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CONTROLLED RELEASE ORGANOTINS AS MOSQUITO LARVICIDES

Nate F. Cardarelli

Environmental Management Laboratory, University of Akron, Akron, Ohio 44325

ABSTRACT. A number of commercially available controlled release organotin molluscicides and their degradation products were evaluated against culicine larvae as a segment of a broad environmental impact and chemodynamics study. Test materials were observed in periodic bioassay, in some cases for over 400 days, and found effective against mosquito larvae at dosages considered to be practical for field application. The LT100 results were comparable to controlled release temephos and chlorpyrifos. Long term efficacy, low biological persistency (once released from the binding matrix), and the suspected proteolytic kill mechanism which may negate the development of tolerance would indicate that tributyltin fluoride and bis (tri-n-butyltin) oxide in sustained release compounds are suitable as mosquito larvicides.

INTRODUCTION

A number of controlled release organotin molluscicides were evaluated against culicine larvae and pupae as a portion of a broad environmental impact study. The bioassay results when coupled with other factors regarding the relative environmental safety indicate that these formulations would have significant merit as mosquito larvicides.

It has been recognized for a number of years that the trialkyltins and triaryltins are larvicidal. Bis (tri-n-butyltin) oxide and other organotins formulated in elastomers were examined in 1965–1966 against *Culex pipiens pipiens, Cx. pipiens quinquefasciatus*, and *Aedes taeniorhynchus* with good results (Smith and Roos 1966, Cardarelli 1976, Schultx and Webb 1969). Boike and Rathburn (1973), examined 23 organotin/elastomer compounds and found high larvicidal activity in clear water and generally poor activity in water laden with organic matter. Further work in this area was curtailed because organic content of field waters was believed too great a burden and the environmental effects were essentially unknown.

In the snail control area, however, a number of materials are commercially available, and the results of field tests have demonstrated not only efficacy but long term control (Cardarelli 1977, Castleton 1974, Shiff 1975, Gilbert, et al. 1973, Gilbert, et al. 1976, Upatham 1976, Arfaa 1976 and Santos 1977). Analysis of materials returned after 2 years field immersion indicated a biological half-life in excess of 4 years (Cardarelli, et al. 1977).

MATERIALS AND METHODS

The commercial organotin molluscicide formulations evaluated against mosquito larvae were BioMet® SRM, 6% bis (tri-n-butyltin) oxide in natural rubber manufactured by M&T Chemicals, Rahway, NJ; CBL–9B containing 20% tributyltin fluoride in natural rubber from the Creative Biology Laboratory, Inc., Barberton, OH; ECOPRO®–1330 and ECOPRO®–1230, both 30% tributyltin fluoride in ethylene propylene

copolymers available from Environmental Chemicals, Inc., Barrington, IL.

Several film-forming organotin polymers used in antifouling paints and commercially available from M&T Chemicals were also evaluated.

In examining the various organotin degradation models, several suspected breakdown products were selected and investigated against mosquito larvae. Materials chosen followed the Sheldon degradation scheme (Sheldon 1974) while recognizing that this is not the only possible mode of environmental decay. Tributyltin carbonate ("TBTC"), monobutyltin trihydroxide ("BTTH") and dibutyltin dihydroxide ("DBDH") were prepared in the laboratory. Dibutyltin oxide and stannic oxide, the ultimate proposed decay product, were also tested. ECOPRO®–1700 controlled release temephos (Abate®) as described by Walker and Cardarelli 1977, and controlled release chlorpyrifos (Dursban®), described by Nelson, et al. 1976, as the Dow Chemical Company 10CR product were evaluated as comparable formulations.

Initially local *Cx. pipiens* were collected as larvae and tested. Later tests were standardized with organophosphorus susceptible, DDT-dieldrin resistant *Cx. quinquefasciatus* reared in the laboratory from eggs supplied by the Center for Disease Control, Atlanta, GA. Larvae, pupae and adults were handled in the conventional manner. Neonatal laboratory mice served as an inexpensive and convenient blood source.

Bioassays were performed in 1 liter plastic beakers using demineralized water with finely powdered, dry dog food added in small amounts. In early tests 20 to 25 larvae per container were used. The number was later decreased to 15 larvae to minimize any possible crowding effect. The first 2 tests reported were run using 2nd and 3rd instar larvae; afterwards, only 1st and 2nd instars were utilized.

