Phytotoxicity Of Four Formulations Of The Alkylamine Salt Of Endothall On *Hydrilla Verticillata* And Fish[1]

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ABSTRACT

The commercial formulation of 7-oxabicyclo[2.2.1] heptane-2, 3-dicarboxylic acid (endothall) was compared with a liquid, technical, granule, and controlled release pellet formulation of the mono (N,N-dimethyl alkylamine) salt of endothall (hereinafter referred to as hydrothol) on the submersed aquatic weed hydrilla (*Hydrilla verticillata Casp.*) and the bluegill sunfish (*Lepomis macrochirus*). The controlled release pellet formulation of hydrothol gave the most effective control of hydrilla. Controlling the release of the herbicide reduced fish toxicity and residue level in the water, and gave longer weed control in small pool tests. The commercial formulation of endothall was very safe to fish, but had little phytotoxicity to hydrilla. The controlled release pellet of hydrothol applied at 2 ppmw in field experiments was more effective on hydrilla than 3 ppmw of liquid, granule, or technical formulations. Phytotoxicity of the controlled release formulation was not as rapid as the other three hydrothol formulations, but there was increased length of control.

INTRODUCTION

The herbicide 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid (endothall) was discovered in 1953 to have aquatic herbicidal properties (9), and is now widely used for the control of submersed aquatic weeds (2). It is not applied in the aquatic environment as the free acid, but is first converted to inorganic or amine salts. The most active formulations on aquatic weeds are the mono and di (N.N-dimethyl alkylamine) salts. However, the long chain amine safts are toxic to fish at concentrations of 0.3 to 1.0 ppmw (11). The amine salts are two to three times more active on aquatic weeds than the inorganic salts, but 200 to 400 times more toxic to fish.

Research emphasis on the rapidly spreading submersed aquatic weed hydrilla (*Hydrilla verticillata* Casp.) has been increased during the past 5 years. One of the more active herbicides evaluated on this aquatic weed is the amine salt of endothall (4). Results have shown that 2 to 4 ppmw of this herbicide will control dense infestations of hydrilla; however, the toxicity of this herbicide to fish has limited its use in the aquatic environment.

The research reported herein was initiated to compare a controlled release formulation of the amine salt of endothall with other existing formulations. Controlled release of the herbicide in the aquatic environment may be considered as a method which will reduce fish toxicity, residue levels, and cost of control when using the amine salts of endothall.

MATERIALS AND METHODS

Laboratory Evaluations: Evaluations of the herbicide formulations in the laboratory were similar to those described by Blackburn (1) for submersed plants. Terminal cuttings, 5 in long, were collected from hydrilla infestations in canals near Fort Lauderdale, Florida. The basal two to three-node portion of three cuttings were placed in a sandy loam soil in a 2-in plastic pot. Two pots were placed in a 3,680 ml glass jar filled with pond water, the water quality of which is shown in Table 1. The top of the jar was covered with a sheet of thin transparent plastic to prevent evaporation. Jars were placed in a controlled environment room with 120 ft-c of light at the jar top for 14 hr followed by 10 hr of darkness. Room temperature was 20±2 C.

At the end of a 3-week growth period, potassium endothall and the technical, commercial liquid, commercial granule, and an experimental controlled release clay pellet formulation of the amine salt of endothall (hereinafter referred to as hydrothol) were added to the jars. At the time of treatment, approximate length of the 3-week-old hydrilla was 10 to 12 in. Each treatment was replicated three times. Visual evaluations of herbicidal activity were made at 2, 4, and 6 weeks after treatment.

Pool Experiment: Hydrilla cuttings were planted in outdoor, circular, plastic pools. A soil mixture (50% peat and 50% sand) was uniformly spread over the 7.2 sq m area of the pool bottom to a depth of 6 in. Terminal cuttings, ca. 8 in long were placed with the basal 3 to 4 in. portion in the soil. The pools were planted with equal quantities of hydrilla and southern naiad. Pond water was used to fill the pools to an average depth of 24 in. After filling, each pool was stocked with 10 fingerling bluegills. The plants at the time of treatment extended to the surface.

