PAPERS AND PROCEEDINGS OF THE 29th ANNUAL MEETING

of the

AMERICAN MOSQUITO CONTROL ASSOCIATION

and the

TEXAS MOSQUITO CONTROL ASSOCIATION

Houston, Texas, March 25–28, 1973

Part II

AN EVALUATION OF SEVERAL TOXIC RUBBER COMPOUNDS AS MOSQUITO LARVICIDES[1]

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Toxic rubber compounds were originally developed by the B. F. Goodrich Aero-space and Defense Products Division of the B. F. Goodrich Company as marine anti-fouling materials. The toxic components, generally compounds of tributyltin, are incorporated in a rubber base and are also very slightly soluble in water. Thus, the surface molecules of the toxicant undergo gradual dissolution in water where they are toxic to various aquatic fauna.

The toxicity of such compounds to mosquito larvae was first shown by Cardarelli and Mercier (1965) and later by Cardarelli *et al.* (1966) who tested tributyltin oxide impregnated neoprene rubber, designated 105B, and found it to give good larval kill for at least 4 months. Schultz and Webb (1969) in laboratory tests with tributyltin oxide impregnated neoprene rubber obtained 100 percent mortality of 2nd instar *Culex pipiens quinquefasciatus* larvae for only 2 to 3 immersions of the material. From these tests they concluded that the tributyltin compounds had little potential as long term mosquito larvicides.

The following research was designed to determine the toxicity of various tributyltin impregnated rubber compounds to eggs and larvae of *Culex* and *Aedes* mosquitoes under laboratory and field conditions.

METHODS. In laboratory tests of tributyltin oxide impregnated neoprene rubber (105B), various pieces of known weight were tested in replicated experiments with untreated controls in circular pans measuring 930 sq. cm. using 25 2nd and 3rd instar *Aedes taeniorhynchus* larvae in 500 ml. of tap water. The toxic rubber was placed in the pans immediately after the larvae were introduced. In studies against mosquito eggs and developing larvae, 3 mm. cubes of 105B were tested in 5000 ml. of water in circular pans measuring 930 sq. cm. or 10 oz. styrofoam cups containing 250 ml. water. The *Aedes* eggs (150–200) were tested in tap and brackish water (salinity 7.4 ppt) and *Culex quinquefasciatus* eggs (1 raft of 150–170 eggs) were tested in tap water. The toxic rubber was placed in the water just prior to the introduction of the eggs.

[1] All test formulations supplied by B. F. Goodrich Company.

Laboratory tests also were made of tributyltin oxide solutions (TBTO), the active ingredient in 105B. In these tests, solutions of TBTO were prepared in acetone to give 4.7, 0.47, 0.047 ppm TBTO when diluted in the test beakers. Two tests replicated four times each were conducted using either 2nd or 3rd instar *Aedes taeniorhynchus* larvae in 600 ml. beakers lined with plastic freezer bags and containing 250 ml. of brackish water (salinity 8–9 ppt.).

Laboratory screening tests were conducted with 26 toxic impregnated rubber compounds. In these tests, one 10 mg. cube of each compound was added to styrofoam cups containing ten 2nd instar *Aedes taeniorhynchus* larvae in 250 ml. of brackish water (salinity 8–9 ppt). This dosage equals 28.7 lb. of the rubber per acre. Five replications of each of the 26 compounds and an untreated control were used at each flooding. Mortalities were determined at 24-hour intervals up to 168 hours. At the end of this time the water and all larvae were discarded, the cups rinsed with clear tap water and allowed to dry overnight. The cubes of toxicant were then reflooded and new 2nd instar larvae were added. Up to 8 floodings of the same cube were used. Five drops of an aqueous infusion of powdered liver and yeast were added as food at the time of flooding and every other day until termination of the test.

