Tests of 15 Experimental Molluscicides Against *Australorbis glabratus*

ERIC A. SEIFFER and HERBERT F. SCHOOF

THE PUERTO RICO Field Station of the National Communicable Disease Center, Public Health Service, conducts laboratory and field studies to evaluate pesticides for use as molluscicides against *Australorbis glabratus*. The tests described in this paper consisted of laboratory evaluations of 15 compounds and field appraisal of the two most promising. The compounds tested are listed by their trade and chemical names; the letters in parentheses are supply references. Bayluscide (2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicyanilide) and sodium pentachlorophenate (NaPCP) were used for comparison standards in the laboratory tests.

GC–405 (*A*) : zinc nicotinyl fluosilicate
GC–2131 (*A*) : 1-chloro-2,4-phenylene-bis (O,O-diethyl) phosphorothiolate
AC–43913 (*B*) : confidential compound
1,2,3,4,tetrachlorobenzene (*C*)
HRS–1622 (*C*) : octachloropropane
Mobam (*D*) : 4-benzothienyl-N-methyl carbamate
carbaryl (*E*)
bis (tri-n-butyltin) oxide (*F*)
triphenyltin chloride (*F*)
tri-n-butyltin acetate (*G*)
tri-n-propyltin oxide (*G*)
WL 8008 (*H*) : N-tritylmorpholine
TD–282 (*I*) : di (N,N dimethyltridecylamine) salt of Endothall
TD–283 (*I*) : mono (N,N dimethyltridecylamine) salt of Endothall
triphenyltin acetate (*G*)

Procedures

Laboratory Tests

In these tests, field-caught *A. glabratus* 10–26 mm. in diameter were brought from various natural habitats throughout the island. The snails were kept in an outdoor holding tank for at least 1 week before testing; infected specimens were discarded. Field-caught snails were used to measure the efficacy of the chemicals against *A. glabratus* normally encountered in field trials.

In each test series the chemical concentrations were established in a geometric series; three series of tests were made on separate dates. In each test 30 snails were exposed to each molluscicide concentration, 10 snails in 2 liters of treated water per glass jar. Each jar had a capacity of 4 liters and was lined with a polyethylene bag. Snails were exposed for 6 hours, and during this period any specimens crawling from the treated water were pushed back. Reactions were observed every 2 hours during the exposure period. At the end of 6 hours, the snails were rinsed and transferred to 2 liters of tapwater inside a polyethylene-lined jar. The water contained no residual chlorine.

In each test series, two control groups of 30 snails each were used. One was kept in un-

At the time of this study, Mr. Seiffer was with the Puerto Rico Field Station, National Communicable Disease Center, Public Health Service, San Juan. He is now with Seiffer Consultants, Inc., Irvington, N.Y. Dr. Schoof is assistant chief of the Center's Technical Development Laboratories, Aedes aegypti *Eradication Program, Savannah, Ga.*

Juan R. Palmer, chief of the vector control section, Puerto Rico Department of Health, cooperated in the field studies. E. A. Kildare, a biological aide at the Puerto Rico Field Station, assisted in much of the work reported.

treated tapwater and the other was exposed to a known chemical standard, Bayluscide. If an organic solvent was required for the candidate compound, a third control of the organic solvent alone was used.

During the post-treatment holding period the snails were fed a leaf of lettuce or a modified Standen preparation (*1*) consisting of 4 parts cerophyl, 2 parts glandex, 2 parts wheat germ, and 1 part powdered milk (suggested by Dr. L. S. Ritchie, U.S. Army Tropical Research Laboratory, San Juan) plus a piece of chalk. Specimens were observed for 96 hours with readings at 24-hour intervals; the 72-hour reading was used for the reporting of data. Results were reported at LC_{50} and LC_{95} following Finney's probit analysis method (*2*). Compounds which gave 100 percent kill in solution at 10 ppm or less were considered acceptable for field studies. All concentrations refer to the active ingredient.

Field Tests

In the initial series of field trials, three still-water tests were made in farm ponds, followed by two stream tests. Streamflow was measured with a V-notch weir placed across the stream section or with a Parshall flume placed in the stream. Because of the limited quantities of test chemicals available, both rapidly flowing and slow-moving streams of low-flow volumes were used. These low-velocity streams usually contained small pools.

