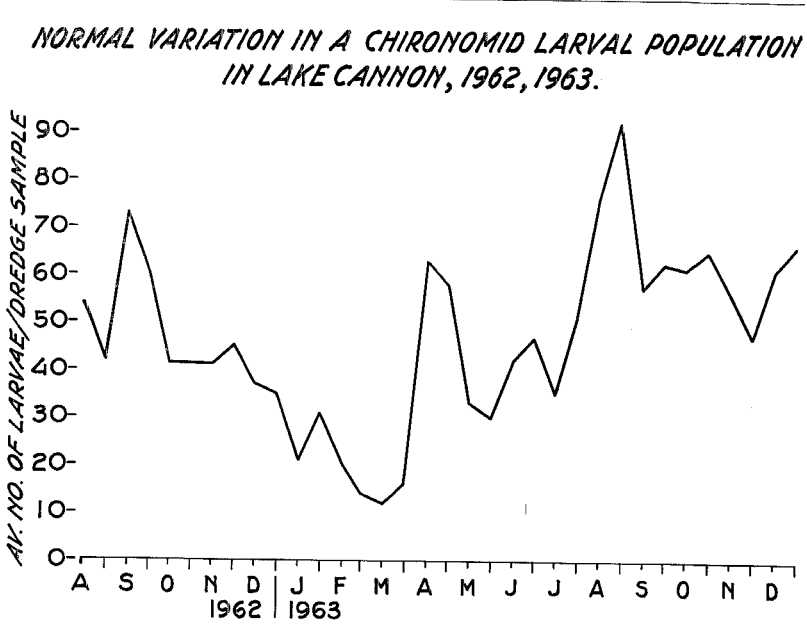
Chironomid midges are a serious nuisance in certain areas of Florida. The principal species, *Glyptotendipes paripes,* becomes so numerous at times that almost all outside activity is prohibited. In the Winter Haven area, several of the lakes that are widely used for recreational purposes produce large numbers of adult midges almost daily from March through November. Many of the lakes have a larval density of 500 or more per square foot in the sandy areas, but usually more than half the lake bottom is covered by muck. Few of the important species of chironomids inhabit this muck area, but *Chaoborus* midges do breed in muck-bottom lakes and appear to be increasing in numbers in several lakes in central Florida.

Of the many compounds tested, only two, BHC and EPN, have been used extensively as midge larvicides. Although each of these chemicals was very successful at the beginning, within a year of their initial applications there were definite indications according to Lieux (1955) that they were no longer effective in certain lakes. Resistance was suspected as reported by Lieux and Mulrennan (1956) but was not substantiated since there is no known method of rearing this species of chironomid or keeping them alive in the laboratory for the time required to establish tolerance levels. Therefore, most of the previous evidence of resistance against these compounds was based on observations of adult midge populations following the application of the insecticide.

BHC was first widely used as a midge larvicide in 1953 but was generally discontinued by 1955 because it was not giving adequate control in certain lakes. However, in a few areas of the state it has been used almost continually with apparent success. This apparent prolonged success of BHC is, however, based purely on observations made by laymen, and is not supported by scientific evaluation of the treatments. EPN was used extensively in lakes at Winter Haven in 1954 but was judged ineffective after 1955 and has been used only sporadically in recent years.

The 1963 investigations were conducted



to determine (1) why chironomids in certain lakes only are apparently tolerant of these chemicals; (2) if there is a true insecticide tolerance, how quickly it takes to develop; and (3) how long the tolerance persists once the insects are released from the pressure of the insecticide.

METHODS. Tests were conducted initially in metal cylinders placed in the lakes in a manner similar to that reported by Jamnback (1954). One Ekman dredge sample from the lake bottom containing the chironomid larvae was placed in one 9" x 12" pan per metal cylinder. Within twenty hours the larvae had oriented themselves to their new environment at which time the insecticide was added. Forty-eight hours later the pans were removed and the live larvae counted in each sample. There were five replicates for each treatment plus five untreated checks for each test.

