RESULTS OF LABORATORY AND FIELD TESTS WITH DURSBAN INSECTICIDE FOR MOSQUITO CONTROL

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Dursban® insecticide (*O, O*-diethyl *O*-3, 5,6-trichloro-2-pyridyl phosphorothioate) has shown outstanding biological activity against many different insects as reported by Kenaga *et al.* (1965). The chemical and physical properties of this material have been reported by Gray (1965). This paper describes the biological activity of Dursban against both adult and larval stages of several species of mosquitoes.

LABORATORY STUDIES. Remarkable activity against *Aedes aegypti* (L.) larvae was noted in the laboratory with Dursban. Once this initial biological activity was observed, a number of laboratory tests were conducted to establish the range of efficacy against adults and larvae of this species.

The method used for evaluation of Dursban was patterned after the method used by the World Health Organization (1960). Mosquitoes were reared in the laboratory using ground dog chow as larval food. Larvae were fed daily until pupation occurred, at which time they were collected and held until adults appeared. Adults were held in a large screened cage 3′ x 3′ x 3′ and allowed to feed on chicken blood daily for 5 days a week. Larvae for test purposes were hatched in well water, and after 24 hours

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the young larvae were placed in plastic beakers and held at 80° F. until they reached the fourth instar. If larvae from several jars were to be used, all larvae were combined prior to use.

In the actual test, 25 to 30 fourth instar larvae were placed in 25 c.c. of water. The transfer of larvae was accomplished by means of a small strainer. Two hundred and twenty-five milliliters of distilled water were placed in another container which was to serve as a test vessel. The test material was dissolved in alcohol and added to the test vessel by means of a pipette. After addition, the solution was stirred for several seconds to insure complete distribution.

Within a few minutes of preparing the test solutions, the mosquito larvae were added by tipping the 25 c.c.'s of water and the 25–30 larvae into the testing vessel. This gave a final volume of 250 c.c., and all dilutions were calculated on that basis.

Since the results against *Aedes* were so impressive (Table 1), a colony of *Culex*

TABLE 1.—Percent mortality of fourth instar *Aedes aegypti* larvae obtained with Dursban and Fenthion at various concentrations.

	Concentration (ppm)	Percent mortality	No. of tests
Dursban	0.004	98.5	18
	0.002	76.5	21
	0.001	42.0	14
	0.0005	24.0	10
Fenthion	0.06	100	6
	0.04	94.5	6
	0.02	64.5	6
	0.01	0	6
Dursban	LD_{50} 0.001	LD_{95} 0.0028	
Fenthion	LD_{50} 0.016	LD_{95} 0.042	

quinquefasciatus (Say) was obtained from Dr. Don Micks, University of Texas Medical School, Galveston, Texas. This species was colonized and then used for bioassay. The results are listed in Table 2.

Since outstanding results were obtained in the larval tests, a decision was made to evaluate the residual activity of Dursban against adult mosquitoes. *Aedes aegypti* were used as test species. The method

TABLE 2.—Percent mortality of fourth instar *Culex quinquefasciatus* larvae obtained with Dursban and Fenthion at various concentrations.

	Concentration (ppm)	Percent mortality	No. of tests
Dursban	0.004	100	12
	0.002	100	15
	0.001	79.8	15
	0.0005	64.7	10
	0.0001	17.0	1
Fenthion	0.032	83.7	12
	0.016	73.3	15
	0.008	42.0	12
	0.004	21.0	12
Dursban	LD_{50} 0.0003	LD_{95} 0.0025	
Fenthion	LD_{50} 0.009	LD_{95} 0.05	

of testing was similar to that developed by the World Health Organization for evaluating resistance in field populations of mosquitoes (1960).