Unlike conventional laboratory bioassay methodology, LD and LC values have little meaning. When toxicant evolution is continuous, the distinction between higher and lower dosages lies in the pre-mortality duration. Consequently the criterion used in this study is the lethal time to effect 100% population mortality; i.e., the LT_{100}. It was early observed that the organotins, and to a lesser extent temephos, dramatically slow or prevent morphogenesis. The normal egg-to-adult time of 8 or 9 days under these test conditions was extended to 21 or more days at sublethal levels.

Pellets were soaked continuously prior to and during the test periods. Water was changed periodically between tests to preclude the establishment of an equilibrium condition. The degradation materials were added directly via serial dilution.

Controls were established for each evaluation. In toto over 2,000 culicine 1st and 2nd instars were examined at 15 per liter of water. The mean larval control mortality was 22%, pupal mortality about 6%, and adult emergence mortality less than 1%.

RESULTS

Table 1 is a compendium of LT_{100} values observed over a 310–day duration with various dosages of the ECOPRO® 1230 and ECOPRO® 1330 materials. Since pupation was significant at the last bioassay, percentages are given. It was noted that the agent involved, tributyltin fluoride, was toxic to pupae at relatively low concentrations. The daily release rate of TBTF was less than 10 ppb and thus below the sensitivity capabilities of in-house instrumentation and that of an outside commercial biotest laboratory. Table 2 depicts values for the controlled release temephos material. In Table 3, long term immersion data are presented for the tributyltin formulations, ECOPRO® 1230 and ECOPRO® 1330. The pellets involved were removed from snail challenge bioassays and examined for larvicidal potency after 480 plus days of continuous water immersion. Controlled release chloropyrifos, BioMet SRM, CBL–9B, and the selected organotin decom-

Table 1. Evaluation of controlled release organotin molluscicides against *Culex quinquefasciatus* larvae

Material	ppm Total Available Toxicant	Days[a] Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Percent Pupation	Percent Pupal Mortality
EC-1330	12.5	30	2	106	4	160	8	225	5	310	12	0	—
	8.0	30	3	106	9	160	10	225	7	310	13	7	100
	4.1	30	7	110	13	160	8	225	9	310	13	15	100
	2.7	30	8	106	13	160	11	225	10	310	15	30	60
	2.3	30	10	110	14	160	9	225	12	310	15	33	20
	1.4	30	10	110	13	160	10	225	12	310	14	40	0
	0.47	30	8	110	13	160	10	225	10	310	13	44	0
EC-1230	3.3	30	6	110	9	160	9	225	11	310	13	10	100
	1.4	30	6	110	12	160	11	225	12	310	13	37	0

[a] Number of days the pellet soaked prior to the addition of larvae.

Table 2. Evaluation of controlled release temephos formulation against *Culex quinquefasciatus* larvae

Material	ppm Total Available Toxicant	Days[a] Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Days Soak Time	Larva LT_{100} Days	Percent Pupation
EC-1700	3.6	30	2	71	2	110	2	160	3	225	2	310	3	0
	3.4	30	2	71	2	110	2	160	1	225	1	310	3	0
	2.2	30	2	71	3	110	3	160	2	225	2	310	3	0
	1.5	30	3	70	3	110	4	160	1	225	3	310	6	0
	1.3	30	2	—	—	110	5	160	3	225	7	310	3	0
	0.84	30	3	—	—	110	3	160	6	225	5	310	5	0
	0.54	30	2	70	3	110	3	160	6	225	4	310	—	33
	0.46	32	2	70	4	110	8	—	—	225	4	310	—	20
	0.23	30	2	70	4	110	8	160	4	225	4	310	—	13
	0.14	30	5	70	6	110	7	160	11	225	5	310	—	33
	0.06	30	5	71	9	110	13	160	9	225	9	310	—	67
	0.035	30	6	71	11	110	10	160	11	225	14	310	—	67

[a] Number of days the pellet soaked prior to the addition of larvae.

Table 3. ECOPRO^R–1330 and ECOPRO^R–1230 bioassay with *Culex quinquefasciatus* after long term pre-immersion (1st and 2nd instar)

Material	ppm Total Available Toxicant	Days[a] Soak Time	Larva LT_{100} Days	Percent Pupation	Percent Adult Emergence
EC–1230	30.20	481	7	0	0
	16.30	481	5	0	0
	6.93	470	10	7	0
	4.29	470	16	20	0
	1.95	495	16	20	0
EC–1330	32.60	487	5	0	0
	16.30	473	5	0	0
	5.94	483	11	0	0
	5.61	512	10	0	0
	5.22	485	8	0	0
	5.19	497	—	13	0
	4.80	492	14	0	0
	2.46	473	—	40	0
	1.92	485	—	13	7
	0.90	485	—	13	13

[a] Number of days the pellet soaked prior to the addition of larvae.

position products were examined at various dosages with the results shown in Table 4. Periodic challenges have not, as yet, been made with these materials.