The pools were treated with potassium endothall and the liquid, technical, granule, and controlled release formulations of hydrothol at a concentration of ppmw. Each treatment was replicated three times and three untreated pools served as controls. Evaluations for herbicide phytotoxicity were made at 4 week intervals for 24 weeks.

TABLE 1. QUALITY OF POND WATER USED IN THE LABORATORY AND POOL EXPERIMENTS

Component	All concentrations in ppmw except pH
pH	7.90
$CaCO_3$	126.00
$MgCO_3$	20.00
Total hardness	152.00
Copper	0.05
Iron	0.10
Nitrate N	0.37
Phosphate	1.17
Sulfate	24.00
Tannins and Lignins	1.66
Chlorinity	0.00

[1]Cooperative investigations of the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture; the Central and Southern Florida Flood Control District, the U.S. Army Corps of Engineers, and the Florida Agricultural Experiment Station, Fort Lauderdale, Florida. Published as Journal Series No. 3695. Florida Agricultural Experiment Station.

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Water samples were removed from the pools at 0, 1, 3, 7, 10, 15, 20, 30, and 40 days after treatment. Water samples were taken from each pool at depths of 12 in. The samples were analyzed for endothall acid and the mono (N,N-dimethyl alkylamine) salt.

Field Experiments: Field experiments were conducted in lakes Baldwin and Virginia, near Winter Park, Florida. These lakes vary in average depth from 10 to 17 ft. Hydrilla was well established in the lakes at the time of treatment. During the period May through October, hydrilla covers 50% of the surface area of these lakes.

Plot size varied depending on the size of the infested area and density of the weed infestation at the time of treatment, and the amount of experimental herbicide made available by the manufacturer. All treatments were replicated three times.

Liquid formulations were applied with a power sprayer mounted in an airboat. The spray tank was equipped with mechanical and by-pass agitation to insure uniform mixing of the herbicide. The herbicide was injected 10 to 12 in. below the water surface at a pressure of 100 psi. The desired amount of herbicide to be applied in each plot was mixed with enough water to equal 150 gpa. The technical formulation of hydrothol was pumped below the water surface without mixing with water.

Granular and pelleted formulations were applied with a motor-driven centrifugal spreader mounted on the bow of an airboat. Care was taken to insure uniform coverage of each plot. The amount of material applied in each plot varied with the average depth and the desired concentration.

Evaluations for herbicidal effects of the various formulations of endothall were made at 2 and 4 weeks, and at monthly intervals until the plot had regrown. Evaluations were made by diving into the treated area using SCUBA equipment. A rating scale of 0-100 was used (0—no effect, 100—complete topkill). Percentage of regrowth of the plants in the plot was also recorded at each evaluation.

Fish Toxicity: Herbicides were tested in the laboratory by modifications of bioassay methods described by many workers (5, 6, 7, 12). Fish used in the bioassay tests were seined from earthen ponds and transferred into circulating tanks in a temperature control laboratory. Test fish were treated with chemicals for disease and fungus control prior to use in bioassay tests. Following treatment, the fish were fed a commercial fish food until needed for the toxicity studies. Food was withheld from the fish 24 hrs prior to their removal for stocking in glass jars. Three bluegill (*Lepomis macrochirus*) were stocked in each one-gallon jar and acclimatized to the water used in the bioassay test. Aeration of jars was terminated before the herbicides were introduced. Quality of water used in the bioassay studies is shown in Table 1.

After endothall and various formulations of hydrothol were introduced, observations were made at periodic intervals on both the test fish, and the controls for a period of 96 hrs. Toxicity results were recorded as number of fish in distress and number dead. Each treatment was replicated six times.

RESULTS AND DISCUSSION

Laboratory Experiments: Hydrothol was more effective on hydrilla than endothall (Table 2). These data confirmed the results of previous experiments conducted with hydrothol at this station (3). The commercial liquid formulation of hydrothol gave a more rapid kill of hydrilla than the technical hydrothol. This was due to the slow solubility of the technical material in water. The granular hydrothol had a more rapid effect on hydrilla than the pellet. This was thought to be related to the controlled release of the herbicide from the pellet.