Laboratory tests against *Culex* egg hatch and larvae were continued in 250 ml. of water in styrofoam cups with the best four compounds from the previous tests; 408A, 443A, 822C and 12–1–HJ326– all tributyltin compounds in a rubber base. Various types of water were used. The first test was conducted against egg rafts of *Culex quinquefasciatus* in a toxic oak leaf infusion water of the four materials with and without a 10 mg. cube of each of the four corresponding compounds. This was water in which 225 mg. (10–12 cubes) of each of the four formulations had been soaked for 10 days in 5000 ml. of tap water containing oak leaves. In the second test, tap water only was compared to 4-day old oak leaf infusion water, the toxic rubber compounds being added a day prior to the addition of eggs of *Culex quinquefasciatus*. In the third test, *C. nigripalpus* egg rafts were added to cups of tap water in which the four materials had been previously soaked for 10 days.

In the field tests of 105B, circular enameled pans containing fresh water and leaf trash were placed in two locations out-of-doors. A set of 3 pans was used in each location, one containing two 3-mm. cubes of 105B, one with 8 cubes and one with no toxicant which served as a check. The pans were inspected periodically for egg rafts, larvae, pupae and emerging adults. Field pan tests also were conducted with the best 4 formulations shown by the laboratory screening tests. In these tests, two sets of the circular pans containing fresh water and leaf trash were placed on the ground under a canopy of trees. One set was covered with a plastic sheet on a wood frame placed about 30 cm. above the pans so that no rain could enter the pans. The other set of pans was uncovered. Ten to 12 small cubes of each compound or a total of 225±5 mg. were placed in each pan, one compound per pan, after the leaf trash had settled. The difference in the number of cubes was due to variations in the thickness of the sheets from which the cubes were cut, the cubes being all of the same linear measurement. The weight and cube number were chosen to simulate the amount used in the laboratory screening tests and represented a dosage of 21.8 lb. of 105B per acre.

Results. Results of the tests of 105B on mosquito larvae and eggs are shown in Table 1. In general, good mortality was obtained in 5 to 7 days in all tests in tap water when treated in the egg stage, and also when 2nd instar larvae were treated with a dosage. When the egg stage was treated in brackish water at low dosage no significant mortality was produced up to 10 days. However, a similar test performed in styrofoam cups gave 95 percent mortality in 10 days. The

TABLE 1.—Laboratory tests of a tributyltin oxide rubber compound (105B) against mosquito eggs and developing larvae.

Type of water	Dosage 105B		Stage treated	Reps.	Mortality after indicated time of exposure	
	No. of cubes	Total wt. mg.			Percent	Days
Tap[1, 2]	1	0.032	eggs	4	95	5
Tap	2	0.17	eggs	6	92	7
Tap	8	0.67	eggs	6	96	7
Tap	2	0.12	2nds	2	64	1
Tap	8	0.53	2nds	2	22	1
Tap	32	2.45	2nds	2	90	1
Tap	1	0.14	3rd-4ths	2	0	1
Tap	1	0.58	3rd-4ths	2	0	1
Tap	1	1.88	3rd-4ths	2	22	1
Brackish[1]	1	0.036	eggs	4	95	10
Brackish	1	0.09	eggs	2	0	10
Brackish	2	0.19	eggs	4	43	9
Brackish	8	0.77	eggs	2	85	7

[1] Tests in styrofoam cups, all others in 930 sq. cm. pans.
[2] *Culex quinquefasciatus*, all other *Aedes taeniorhynchus*.

material 105B did not appear to prevent egg hatch, although in some instances a delayed hatch was noted. In all cases there was an obvious retardation of larval development. Where the treatment had any effect at all, most larvae failed to develop beyond the 2nd instar.

The results of the laboratory tests with tributyltin oxide solutions in acetone are shown in Table 2. In the first test, pupation in the 0.047 ppm dosage and untreated controls occurred at 96 to 120 hours and normal adults were produced. The minimum dosage at which satisfactory mortality was obtained was 0.47 ppm. The mortality obtained with the 2nd instar larvae was somewhat greater than that with the 3rd instar larvae. When compared to results with other mosquito larvicides, this dosage of TBTO is excessively high.