Evaluation of the field applications was made by density measurements of the natural snail population or by the mortality of caged field snails if the indigenous population was too low or absent. Snail populations were sampled with a wire mesh (1/8 inch) sampler 8.5 inches square. At least 100 dips were taken around the periphery of the ponds. In streams, at least 10 dips were taken every 50 feet, and visual observation was made between sampling stations while walking along the bank for the entire length of the test stream.

When caged snails were required, the cages, 6 by 6 by 12 inches, were immersed to half their depth in water and attached by pieces of inner tubes to wooden stakes placed in the marginal vegetation. The distance between cages was determined by the particular characteristics of the stream or pond since the cages could not be placed in shallow, rapidly flowing stream sections or in deep holes in a pond. The cages were made of nylon mesh window screening fastened on a framework of 1-inch wood strips. The top flap of screening was held closed with masking tape.

The snails (usually 10 or more per cage) and pieces of lettuce for their food were put in cages when they were placed in the water just before the test. In pond tests the control snails were placed in untreated pond water in a polyethylene-lined tub which usually was kept in the shade during the test. In stream tests caged control snails were placed upstream from the point of chemical application.

Streams were treated by the drip method. Ponds were treated with either a backpack sprayer or a bucket trailed behind a boat. The concentration of chemical was estimated on the basis of water volume measurements of still and flowing waters. The applied dosage in each case was calculated from the results obtained from laboratory tests (LC_{50}, LC_{95}, and 100 percent mortality) plus consideration of the physical factors present in any particular pond or stream.

In ponds where exposure for 48 hours was possible, low concentrations of the chemicals were used. In streams that involved additional factors, that is, length of treatment time and reach of stream effectively treated, chemical concentrations were estimated in relation to field data obtained with Bayluscide treatments (15 ppm for an application period of 1 hour).

Results

Laboratory Tests

Seven compounds (GC–405, GC–2131, AC–43913, 1, 2, 3, 4 tetrachlorobenzene, HRS–1622, Mobam, and carbaryl) failed to meet the criterion of acceptability, that is, complete kill after 6 hours exposure to 10 ppm. Table 1 lists the eight promising chemicals and comparable values for sodium pentachlorophenate and Bayluscide.

Five of the eight chemicals approached the effectiveness of Bayluscide. Activity of these five toxicants depended greatly upon formulation of the chemical. Only one, bis (tri-n-butyltin) oxide, was soluble in water. Four chemicals (triphenyltin chloride, tri-n-butyltin acetate, tri-n-propyltin oxide, and WL 8008) were insoluble or only slightly soluble in water but dissolved in 95 percent ethyl alcohol.

After triphenyltin chloride was dissolved in alcohol, the solution was diluted with water, a procedure that reduced the LC_{95} value fifteenfold. Only half as much tri-n-propyltin oxide was required for 100 percent mortality when it was first dissolved in alcohol; the same procedure produced a thirty-threefold decrease in the LC_{50} value for WL 8008. When formulated as a 10 percent emulsifiable concentrate, WL 8008 was highly toxic and gave 100 percent mortality at 0.03 ppm, the results being superior to

Table 1. Toxicity of eight compounds against *Australorbis glabratus* compared with Bayluscide and sodium pentachlorophenate [1]