TABLE 2. COMPARISON OF THE PHYTOTOXICITY OF ENDOTHALL AND FOUR FORMULATIONS OF THE AMINE SALT OF ENDOTHALL (HYDROTHOL) ON HYDRILLA IN LABORATORY TESTS.

Chemical	Concentration (ppmw)	Percent Control[a]/ Weeks after treatment 2	4	6
Endothall	1.0	45	68	70
	2.5	55	78	83
	5.0	75	100	100
	10.0	98	100	100
Hydrothol (liquid)	0.5	75	100	100
	1.0	90	100	100
	2.5	100	100	100
Hydrothol (technical)	0.5	55	100	100
	1.0	75	100	100
	2.5	95	100	100
Hydrothol (granule)	0.5	50	98	100
	1.0	78	100	100
	2.5	85	100	100
Hydrothol (pellet)	0.5	45	78	95
	1.0	50	88	99
	2.5	55	90	100

[a]Percent control (0—no control; 100—complete control).

The formulation of hydrothol used in the experiment did effect the herbicidal activity on hydrilla. The release rate of the herbicide into the water effected the speed of herbicidal activity. Plants treated with the pelleted formulation began to show herbicidal effects first near the soil and gradually died from the bottom of the test container to the top. This was an indication that the toxic concentration of the herbicide was building up near the area where the pellets lay in the test containers.

Pool Experiments: Endothall did not control hydrilla at the 2.0 ppmw concentration (Table 3). The liquid and technical formulations of hydrothol gave a rapid topkill of hydrilla. The granular formulation did not give complete control. The most effective formulation of hydrothol in length of control was the pellet.

All fish were killed in the pools treated with the liquid and technical formulations of hydrothol. The fishkill could

TABLE 3. COMPARISON OF THE PHYTOTOXICITY OF ENDOTHALL AND FOUR FORMULATIONS OF THE AMINE SALT OF ENDOTHALL (HYDROTHOL) ON HYDRILLA IN PLASTIC POOLS. EXPERIMENT INITIATED ON SEPTEMBER 19, 1969.

Chemical	Concentration (ppmw)	Percent Control[a]/ Week after treatment 4	8	12	20	Percent Fish Mortality
Hydrothol (liquid)	2	100	100	95	80	100
Hydrothol (technical)	2	100	100	100	80	100
Hydrothol (granules)	2	72	80	85	40	65
Hydrothol (pellets)	2	88	98	100	100	0
Endothall	2	75	85	70	30	0

[a]/ Percent control (0 - no control; 100 - complete control)

have been caused by the toxicity of the herbicide or the rapid kill of the aquatic vegetation which depleted the oxygen in the water. No fish mortality occurred in the endothall and hydrothol pellet treatments. The slow release of the herbicide and slow plant kill prevented fish toxicity.

The concentration of endothall found in the water at various intervals after treatment with hydrothol is shown in Figure 1. The water samples were analyzed for endothall and the amine salt. A different analytical procedure is required for each material. The concentration of endothall in the water varied with the formulation. The greatest concentration and longest persistence was in the pools treated with the liquid formulation. The difference in concentration and persistence of the granular and pelleted formulation is related to the release rates of the herbicide from the carrier. The slow solubility of the technical material in water would cause the same effect.

The concentration of the mono (N,N-dimethyl alkylamine) salt found in the water at various intervals after treatment is shown in Figure 2. The amine portion is the fish toxic part of this herbicide. The highest concentration of the amine salt was found in pools treated with the liquid formulation. This formulation is highly water soluble, while the technical material has a low water solubility. The low concentration of the amine in the pools treated with pellets and granules can be correlated with the slow release from the carrier.

No attempt was made to measure the concentration of endothall or amine salt in plants or soil. It must be assumed that part of the herbicide was absorbed into the plants and bottom muds. Laboratory results have shown that the release of the amine from the pellet continues after 5 days in water containing no plants or soil, but in this experiment, none could be detected at 5 days. The amine must have been absorbed by plants or bottom muds.