The toxicant concentration given is the total available based upon pellet weight and the known amount within that pellet. It is *not* the concentration of the toxicant in the test water.

DISCUSSION

The data indicate long-term efficacy for several controlled release organotin materials when tested against culicine larvae under laboratory conditions. The amount of toxicant present in the test water at any given time was unknown since our instrumental capabilities were not sufficiently sensitive. In liquid scintillation counter studies using C^{14} labled TBTO formulated as BioMet SRM, the emission rate appeared to be less than 0.0008 $\mu g/cm^2$-day (to be published). However, the extreme hydrophobicity of both TBTO and TBTF results in rapid loss of the agent, through adsorption and absorption by organic and inorganic matter.

Whereas considerable data concerning the environmental impact, system chemodynamics, lethal mechanisms, toxicity, and dosage regimens of organotins have been amassed, information is keyed towards snail control and not mosquito abatement. Within this context several salient properties of TBTO and TBTF may be of interest. In general, water quality does not affect the net result over a given dosage profile, but the presence of certain ions and suspendents will retard or accelerate achievement of the LT_{100} (Cardarelli, Gingo, and Walker 1977; Walker and Cardarelli 1976[1]). Both organotins, TBTO and TBTF, examined in microecological systems show a major tendency towards adsorption on soil (Cardarelli 1978[2]). At any given time in the life of a heavily dosed water-soil system an approximate 1:15 organotin content ratio is noted by conventional analytical means. Removal of the organotin molecule from soil requires considerable energy and would likely not occur under natural conditions.

Both TBTO and TBTF display selective phytotoxicity e.g. *Elodea canadensis*, at dosages considerably higher than that es-

sential for mosquito or snail kill. Mammalian and fish toxicity has been described elsewhere (Cardarelli 1977; Cardarelli, Gingo, and Walker 1977). Molluscicidal dosages are considerably higher than those needed for mosquito control, and by careful control of the concentration used in a given aquatic system snails are destroyed without significant effect on fish or elements of the food chain (Cardarelli 1976, Shiff 1974).

Snail control is *not* affected by organic matter or various clay suspendents, although molluscicidal action may arise from ingestion of organotin ligands which may not occur with mosquito larvae

Table 4. Bioassay data for several controlled release organotin formulations, organotin decomposition products and controlled release chlorpyrifos (5 replicates of 15 larvae)[a]

Material	ppm Total Available Toxicant	Larva LT_{100} Days	Percent Pupation
BioMet SRM	10.00	5	0
	1.00	8	18
	0.10	9	15
	0.01	9	25
Control	0.00	9[b]	88
CBL–8B	10.00	4	0
	1.00	5	0
	0.10	8	0
	0.02	—	16
Control	0.00	—	86
Dursban 10 CR	1.37	1	0
	10.8	2	0
	0.90	4	0
	0.33	2	0
	0.15	1	0
	0.13	2	0
			0
TBTC	10.00	9	0
	1.00	—	13
DBTO	10.00	14	0
	1.00	14	2
	0.10	11	0
	0.01	10	0
BTTH	10.00	15+	—[b]
	5.00	15+	—
	1.00	15+	—
	0.10	15+	—
	0.01	15+	—
DBDH	10.00	8	0
	5.00	12	1
	1.00	14	5
	0.10	15+	
	0.01	15+	—
SnO_2	100.00	15+	50
	10.00	15+	52
	1.00	15+	50
Control	0.00	15+	76

[a] Tests were usually terminated on day 15.
[b] Not measured.

(Cardarelli 1977: Walker and Cardarelli 1976). The lethal mechanism of organotins for snails or mosquitoes has never been elucidated. Histological examination of exposed snails strongly indicated a proteolytic activity in connective and other tissue. Trialkyl organotins react readily with select amino acids, peptides and fatty acids *in vitro* (to be published). Resistance studies with snails exposed at an LT_{50} over three generations have failed to detect the development of tolerance to bis (tri-n-butyl-tin oxide (Walker and Cardarelli 1976). It is therefore believed that controlled release organotin formulations, prepared and evaluated as molluscicides, may be of considerable merit as mosquito larvicides.

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