Compound	Primary solvent	LC_{50} (ppm)	LC_{95} (ppm)	Concentration (ppm)	Percent kill	Remarks
Bis (tri-n-butyltin) oxide, technical grade.	Water	0.41	0.84	2.0	100	Snails retracted immediately upon contact with 1.0 ppm or greater. At 0.5 ppm or less snails crawled from solution.
Triphenyltin chloride, technical grade.	Water	5.5	19.0	10.0	78	Snails crawled from solution at all concentrations.
Do	1.5 percent ethyl alcohol.	.46	1.25	2.0	100	Do.
Tri-n-butyltin acetate, technical grade.	1.5 percent ethyl alcohol.	.29	.57	1.0	100	At 1.0 ppm all snails retracted at end of 6 hours' exposure, no recovery observed. At concentrations lower than 1.0 ppm snails crawled from solution.
Tri-n-propyltin oxide, technical grade.	Water	.65	1.7	2.0	97	Odor of chemical offensive.
Do	10 percent ethyl alcohol.	[2]	[2]	1.0	100	Snails crawled from solution.
Triphenyltin acetate, 20 percent wettable powder.	Water	.4	1.4	[3] 2.9	99	In some tests, snails crawled from solution.
TD-282, 1.6 pounds per gallon emulsifiable concentrate.	Water	[2]	[2]	5.0	100	Unpleasant odor. Snails extended from shells at concentrations up to 5.0 ppm.
TD-283, 2 pounds per gallon emulsifiable concentrate.	Water	3.8	6.2	10.0	100	At 10.0 ppm all snails retracted after 4 hours' exposure, no recovery observed. At lower concentrations numerous snails crawled from solution.
WL 8008, 10 percent emulsifiable concentrate.	Water	[2]	[2]	.03	100	Snails bled profusely at concentrations of 0.03 ppm and higher after 1 to 4 hours' exposure. Retracted when exposed to lethal concentrations, no recovery observed.
WL 8008, technical grade	Water	10.0	[4]	15.0	66	Do.
Do	2.5 percent ethyl alcohol.	.3	.83	1.0	100	Do.
Sodium pentachlorophenate.	Water	2.0	12.0	15.0–18.0	[5] 100	
Bayluscide	Water	.3	.75	0.8–1.0	[5] 100	

[1] 6-hour exposure period. [2] Variable activity. [3] Calculated. [4] Not done. [5] Data from reference 7.

those for Bayluscide. Shiff and Ward (*3*) also reported that WL 8008 showed greater toxicity than Bayluscide to three species of snails.

TD–282 and TD–283 were considered candidate molluscicides when both gave 100 percent mortality at 10 ppm or less. These compounds are used as herbicides at concentrations of 1 to 2 ppm and may be of value for the dual purpose of molluscicidal-herbicidal use.

Field Tests

Triphenyltin acetate. In four pond tests with triphenyltin acetate, 100 percent mortality of *A. glabratus* resulted after 48 hours' exposure to applications of 1 or 2 ppm. In the one pond not subject to intermittent drying there was some evidence of a residual effect from the treatment (1 ppm). Forty caged snails placed in this pond 3 days after the treatment were dead 14 days later. Of 40 caged snails placed in the pond 17 days after chemical addition, 15 were found dead 4 days later.

One of the two ponds treated at 1.0 ppm had a large sunfish population; a total kill of the fish resulted within 48 hours after application even though at 24 hours there had been no visible evidence that any fish had been affected. The other three ponds were shallow farm stock watering units, subject to intermittent drying. Control snails used only in the final pond trial had a mortality of 35 percent.

In the first flowing water trial a concentration of 5.0 ppm of triphenyltin acetate was applied for 2 hours (table 2). In the initial 1,000 feet of stream, 93 percent of the naturally occurring snails were killed, and it is possible that the snails found were deposited in the treated stretch by a storm. Mortality of caged snails was 100 percent 4,750 feet from the point of chemical application, the furthest distance that caged specimens were exposed. The caged control snails held above the point of treatment showed a mortality of 2.5 percent.

This stream, Quebrada Faria, is a natural earth-banked channel with considerable vegetation including malanga (elephant ear) along its banks. The water had a pH of 7.2 and dissolved oxygen content of 3 ppm. Volume of streamflow was 3.27 cubic feet per second (c.f.s.), no pools were in the treated reach, and time of flow for the 4,750 feet was approximately 2 hours.