The test kits were obtained from W.H.O. and were constructed as follows: Plastic tubes 125 mm long by 44 mm in diameter were used to expose the mosquitoes to the insecticide. Each tube was fitted at one end with a 32-mesh screen. A slide unit into which two of the above tubes were fastened in place constituted the other end. The sliding door in the slide unit had a 20 mm filling hole to allow introduction of the insects. The inside of each tube was fitted with special paper, which was received with the W.H.O. Adult Test Kit. Paper sheets were treated with Risella 17 mineral oil and measured 12 by 15 cm. Papers were treated by dipping in solution of Dursban in either acetone or alchol. After dipping, the papers were allowed to dry and were then placed in the plastic tube. This plastic tube was then joined to the slide unit and another tube with untreated paper in it was fastened to the other end of the slide unit.

The procedure used for the test was as follows: Mosquitoes were collected from stock (approximately 4 days old) and placed in the side of the tube with the control paper. The mosquitoes were allowed to remain on the untreated paper for at least one hour to insure good in-

sects for the test. At the end of this period, the tube with the treated paper was placed on the slide unit, the sliding door opened and the mosquitoes gently blown into the chamber with the treated paper. The exposure to the treated paper was one hour, after which the slide was again opened and the mosquitoes blown back into the control chamber. Knockdown was noted if any occurred. The mosquitoes were held in the test chamber for 24 hours, at which time final mortality counts were made. During the holding period, a honey-water solution was made available to the adult mosquitoes. The plastic chambers with the test papers inside were stored in the laboratory at room temperature during the intervals between tests.

The results of these tests are given in Table 3.

TABLE 3.—Residual activity of Dursban against *Aedes aegypti* adults.

Concentration (ppm)	No. of weeks obtained 100% mortality	Additional no. of weeks obtained 99 to 85% mortality	Total no. weeks with greater than 85% mortality
1000	71	9 terminated	80+
1000	50	20	70
500	52	4	56
500	29	16	54
250	3	21	24
200	3	7	10
100	0	0	0
50	0	0	0

These data indicate that Dursban insecticide is highly effective as a residual insecticide for *Aedes aegypti*. Concentrations of 1,000 ppm gave 70 to 80 weeks of excellent kill. To evaluate these data in terms of other materials available, similar tests were conducted with several commercial insecticides. Results are given in Table 4.

The standard materials tested at the same concentration as Dursban gave far less residual activity. Only Fenthion gave control for two weeks; all others, even DDT at 50 times the concentration used for Dursban, were not effective.

FIELD STUDIES. Since these laboratory tests showed a high degree of activity, it was desired to evaluate Dursban under field conditions to determine if the efficacy noted in the laboratory would display itself under practical field conditions.

The field research probes were in three directions: (1) to evaluate hand application of granular formulations containing Dursban; (2) to evaluate the aerial application of an emulsifiable concentrate; and (3) to evaluate an oil solution of Dursban dispersed via a thermal fog generator.

I. Granular applications of Dursban for control of salt-marsh mosquito (*Aedes sollicitans*) larvae.

An area in the marshland of Brazoria County was selected as the test site. The area had a history of high infestations of *Aedes sollicitans* when inundated by high tides. Six small plots (10 ft. x 40 ft.) were laid out side by side. Treatments were applied on April 2, 1964, at which time the ground was moist but not covered with water. All the treatments were made with granular material and applied with a small cyclone seeder. An untreated plot was spaced in between each treatment so that if and when flooding occurred, some separation of treatments would be maintained.

The plots were not checked for several months, during which time the entire area was covered by salt water two to three times. On June 17, 1964, a systematic

TABLE 4.—Residual activity of several commercial insecticides against *Aedes aegypti* adults.

Material	Concentration (ppm)	Percent mortality at			
		1 Day	1 Week	2 Weeks	3 Weeks
DDT	10,000	18	5	..	..
Dieldrin	1,000	18	5	..	..
Fenthion	1,000	100	100	100	40
Dimethoate	1,000	15	..	..	..
Lindane	1,000	73	69	..	..
Zectran®	1,000	73	85	..	..
Dursban	200	100	100	100	100

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search for mosquito larvae was made by use of the dipping technique. With this method, a long-handled dipper is used to sample the water at various locations in the plots. Seven dips were made in each plot, and the number of larvae found was recorded. The results of these observations are found in Table 5.