The reduced fish toxocity and the longer lasting control makes the slow release pellet superior to other hydrothol formulations evaluated in the pools. The advantage for the pelleted formulation was greater in pool tests than in the laboratory.

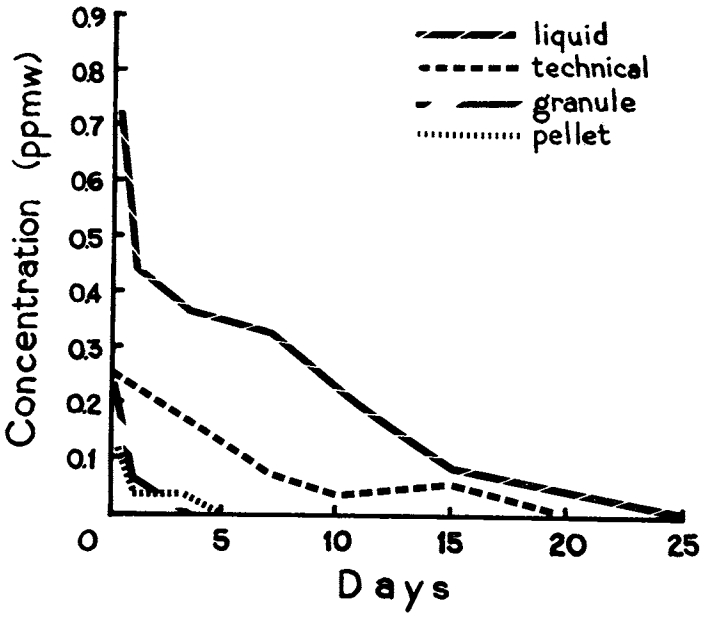

Figure 1. Residue of endothall acid after treatment of submersed weeds with 2 ppmw of the amine salt of endothall.

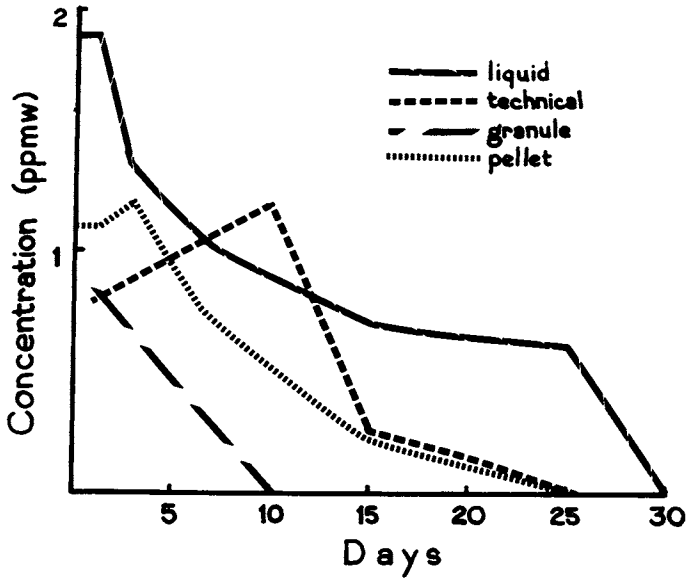

Figure 2. Concentration of the mono (N,N-dimethyl alkylamine) salt remaining in the water after treatment of submersed weeds with 2 ppmw of the amine salt of endothall.

Field Experiments: The results of experiments conducted in Lakes Virginia and Baldwin, using endothall and various formulations of hydrothol, were similar to pool experiments. The pelleted formulation of hydrothol was the most effective one evaluated in the experiments (Tables 4 and 5). A 2 ppmw rate of the pellet was more effective than a 3 ppmw treatment of granule in the Lake Virginia treatment, and a 3 ppmw treatment of the liquid in Lake Baldwin. Satisfactory weed control was obtained for 5 months in both experiments when using the pelleted formulation.

Hydrothol pellets were found on the bottom of the lake 3 months after treatment. The slow dissolving of the pellets in the treated area would account for the longer lasting control. Since the plots were located in an area of the lake where there was no apparent water movement, the herbicide released into the water is likely to have remained in the treated area.