In the second trial, 5.0 ppm of triphenyltin acetate were applied to another stream, Quebra-

Table 2. Effectiveness of triphenyltin acetate and WL 8008 against *Australorbis glabratus* in streams

Compound and test number	Concentration (ppm)	Application time (hours)	Mortality of caged snails: Percent	Mortality of caged snails: Feet downstream from application	Remarks
Triphenyltin acetate:					
1	5.0	2	100	4,750	Caged field snails.
			93	1,000	Used only natural snails occurring in first 1,000 feet (7 percent of snails cast on higher dry areas by increased flow, then returned to water).
2	5.0	1	80	4,750	Used caged field snails only.
WL 8008:					
1	5.0	3	100	728	Used caged field snails.
			80	1,605	
			30	4,438	
2	20.0	2	100	461	Do.
			10	1,631	
3	12.5	2	93	[1] 3,672	Caged and naturally occurring snails used.
			40	4,452	
			80	5,813	

[1] All naturally occurring snails were killed as far as 3,672 feet downstream from the point the chemical was applied. Before treatment, snails were not found beyond that point.

da El Toro, for 1 hour (table 2). Eighty percent mortality occurred in the caged snails positioned in a concrete-lined section of the stream at 4,750 feet below the point of chemical application. Mortality of caged snails in untreated water was 10 percent. Beyond 4,750 feet the kills were less, which may have been due to the dilution of the treatment by a tributary. The volume of streamflow was 0.28 c.f.s., the pH 7.3, and the dissolved oxygen content 2.9 ppm.

In both tests considerable faunal kill (guppy, tadpole, toad, and crayfish) was evident, but no harm to vegetation was discernible. Triphenyltin acetate was difficult to handle and caused extreme irritation of one worker's skin. When inhaled, the dust caused gross irritation of the nasal and throat passages and resulted in severe sneezing.

WL 8008. This chemical as an emulsifiable concentrate (18 percent) was tested in three ponds and in three streams. In the first two pond tests at a concentration of 0.25 ppm, practically complete caged snail kills (99 and 91 percent mortality) resulted after 48 hours' and 24 hours' exposure, respectively.

In the test with 99 percent kill, mortality of the untreated control snails also was high, but in the second test survival of the control snails was satisfactory. In the third pond treated at 0.125 ppm, 99 percent of the snails were killed after 48 hours, but the control snails again showed unsatisfactory survival. Exposure of the control snails to direct sun presumably caused too high a temperature in the control bucket. No residual effects on the snails were observed, and only in the test at 0.125 ppm were a few guppies killed. The test ponds contained little other animal life.

In the first flowing stream application a concentration of 5.0 ppm of WL 8008 was applied to the Quebrada Pasto in Aibonito for 3 hours (table 2). Caged snails showed 100 percent mortality 728 feet downstream from the point of chemical application; mortality decreased to 80 percent at 1,605 feet and to 30 percent at 4,438 feet.

In the Quebrada Pasto, the volume of flow was 0.051 c.f.s. The initial stretch of the stream below the point of chemical application was rather wide, shallow, and slow flowing, and the rate of flow was only 164 feet per hour. The shallowness and a slow flow of the stream meant that mud adsorption, organochemical reactions, and light inactivation factors could have exerted maximum detrimental effects upon the chemical. Vegetation was minimal throughout this reach of stream, the pH of the water was 7.8, and the concentration of dissolved oxygen was 2.5 ppm. Beyond station number 4, at 4,438 feet, successive ponding of the streamflow resulted in a high dilution of the pesticide. Obviously, no appreciable concentration of chemical moved this far downstream.

The second trial was made in the Rio Aibonito where a concentration of 20.0 ppm was applied for a 2-hour period (table 2). One hundred percent mortality of caged snails was obtained 461 feet downstream from the point of application. It decreased to 10 percent at 1,631 feet.

Guppies abounded in the water and considerable *Sphaerotilus* growth was observed. The guppies showed immediate distress upon addition of the chemical.

The test reach was downstream from the outfall of a sewage treatment plant and had a volume of flow of 0.34 c.f.s. and a pH of 7.4. The rate of flow through the first 461 feet was 170 feet per hour. Approximately 10 percent of the streamflow was through vegetation along the banks. Between the stations at 461 feet and 1,631 feet there were many deep pools (this reach of stream is in a rock outcrop formation) which afforded approximately a hundredfold dilution. Apparently the concentration applied was not adequate to cause high snail mortalities after its dilution within the pools.