Results of the screening tests of the 26 compounds are shown in Table 3. Since under normal conditions emerging adults would be expected after 96 hours, larval mortality at the end of this period was selected for comparison of all compounds. Larval mortality decreased with time but generally leveled off at about 96 hours. The best of the 26 compounds tested were

TABLE 2.—Results of laboratory tests of tributyltin oxide solutions in acetone against larvae of *Aedes taeniorhynchus* Wied.

Dosage ppm	Larval instar	Percent mortality[1] @ indicated hours				
		4	24	72	96	100
4.7	3	..	100	100	100	100
.47		..	0	87	100	100
.047		..	0	0	4	6
Check		..	0	1	2	2
4.7	2	94	100	...	100	100
.47		9	64	...	100	100
.047		0	5	...	64	64
Check		1	3	...	39	42

[1] Corrected for control mortality by Abbott's formula, average of four replications of each dosage.

TABLE 3.—The matrix, toxicant, concentration and effectiveness after repeated floodings of 26 toxic rubber compounds used in laboratory screening tests.

B. F. Goodrich no.	Matrix	Toxicant[1]	Concentration		Percent mortality of 2nd instar *Aedes taeniorhynchus* @ 96 hours for indicated number of floodings[2]							
			pph in rubber	percent toxicant	1	2	3	4	5	6	7	8
105B	Neoprene	TBTO	6.0	4.8	67	83	58[3]	77	..	..	..	..
345B	Neoprene	PMO	10.0	7.1	0	4	0	0	..	..	..	..
350A	Neoprene	TBTC	10.0	7.1	100	100	98	100	86	81	43	8
366B	Neoprene	TBTC	6.5	4.8	4	0	6	6[4]	..	..	..	..
374A	PVC	TBTO	4.0	2.9	98	92	71[3]	68	..	..	..	..
374E	PVC	TBTS	4.0	2.9	63	79	48[3]	69	..	..	..	..
376A	Neoprene	TBTO	6.5	4.8	0	0	6[3]	0	..	..	..	..
376B	Neoprene	DBTO	6.0	4.4	0	11	16	2	8	..	0	0
376D	SBR	TBTO	6.5	4.3	98	100	90	89	77	44	69	28
376F	NRX	TBTO	6.0	3.3	100	97	92	62	50	62	57	41
378A	Hycar	TBTO	5.0	3.4	63	94	60[3]	91	..	..	..	..
396C	SBR	TBTO	6.0	4.0	96	90	90	63[4]	..	..	..	..
396M	SBR	TBTS	4.0	2.5	0	49	50[3]	57	..	..	..	..
397A	Neoprene	TBTO	7.0	5.1	97[3]	75	72	84	72[3]	..	..	..
397B	Neoprene	TBTO	7.0	5.1	100	discon'd, same as 397A except color						
397D	Neoprene	TBTO	7.0	5.1	100	discon'd, same as 397A except color						
402A	Neoprene	TBTF	8.0	5.7	100	85	75[3]	86	56	61	63	14
408A	Neoprene	TBTO	14.0	9.6	100	100	100	100	91	81	69	38
419A	Neoprene	TBTAd	8.0	5.7	100	92	98	..	70	81	69	24
419B	Neoprene	TBTR	8.0	5.7	30	38	94	..	..	..	..	..
431C	Butyl	TBTO	20.0	11.1	100	100	100	98	79	53	55	26
443A	Neoprene	TBTO	8.0	5.8	100	100	95	98	67	47	49	26
822C	Hydrin	TBTO	8.0	5.8	100	98	100	94[4]	74	78	57	40
895B	Neoprene	Bayluscide	6.0	4.4	0	8	2	0[4]	..	..	..	..
895C	Neoprene	Bayluscide	8.0	5.7	0	0	6	..	..	..	..	..
12-1-HJ326	Neoprene	TBTO+ TBTA	8.0 2.0	5.7 1.4	100	100	100	..	84	61	82	26

[1] TBTO-tributyltin oxide, TBTC-tributyltin chloride, TBTS-tributyltin sulfide, TBTF-tributyltin fluoride, TBTAd-tributyltin aldehyde, TBTA-tributyltin acetate, DBTO-dibutyltin oxide.