Since BHC previously was effective at 0.15 pound per acre of the gamma isomer, this dosage was used in all tests in this study. The BHC tests were made in Lake Cannon and Lake Bonny, Polk County. The later EPN studies made in the cylinders were run at 0.125 pound per acre. These tests were run in Lake Barton, Orange County and Lake Cannon, Polk County. Baytex applied at 0.2 pound per acre was included in this last series of tests.

BHC was applied to the entire surface of Lake Bonny at the rate of 0.15 pound per acre of the gamma isomer. During the time the BHC tests were in progress, a group of business establishments on the shore of Lake Barton in Orlando (Orange County) purchased some EPN for emergency treatment of that lake. Therefore it was decided to utilize this opportunity to secure data on EPN in this study.

Lake Barton had never been treated with any insecticide; therefore, it was recommended that EPN be applied at 0.125 pound per acre, the dosage previously effective at Winter Haven. The Orange County people applied the insecticide and the treatments were evaluated by the writer, based upon larval sampling with an Ekman dredge before and at regular intervals after each treatment. The dosage used in each EPN treatment was greater than that recommended and time

TABLE 1.—Percent mortality[1] of *Glyptotendipes paripes* larvae, Lake Cannon (BHC 0.15 lb./A)

Date of test	Percent mortality in treated cylinders	Percent mortality in untreated cylinders
11–21–62	79	7
11–21–62	75	5
11–23–62	92	1
11–23–62	93	7

[1] Data based on five replications per treatment.

of treatment was based more on nuisance levels of adult midges than on larval counts. A one percent Baytex granular formulation was applied at the rate of 0.27 pound per acre to Lake Barton following the six EPN treatments.

Both the BHC and EPN were distributed throughout the lakes by pumping them from the mixing tank into the propeller wake of a 40 horsepower outboard motor. The Baytex granules were applied by plane utilizing a granular applicator.

RESULTS. Although tolerance to BHC was suspected in Lake Cannon seven years ago, data in Table 1, from container tests in this study, show that larvae of *Glyptotendipes paripes* presently are susceptible to BHC in this lake.

Container tests also were conducted in Lake Bonny, where a BHC-tolerant strain of chironomids was reported to exist only three years previously. Again, results of container tests indicate no resistance in the present population (Table 2). Since no insecticides have been used in Lake Bonny in recent months, any BHC tolerance that might have existed there three years ago apparently no longer exists.

Previously it had been reported that *Glyptotendipes paripes* was the principal species in Lake Bonny, but *Tendipes crassicaudatus* far outnumbered the other species at this time. It should be pointed out that records from other lakes in Florida have shown definite indications that *Tendipes crassicaudatus* larvae are more numerous during the winter.

It was decided to try to recreate this tolerance by treating the lake with BHC at set intervals. Treatment data and results are shown in Table 3.

As shown in Table 3, BHC was very

TABLE 2.—Percent mortality[1] of *Tendipes crassicaudatus* larvae, Lake Bonny (BHC 0.15 lb./A)

Date of test	Percent mortality in treated cylinders	Percent mortality in untreated cylinders
12–6–62	93	5
12–8–62	94	5
12–14–62	94	4
12–17–62	99	9
12–21–62	91	8
1–7–63	95	3
1–10–63	89	–
1–14–63	92	4

[1] Data based on five replications per treatment.

TABLE 3.—Effects of BHC on chironomid larvae in Lake Bonny.