In the third trial with WL 8008 (table 2) a concentration of 12.5 ppm WL 8008 was applied to the Quebrada Faria for 2 hours. A pretreatment survey showed a natural resident snail population of two *A. glabratus* per foot of stream length from the application point to 3,672 feet, but natural *Australorbis* were not present beyond 3,672 feet. Total natural snail kill occurred throughout the 3,672 feet inhabited by *A. glabratus*.

Beyond 3,672 feet live *Physa* were detected and beyond 5,813 feet live but no dead *A. gla-*

bratus were found. Caged snail kill at 3,672 feet was 93 percent, at 4,452 feet it decreased to 40 percent, and at 5,813 feet rose to 80 percent. The reason for the high mortality at the greater distance was not apparent.

In the Quebrada Faria the volume of flow was 0.44 c.f.s., the average velocity of flow was approximately 750 feet per hour, and the pH was 7.0. Guppies, tadpoles, and *Sphaerotilus* were abundant. The guppies and tadpoles were in immediate distress upon addition of the chemical. For the first 1,320 feet, approximately 50 percent of the streamflow was through malanga; beyond, approximately 25 percent of the streamflow was through mixed vegetation.

In the three stream trials all guppies, tadpoles, and crayfish were killed.

Discussion

Snail behavior during the laboratory tests may provide data that either enhance or decrease the potential of the compound when used under field conditions (table 2). Certain chemicals repel or irritate the snails causing them to crawl out of solution. Other chemicals stimulate another avoidance mechanism of the snail, body retraction, which also lowers snail mortality, particularly when the snail recovers after removal from the chemical treatment or the chemical dissipates. However, if the chemical of low toxicity to the snail causes it to extend its body from the shell, such a reaction may be desirable inasmuch as it may be possible to combine that chemical with a known toxicant, thereby reducing the concentration of toxicant required for effective kill.

Bleeding of the snail is considered a desirable reaction because it weakens the snail, possibly fatally, or it makes the specimen more susceptible to parasitic invasion. During the laboratory tests none of the snails laid eggs, either during exposure to the chemical (including tapwater control) or during the recovery period.

Organotin compounds tested showed good molluscicidal activity with kills of 90 percent or more in the laboratory tests at concentrations of 1 and 2 ppm. Frick and de Jimenez (*4*) reported similar findings for tri-n-propyltin oxide and tri-n-butyltin acetate, but they indicate triphenyltin acetate is far less effective.

However, field trials with triphenyltin acetate showed that it could produce high mortality of *A. glabratus* in both ponds and streams. Ponds required a concentration of 1.0 ppm; flowing streams needed at least 5.0 ppm which had to be applied for 2 hours to be effective downstream. At the concentration tested, triphenyltin acetate did not appear to be harmful to the vegetation, but it apparently was lethal to the macrofauna in the test ponds and streams.

Crossland and co-workers (*5*) found triphenyltin acetate effective against *Biomphalaria pfeifferi* and *Bulinus tropicus* in small canals when applied at 0.5 ppm for 8 hours. In these trials the compound was exceptionally effective as an ovicide. As triphenyltin acetate is highly toxic, it must be handled with extreme care. It should be applied only by field crews experienced in handling toxic materials.

Field trials with WL 8008 revealed it highly effective against naturally occurring *A. glabratus* in Puerto Rico. The data indicate that concentrations of 0.25 to 0.50 ppm active ingredient in impounded waters should cause 100 percent mortality. In flowing waters concentrations of 12.5 to 15.0 ppm for 2 hours would be necessary to obtain mortalities of 80 percent or more for approximately three-fourths of a mile in streams where large pools or masses of impeding vegetation do not occur.

The effective length of a treatment in a stream decreases considerably when pools are present. The fact that WL 8008 visibly sinks to the bottom must be considered in its application. Presumably the level of 12.5 ppm could be reduced if the exposure time was increased since in Africa dosages of WL 8008 applied continuously to maintain concentrations of 0.015 to 0.02 ppm in experimental canals have caused 100 percent kill of *B. pfeifferi* from 48 to 72 hours (*6*).