From the above results, it is apparent that granules of Dursban at rates as low as 2.0 lb. active ingredient per acre gave perfect control for at least 11 weeks.

application of the granules. Treatment rates and results are given in Table 6.

The final test in 1964 consisted of a granular treatment in the late fall (November 4) for the purpose of observing the results the following spring as the area became flooded. Three plots (40 ft. x 50 ft.) were treated, and again each treated area was separated by a control area. Plot No. 1 was treated with a .3 percent granular on ground corncob and was applied at the rate of approximately 10

TABLE 5.—Larval dip counts (*Aedes sollicitans*) obtained 11 weeks after treatment with granular Dursban.

Plot no.	Treatment	Active ingredient lbs./acre	Counts per dip							Average
1	Dursban	1.0	0,	0,	0,	10,	5,	0,	0	2.1
2	Control	..	10,	12,	11,	6,	8,	7,	5	8.7
3	Dursban	2.0	0,	0,	0,	0,	0,	0,	0	0.0
4	Control	..	5,	3,	10,	4,	20,	12,	15	9.8
5	Dursban	2.0	0,	0,	0,	0,	0,	0,	0	0.0
6	Control	..	10,	12,	15,	7,	8,	30,	17	14.1

In order to evaluate this activity, additional plots at lower concentrations were placed in another area having similar terrain. The plot sizes were increased to 50 ft. x 40 ft., and treated in the same manner and with the same materials. Treated plots were again separated by a control or check plot, and treatments were applied on June 4, 1964. At the time of treatment water was standing in the sunken areas, but the area was not completely inundated. Larval counts ranged from 50–100 per dip immediately prior to

pounds of granules per acre, or .03 lb. active per acre. Plot No. 2 was treated with the same material, but at double the rate used above, giving a dosage of 0.06 lb. active ingredient per acre.

Plot No. 3 was treated at the rate of 0.1 lb. per acre active ingredient using a clay granule containing 1.0 percent Dursban, and applied at 10 pounds per acre. Plots were not flooded at the time of treatment, but were flooded many times during the winter months by tides and heavy rains.

TABLE 6.—Results of test with granular treatments of Dursban for the residual control of *Aedes sollicitans* larvae (treated June 4, 1964).

Plot no.	Treatment	Rate, lb./acre	Larval counts average 10 dips/plot	
			June 17, 1964*	July 17, 1964**
1	Dursban	0.5	0	0.2
2	Control	..	10	16.2
3	Dursban	0.25	2	0.1
4	Control	..	10	8.9
5	Dursban	0.20	0	0.0
6	Control	..	0	12.8
7	Dursban	0.5	0	0.0
8	Control	..	0	50.0

* Larval counts outside plot area (0–15 per dip) low also.
** Larval counts outside plot area were averaging 50, with a range of 30–100 larvae per dip.

No readings were taken until May 10, 1965, or 7 months after materials were applied. At this time, Plots No. 1 and No. 2 had many larvae present, while Plot No. 3 had no larvae at all. Demarcation line between treated area and control area could be noted by the presence or absence of larvae. The next observation was made on June 4, 1965, at which time larvae were found in Plot No. 3.

From these data, it appears that a granular treatment with clay granules at the rate of 0.1 lb. active ingredient per acre may provide a residual control of at least 7 months, even when floodings occur.

II. Aerial spray applications of Dursban M for the control of salt-marsh mosquito (*Aedes sollicitans*) larvae.

In Brazoria County, there are approximately 300 square miles of marshland with an average elevation of 1–2 feet above sea level. This area is so extensive, and being isolated by permanent waterways, it is impossible to reach the vast mosquito breeding area by any means other than by air. Therefore, several small plot tests were initiated to evaluate critically the field performance of Dursban when applied by airplane.