[2] Five replications of each compound at each flooding.

[3] 120 hr.—% Kill @ 96 hrs. probably about the same.

[4] 72 hrs.—% Kill @ 96 hrs. probably slightly higher.

350A, 408A, 431C, 443A, 822C, and 12-1-HJ326. All of these were approximately equal in effectiveness, giving 98 percent or better mortality after four floodings.

Results of tests using the best 4 materials from these screening tests against egg hatch and larval mortality in styrofoam cups were as follows: In the first test, using the toxic oak leaf infusion water no effect on egg hatch or larval development was noted; however, when toxic rubber cubes were added to the toxic oak leaf infusion water egg hatch was observed, but almost 100 percent larval mortality occurred after about 72 hours. In the second test, when the compounds were added to tap water none of the materials prevented egg hatch; however, most of the 1st instar larvae were killed within a few hours, and there was almost 100 percent larval mortality after 2 days with all compounds. When the compounds were added to 4-day old infusion water variable results were produced. In some replications of the same compound, larvae reached the 3rd instar, while in others they died in the 1st instar.

Untreated control larvae appeared normal and 4th instar larvae, pupae, and adults were noted before termination of the test. In the third test in which *C. nig-*

ripalpus egg rafts were added to tap water in which the 4 materials had been previously soaked for 10 days, eggs hatched normally. However, almost all 1st instar larvae were killed initially, and 100 percent mortality was obtained after 3–4 days.

In the pan test of 105B in the field, eggs or larvae were noted in all pans except one (Area 1 with 2 cubes) the initial day after treatment. However, early instars were observed in that pan 9 days after treatment. All pans contained developing larvae when the tests were discontinued 50 days after treatment. Although no emerging adults were noted in any pan, all larvae appeared normal and no dead larvae were noted. The species involved were *Culex salinarius, C. restuans,* and *C. quinquefasciatus.* In the field tests with 408A, 443A, 822C, and 12–1–HJ326 the date of the first indication of mosquito breeding in the open and sheltered pans respectively for the 4 materials were: 408A, 1 and 1; 822C, 1 and 8; 443A, 4 and 17; 12–1–HJ326, 4 and 4; and the check pan 3 and 17 days. All pans had some stages present when the tests were concluded 36 days after treatment. All emerging adults appeared normal and no dead larvae were noted. Water in the check pans appeared cloudy shortly after the test was started with some surface scum and algae, whereas, the treated pans remained clear. Pupal cases were noted on the shelter check pans after 17 days.

DISCUSSION. In the laboratory there was a noticeable retardation of growth of larvae exposed to lethal levels of the toxicant. The larvae usually failed to develop beyond the 1st or 2nd instar even during periods of up to 6 or 7 days. Larvae exposed to the toxicant but not killed developed normally and produced apparently normal adults. In laboratory tests tributyltin oxide impregnated rubber apparently was not toxic to either *Aedes taeniorhynchus* or *Culex nigripalpus* eggs but was generally highly toxic to the larvae of both species in clear tap water at feasible field dosages and after repeated floodings. In brackish water or tap water containing organic debris, results were variable but generally poor against larvae.

Stockman, *et al.* (1970) discussed this phenomenom and stated that there are competing reactions occurring in water containing organic debris which result in the removal of the larvicide from the water at the same time that the larvicide is coming out of the polymer. These reactions are decomposition and absorption, the rate of the former being dependent upon the concentration of the larvicide at any time, the temperature of the water, the pH, and the amount of light. Therefore, based on results of this study, field application would not be feasible since organic material in field water apparently counteracts the effectiveness of the tributyltin compounds rendering them inactive.

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