Date	BHC lb./acre	Average number of larvae per dredge sample
1–22–63 pretreatment[1]	0.15	16.5
1–24–63		11.0
1–28–63		1.0
2–7–63		0.5
2–22–63		1.0
3–8–63		2.0
3–22–63		2.0
4–5–63		4.0
4–19–63		4.0
5–9–63		21.0
5–23–63 pretreatment[1]	0.15	9.0
5–28–63		4.0
5–31–63		6.0
6–7–63		8.0
6–14–63		5.0
6–20–63		5.0
7–18–63		1.0
8–1–63		0.4
8–15–63		0
8–30–63		0
9–13–63		0
9–30–63		0
10–11–63		0.1
10–25–63		2.5
11–8–63		3.7
11–22–63		2.0
1–8–64		0.4

[1] Larval samples taken immediately prior to insecticide application.

effective in reducing the chironomid population in the lake. The average number of larvae per dredge sample in the entire lake, based on 100 samples, fell from 16.5 to a low of 0.5 within two weeks. Almost three months elapsed before the general midge population re-established itself to its pretreatment level. It should be noted, however, that this test was conducted during the winter months, when reproduction and development proceed at a slow rate. As previously stated, when the BHC treatment was initiated in January, *Tendipes crassicaudatus* was the predominant species; two months later *Glyptotendipes paripes* was the principal species, and after four months *Tendipes decorus* became the predominant species in the lake and has remained so to date.

By May 9 the chironomid population had surpassed the pretreatment count (16.5) in January; however, by May 23, when the lake was retreated with BHC, the average larval population had dropped to 9 per dredge sample. As indicated by the data in Table 3, this BHC application seemed to have little effect on the midge larvae. At the time of the second application there was a very heavy bloom of unicellular (*Microcystis* sp.) blue-green algae in the lake. Previous experiences have indicated that BHC is not as effective in water having a heavy algae content as in clear water.

Since the lake's esthetic value was lowered by this dense algal bloom, 2400 pounds of $CuSO_4$ was applied by park personnel in June. The $CuSO_4$ was very effective in killing the blue-green algae but there was a tremendous increase in the growth of filamentous green algae, *Oedogonium* sp. and *Spirogyra* sp.; they literally covered the bottom of the shallow areas of the lake. Following the growth of this filamentous algae there was a reduction in the larval population until it numbered less than 0.04 individuals per dredge sample on August 15. This dense growth of algae persisted throughout the year. The larval population remained very low from August 15 to January 8, 1954. Normally there is an increase in the larval population in the fall of the year followed by heavy emergence and then a decrease during the winter, as shown in the graph.

The sudden and prolonged presence of the filamentous algae clouds the treatment effect of BHC; therefore, no firm conclusions can be made on possible tolerance as a result of these treatments.

Neither of the two BHC applications had any noticeable adverse effects on the other aquatic biota in Lake Bonny. *Chaoborus* midges were numerous throughout the duration of the test and were the chief insect nuisance after May.

Rosen and Middleton (1959) reported that chlorinated insecticides at sub-lethal dosages may cause off-flavor in the flesh of fish and thus render it unpalatable. A week after the second BHC treatment, four large-mouth bass, weighing about a pound each, were taken from Lake Bonny to test for off-flavor. Bass taken from an untreated lake served as checks. The fish were cleaned immediately and frozen, then just prior to testing, they were thawed, wrapped in aluminum foil and baked.

Six samples were set up in a random design. Five people, two women and three men, were used as judges. The results indicate that the BHC did cause a very slight objectionable flavor in the flesh of bass.

Other fish which were taken from the lake at the same time were given to various individuals, none of whom knew of the possible contamination. The following day they were questioned about the flavor of the fish. None of the people reported any off-flavor. Thus any possible off-flavor caused by the BHC is masked by normal cooking procedures.

Bass taken from Lake Bonny four months after the first application (the day before the second BHC application) contained a BHC gamma isomer residue of 0.05 p.p.m. Ten days after the second application the residue in the fish had increased to 0.15 p.p.m. Three months later when the bass were again sampled they contained no detectable amount of gamma

isomer but contained traces of other isomers of BHC.