All applications were made by a Piper Pawnee B fitted with a 235 h.p. engine. The spraying system was composed of 24 D 8–45 Spraying Systems nozzles fitted with an Agavenco 25-pound-per-square-inch pump set to supply 18.4 gallons per minute. The plane was operated at the speed of 90 m.p.h., and at this speed the volume of spray was 2 gallons per acre. Estimated swath width was 60 ft. when the aircraft was flown at 10–12 ft. above the surface.

The first application was made on April 16, 1965 to a plot 60 yards by 120 yards. Three passes with the aircraft were made over the plot. Dursban in the form of a 2-pounds-per-gallon emulsifiable concentrate was mixed with water at the rate of 1 quart to 20 gallons of water. When applied at the rate of 2 gallons per acre, the dosage of insecticide was 0.05 lb. per acre.

Dip samples prior to treatment were averaging 50–75 larvae per dip. Most of the larvae were late fourth instar, with approximately 25 pupae per dip. Prior to spray application, a sample of the larvae was collected and stored in a 2-quart fruit jar. The spray was applied at 8:00 a.m. with a heavy crosswind at 10–15 m.p.h. Larvae were collected from the test plot after treatment and they were dead within one hour after application. No mortality was observed in larvae collected prior to application. Recheck of the plot on April 21, 1965 revealed no live larvae. On April 28, 1965 (12 days post application), live larvae were found in the test area. It is felt that only a small portion of the spray volume actually reached the ground due to the high wind velocity.

A second test plot was initiated in an

adjacent area on April 28, 1965, at which time larval counts were averaging 50–100 per dip. Application was made at 9:00 a.m. and environmental conditions were much more favorable than at time of the first treatment.

Wind velocity was approximately 1 mile per hour, directly down the plot. Concentration used was again 0.05 lb. active ingredient per acre dispensed in 2 gallons of liquid. Larval samples were again collected prior to and after spraying. All larvae collected after treatment were dead within two hours. No mortality occurred in the untreated sample.

Dip samples were taken at intervals following treatment to evaluate residual effects. Samples taken on April 29 and 30 were negative. Shortly after the reading on April 30, the entire plot was flooded by a high tide and no further readings were taken until May 10, 12 days after application. At this time, no live larvae could be found in the plot area. The plot was then examined periodically and results recorded. No larvae could be found on May 17 nor after inundation on May 24. On May 31, a few live larvae were found in the plot. These data indicate four weeks residual control.

To evaluate further the application of Dursban, a series of three single plot treatments were established on May 12, 1965. The pertinent data for this test are included in Table 7.

Final evaluation of aerial application was made on June 9, 1965. This plot was in the same general area as the other test plots, but was separated from them by a permanent bayou. The plot size was 30 acres and was treated with 60 gallons of spray. Dursban in the form of a 2-pound-per-gallon emulsifiable concentrate was added at the rate of 1 quart per 30 gallons. At 2 gallons per acre, this converts to 0.033 lb. per acre. The average water depth at time of treatment was 3 to 4 inches. Pre-treatment landing counts were too numerous to count, and larval counts were averaging 100–200 per dip. The spray was applied between 6:45 and 7:00 a.m. with a very slight breeze blowing down the plot.

Within 30 minutes after the application, no live adults could be found in the plot area, as determined by landing counts. Larvae collected immediately after spraying were dead within one hour.

Since the treated area was slightly higher in elevation, it did not become flooded for several months, and no further data were available from this plot.

III. Application of Dursban insecticide as a mosquito adulticide by use of thermal fog.

Since previous tests conducted with Dursban applied by airplane had shown that this material was an excellent mosquito adulticide, it was decided to evaluate the material as a thermal fog.