Fish Toxicity: Endothall was non-toxic to bluegills. Concentrations of 20 ppmw had no visible effect on the fish in bioassay tests extended over a 96 hr period (Table 6). These data agree with the findings of other workers (8, 10).

The toxicity of hydrothol in laboratory bioassay tests varied with the formulation. The toxocity of the various

TABLE 4. A COMPARISON OF THE PHYTOTOXICITY OF ENDOTHALL AND FOUR FORMULATIONS OF THE AMINE SALT OF ENDOTHALL (HYDROTHOL) ON HYDRILLA IN LAKE VIRGINIA, WINTER PARK, FLORIDA. EXPERIMENT INITIATED APRIL 17, 1969.

Chemical	Concentration (ppmw)	Percent Control[a]/ Months after treatment 1	2	3	4	5	6
Hydrothol (liquid)	2.0	55	18	4	10	0	0
Hydrothol (technical)	2.0	92	45	39	20	0	0
Hydrothol (granules)	3.0	99	98	92	88	38	0
Hydrothol (pellets)	2.0	94	99	94	92	68	50
Endothall	3.0	50	30	15	0	0	0
	4.5	68	55	50	10	0	0

[a]/ Percent control (0 - no control; 100 - complete control)

TABLE 5. A COMPARISON OF THE PHYTOTOXICITY OF ENDOTHALL AND THREE FORMULATIONS OF THE AMINE SALT OF ENDOTHALL (HYDROTHOL) ON HYDRILLA IN LAKE BALDWIN, ORLANDO, FLORIDA. EXPERIMENT INITIATED APRIL 20, 1969.

Chemical	Concentration (ppmw)	Percent Control[a]/ Months after treatment 1	2	3	4	5
Hydrothol (liquid)	3	95	97	60	50	15
Hydrothol (technical	2	90	65	54	50	0
	3	95	90	58	72	32
Hydrothol (pellets)	2	99	95	96	82	85
Endothall	3	62	60	38	28	0

a/ Percent control (0 - no control; 100 - complete control)

formulations in decreasing order was liquid, technical, granule, and pellet. Bluegills were killed with 0.2 ppmw of the liquid formulation in 24 hrs while it required 10 times this concentration of pelleted material. The granular material was twice as toxic as the pelleted formulation. The toxicity of the liquid formulation to bluegills agrees with the findings of other investigators (11).

On the basis of this data, it is apparent that the likelihood of a fishkill in field applications of hydrothol is greatly reduced when using the controlled release pellet formulation. The slow release of the amine salt from the pellet and the granule can be correlated with phytotoxicity as demonstrated in pool and field experiments.

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TABLE 6. TOXICITY OF ENDOTHALL AND FOUR FORMULATIONS OF THE AMINE SALT OF ENDOTHALL (HYDROTHOL) TO BLUEGILL IN STILLWATER LABORATORY TESTS. RESULTS EXPRESSED AS PERCENT MORTALITY.

Chemical	Concentration (ppmw)	Hours after treatment 2	4	8	16	24	48	72	96
Hydrothol (liquid)	0.20	0	0	0	10	25	100	100	100
	0.25	0	8	25	100	100	100	100	100
	0.50	33	100	100	100	100	100	100	100
	0.75	75	100	100	100	100	100	100	100
Hydrothol (technical)	0.20	0	0	0	0	10	75	100	100
	0.25	0	0	0	0	50	100	100	100
	0.50	25	33	75	100	100	100	100	100
	0.75	50	100	100	100	100	100	100	100
Hydrothol (granules)	0.5	0	0	0	10	33	33	33	33
	0.75	0	0	0	50	100	100	100	100
	1.00	0	0	50	100	100	100	100	100
	2.00	33	42	66	100	100	100	100	100
Hydrothol (pellets)	1.00	0	0	0	0	0	0	10	25
	1.50	0	0	0	0	0	10	33	50
	2.00	0	33	40	50	100	100	100	100
Endothall	10.00	0	0	0	0	0	0	0	0
	20.00	0	0	0	0	0	0	0	0