In the field trials both triphenyltin acetate and WL 8008 were highly toxic to adult *A. glabratus* and to aquatic life, such as guppies, tadpoles, and crayfish. The length of stream at which at least 80 percent mortality of snails resulted makes them biologically competitive with the chemicals presently in use. On the basis of these results further field testing of both compounds against *A. glabratus* is warranted. Each should also be tested against snail species useful in biological control, such as *Marisa cornuarietis*.

Summary

Of 15 toxicants tested in the laboratory against the snail, *Australorbis glabratus*, WL 8008, TD–282, TD–283, and five organotin compounds were considered as potentially useful molluscicides when each gave 100 percent mortality at concentrations of 10 ppm or less with a 6-hour exposure period. Compounds that did not meet this criterion were GC–405, GC–2131, AC–43913, 1,2,3,4, tetrachlorobenzene, HRS–1622, Mobam, and two carbaryl formulations.

Two compounds, WL 8008 and triphenyltin acetate, were tested under field conditions. Triphenyltin acetate produced high mortalities of *A. glabratus* in four ponds at a concentration of 1 or 2 ppm. In flowing streams concentrations of 5.0 ppm applied for 1 or 2 hours gave effective kills.

WL 8008 yielded mortalities above 90 percent in ponds treated at 0.25 ppm. In stream tests WL 8008 at 5.0 ppm for 3 hours gave 80 percent kills of caged snails up to 1,605 feet from the point of treatment.

At 20 ppm for 2 hours 100 percent kills were obtained for 461 feet from the point of application, but at 1,631 feet the mortalities dropped to 10 percent. Dilution of the toxicant level by pools in this stream presumably caused the lowered kill at 20 ppm since in another stream WL 8008 at 12.5 ppm for 2 hours produced mortality of 93 percent of caged snails and 100 percent mortality of naturally occurring snails, up to a distance of 3,672 feet from the treatment point. The triphenyltin acetate and WL 8008 applications in streams caused considerable kill of guppies, tadpoles, and crayfish.

REFERENCES

(*1*) Standen, O. D.: Some observations upon the maintenance of *Australorbis glabratus* in the laboratory. Ann Trop Med Parasit 45: 80–83 (1951).

(*2*) Finney, D. J.: Probit analysis. A statistical treatment of the sigmoid response curve. Ed. 2. Cambridge University Press, Cambridge, England, 1952.

(*3*) Shiff, C. J., and Ward, D.: Variation in susceptibility to three molluscicides shown by three species of aquatic snails. Bull WHO 34: 147–150 (1966).

(*4*) Frick, L. P., and de Jimenez, W. Q.: Molluscicidal qualities of three organo-tin compounds revealed by 6-hour and 24-hour exposures against representative stages and sizes of *Australorbis glabratus*. Bull WHO 31: 429–431 (1964).

(*5*) Crossland, N. O., et al.: A field trial to compare the molluscicidal properties of I.C.I. 24223 (isobutyltriphenylamine), Bayer 73 (5,2′-dichloro-4′-nitrosalicylicanilide), and triphenyltin acetate. Tropical Pesticides Research Institute, Arusha, Tanzania, Miscellaneous Report No. 334, 1962.

(*6*) Crossland, N. O., Beesley, J. S. S., and Mesmer, E. T.: Small-scale field trials to evaluate the effectiveness of low, continuous dosages of the molluscicide WL 8008. Tropical Pesticides Research Institute, Arusha, Tanzania, Miscellaneous Report No. 499, 1965.

(*7*) Jobin, W. R., and Unrau, G. O.: Chemical control of *Australorbis glabratus*. Public Health Rep 82: 63–71 (1967).

SUPPLY REFERENCES

(*A*) Allied Chemical Corp., Morristown, N.J.
(*B*) American Cyanamid Company, Princeton, N.J.
(*C*) Hooker Chemical Corp., Niagara Falls, N.Y.
(*D*) Mobil Chemical Company, Metuchen, N.J.
(*E*) Union Carbide Corp., New York City.
(*F*) Metal and Thermit Chemicals, Inc., Rahway, N.J.
(*G*) Pure Chemicals, Ltd., Liverpool, Eng.
(*H*) Shell International Chemical Co., Ltd., London, Eng.
(*I*) Pennsalt Chemicals Corp., Montgomery, Ala.