Results of the treatments with EPN in Lake Barton are shown in Table 4. EPN appeared to be effective the first four times it was used. However, following the fifth application there was a large increase in the larval population. On September 25 the count ran as high as 43,000 larvae per square meter. Fortunately most of the larvae were concentrated in the sandy areas, although some were found throughout the lake bottom. Normally there is an increase in the number of midge larvae present in the lakes in September. After the sixth application there was a drop of over 80 percent in the larval population, still it was about three times larger than the population prior to the fifth application.

Following the sixth treatment, container tests were set up in Lake Barton to try to determine if the midge population had become tolerant of EPN (0.125 lb./acre). Similar tests were also set up in Lake Cannon since this lake had not been treated with EPN in the previous eight years. The results of these tests are given in Table 5. Baytex (0.2 lb/acre) was also included in the same tests to determine if a general organo-phosphate insecticide tolerance existed now in the insect population.

TABLE 4.—Effects of EPN on midge larvae in Lake Barton.

Date		EPN lb./acre	Avg. no. larvae	Percent of dredge samples positive	Percent of larval population *G. paripes*
6–17–63 pretreatment[1]		0.275	10.4	76	..
6–25–63			2.3	32	..
7–1–63		0.15			..
7–9–63			0	0	..
7–16–63			0.1	12.5	..
7–30–63			6.0	79	..
8–2–63		0.15			..
8–7–63			0.25	19	0
8–16–63			7.6	72	88
8–22–63		0.2			
8–29–63			0.5	20	50
9–10–63		0.175			
9–13–63			9	77	90
9–25–63			226	100	100
10–3–63		0.20			
10–4–63			109	100	100
10–8–63			31	100	..
10–24–63			49	100	90
11–7–63			63	100	93
11–19–63			52	100	90
12–2–63	Baytex	0.27	51	100	96
12–6–63			0.26	20	..
12–10–63			0.20	10	70

[1] Larval samples taken 6–17–63 and 12–2–63 immediately prior to insecticide application.

It was found that EPN was only about 50 percent effective against the midge larvae in Lake Barton, whereas it gave 98 percent control at the same dosage rate to the same species of midge larvae (*G. paripes*) in Lake Cannon. Baytex was very effective against the larvae in both lakes, indicating that there was not a general organo-phosphate insecticide tolerance established in the population. This was further substantiated when Lake Barton was treated with Baytex which gave excellent control of the chironomid midges as shown in Table 4. The results from recent container tests in Lake Barton

TABLE 5.—Results of drum tests on Lakes Cannon and Barton utilizing EPN and Baytex.

Lake	Date	Percent mortality	
		EPN	Baytex
Cannon	10–21–63	96	99
	10–25–63	99	99
Barton	10–21–63	58	99
	10–24–63	34	99
	4–27–64	90	98
	5–4–64	99	99

(Table 5) indicate that the present chironomid population in this lake is not tolerant of EPN.

DISCUSSION. Although the chironomid population in central Florida lakes may have become tolerant of BHC (0.15 lb./acre) and EPN (0.125 lb./acre) in 1955, this tolerance was not maintained over the years. Both these chemicals were very effective as a midge larvicide when first used in 1963.

The apparent failure of a second BHC application in one lake in 1963 may have resulted from environmental changes in the lake caused by a heavy algal bloom; also there was a change in the predominant species of midge present in the lake between the first and second BHC treatment.

Within a week following the second BHC application enough of the insecticide had been absorbed into the tissues of large-mouth bass to cause a very slight off flavor. Small residues of BHC were recovered from fish up to four months after the first treatment and up to three months after the second application.

Some selection for tolerance in the chironomid midge, *Glyptotendipes paripes,* may have occurred within a four-month period during four applications of EPN in one lake in this study. If this tolerance did exist, it was of short duration and was not a general resistance to organo-phosphate insecticides, for Baytex was very effective against this midge population. Before any definite conclusions can be made concerning insecticide resistance in midges, more research must be done on this problem.

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