The efficacy of the insecticide fog was determined by placing screened cages containing *Aedes aegypti* adults downwind so that generated fog would pass through them. The *Aedes* adults were laboratory-reared and were placed in the screen cages the evening prior to use. A piece of cotton soaked in 5 percent honey-water solution was placed on top of each cage to furnish food. The following day, the cages were placed on stakes 4 feet

TABLE 7.—Results of aerial spray applications of Dursban against *Aedes aegypti* larvae

Dosage, lb. per acre	Pre-treatment counts*	5–13–65		6–4–65		6–9–65		7–6–65
		* Counts	% Control	* Counts	% Control	* Counts	% Control	* Counts
0.025	30–50	1	98.5	0	100	0.3	99.2	25–30
0.05	30–40	0	100	0	100	0	100	25–30
0.1	75–100	0	100	0	100	..**	..	..**

* Mostly fourth instar and pupae. Average number larvae per dip.
** This area not flooded on 6–9–65 or 7–6–65.

above ground and 30 feet apart, directly in line with the wind.

A TIFA-TODD fog generator was used to disburse the Dursban insecticide. Technical material (66 percent in xylene) was added to No. 2 diesel fuel at various rates and then dispersed. The generated fog was observed closely to note any unusual pattern when applied.

The specific machine which was used in the tests described was operated at 5 m.p.h. and dispensed No. 2 diesel oil at the rate of 40 gallons per hour.

The mosquitoes were observed at the end of one hour and mortality noted. The cages were then removed from the stakes and returned to the laboratory. Fresh honey-water pads were placed on the cages and final mortality counts made at 12 hours after treatment.

All treatments were applied in the early morning when wind velocity was low to moderately low. The results are given in Table 8.

There were no dead mosquitoes in control cages.

SUMMARY. The results show that Dursban has a high degree of biological activity against several species of mosquitoes, both larvae and adults. Laboratory data indicate extremely low LD_{50} and LD_{95} values for the larval forms of *Aedes aegypti* and *Culex quinquefasciatus*. Field tests with Dursban against *Aedes sollicitans* also indicate a high degree of activity.

Granular applications at 0.1 pound Dursban per acre appeared to be highly efficacious for several months, even though test plots were flooded frequently. Liquid application of Dursban by airplane at dosages of 0.025, 0.05 and 0.1 pound per acre gave complete control of *Aedes sollicitans* larvae. Residual control of larvae lasted for 4 weeks at the 0.05 lb./A. rate. Dosage of 0.03 pound per acre was highly effective against the adult mosquitoes of the same species.

Thermal generated aerosols containing Dursban were also very effective when evaluated against *Aedes aegypti* adults. When technical material was added to No. 2 diesel oil at ¼, ½, ¾ and 1 ounce per gallon, control values of 73.5, 82.0, 82.5 and 95.6 percent, respectively, were recorded. The one ounce per gallon is estimated to give approximately 0.02 pound of Dursban per acre.

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TABLE 8.—Percent mortality of *Aedes aegypti* obtained at various concentrations of Dursban dispersed with a thermal fog unit.

Distance (feet) of cage from fogger	Ounces technical Dursban per gallon			
	1/4 (.005 lb./A.*)	1/2 (.01 lb./A.)	3/4 (.015 lb./A.)	1.0 (.02 lb./A.)
	(Percent mortality at 12 hours)			
0	68	74	41	100
30	82	79	100	50
60	72	72	100	100
90	53	92	47	100
120	68	72	96	100
150	92	85	90	94
180	86	72	74	100
210	60	64	67	100
240	83	81	100	100
270	60	..	90	100
Average percent mortality	73.5	82.0	82.5	95.6

* Figures in parenthesis indicate estimated amount of technical Dursban per acre.

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References

GRAY, H. E. 1965. Dursban—A new organophosphorus insecticide. Down To Earth, Vol. 21 (3), p. 2.

KENAGA, E. E., WHITNEY, W. K., HARDY, J. L., and DOTY, A. E. 1965. Laboratory tests with Dursban. J. Econ. Ent., Vol. 58(6), pp. 1043–1050.

WORLD HEALTH ORGANIZATION. 1960. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Technical Report Series No. 191, pp. 25–29.

WORLD HEALTH ORGANIZATION. 1960. Instructions for determining the susceptibility or resistance of adult mosquitoes or mosquito larvae. Technical Report Series No. 191, pp. 15–24.