

## MOLLUSCICIDAL ACTIVITY OF ABBOTT'S COMPOUND MC-688 ON BIOMPHALARIA SNAILS

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### S U M M A R Y

A remarkably active molluscicide was found while screening about 2,500 compounds from Abbott Laboratories. Its chemical name is 2,5 Bis(1 aziridinyl)-p-benzoquinone (MC-688). MC-688 is a brick-colored powder of low solubility in water but soluble in Ritchie's solvent. The activity of MC-688 against *Biomphalaria glabrata* was assayed and compared to that of Pentachlorophenol (PCF). With adult snails the values for LC<sub>50</sub> and LC<sub>90</sub> were 0.13 and 0.21 ppm. for MC-688 and 0.21 and 0.46 ppm. for PCF. MC-688 is extremely active on young snails and especially on egg masses (one-day old embryos). For the latter, LC<sub>50</sub> and LC<sub>90</sub> were 0.0035 and 0.0060 ppm. for MC-688 and 0.17 and 0.30 for PCF. The molluscicidal activity of MC-688 in the presence of mud and CaCO<sub>3</sub> was not altered: a suspension of yeast produced a 45% decrease in activity. In the range of 0.1 to 0.2 ppm., MC-688 was lethal to *Lebistes reticulatus* (24-hour exposure). Preliminary field trials in breeding places of *B. glabrata* and *B. tenagophila* showed that MC-688 is very effective in stagnant waters.

### I N T R O D U C T I O N

The simple and sensitive method here-in described allowed the screening for molluscicidal activity of about 2,500 compounds of Abbott Laboratories. Although several compounds showed a variable degree of molluscicidal action on adult specimens of *Biomphalaria glabrata*, compound MC-688 was the most active. The activity of this compound, 2,5 Bis (1-aziridinyl)-p-benzoquinone (Fig. 1), was studied in the laboratory and in the field. The results obtained are presented.

### MATERIALS AND METHODS

#### Screening for molluscicidal activity

Snails used were 2 to 6-day-old and adult

(8-12 mm in shell diameter) *B. glabrata* from a colony maintained in the laboratory.

Drugs were received from Abbott's Laboratories in amounts of 100 mg.

A preliminary screening was conducted on 2 to 6-day old snails. For this purpose an amount of 20 mg was weighed in small plastic bags. A drop of Ritchie's solvent (ethyl alcohol, methyl alcohol, acetone, carbon tetrachloride, xylol; equal parts) was then added. By pressing and rubbing with the fingers on the place the drug was located almost all drugs dissolved. Water was added in order to have a final concentration of 10 ppm. Time of exposure to the drugs was 24 hours. Twenty snails were used for each compound. Active drugs (mortality > 80%)

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Contribution number 30 of the Schistosomiasis Research Unit, Federal University of Minas Gerais. This work was supported, in part, by the "Conselho Nacional de Pesquisas" (MINIPLAN) and Abbott Laboratories

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were retested on adult snails at the concentrations of 10 and 1 ppm. (groups of 10 snails for each dilution).

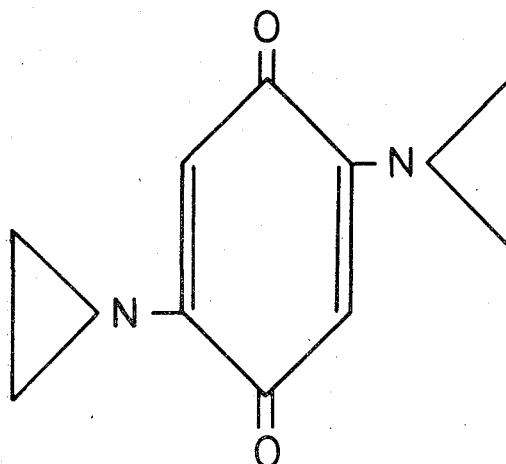


Fig. 1 — Chemical structure of MC-688.

#### Compound MC-688

When pure, MC-688 appears as a brick-colored powder, poorly soluble in water but dissolving in Ritchie's solvent.

MC-688 was used in laboratory studies as the pure chemical or in a formulation as wettable powder containing 25% of the active product. In field trials MC-688 was used in several formulations: a) wettable powder; b) wettable granular bait containing 25% of the active ingredients; c) emulsifiable concentrate containing 12.5% of Abbott MC-688. One liter of commercial alcohol was used to dissolve the emulsifiable concentrate.

#### Molluscicidal activity

The molluscicidal activity of MC-688 was assayed against *B. glabrata* (adult snails and egg masses containing 1-day old embryos). The time of exposure was 24 hours and the period of observation was 4 days for the adult snails and 8 days for the egg masses.

Determinations of the LC<sub>50</sub> and LC<sub>90</sub> were made by plotting the data on a log-prob paper (MILLER et al.<sup>2</sup>). Pentachlorophenol (PCF) was used for comparison.

The influence of mud, calcium carbonate and yeast suspension on the activity of MC-688 was determined by leaving 1 gr of each in 1,000 ml of water containing 1 ppm. of the molluscicide (SOUZA et al.<sup>4</sup>).

#### Piscicidal activity

The activity on *Lebistes reticulatus* (fishes of 19-38 mm) was used as a parameter. The time of exposure was 24 hours and the period of observations 3 days.

#### Field trials

A breeding site of *B. tenagophila* with an area of 480 m<sup>2</sup> and depth up to one meter was selected for treatment with MC-688 wettable granules. One gram of formulation per m<sup>2</sup> was applied with a Hatsuta portable mistblower equipped with a granule spreader and operated with the volume control lever in position 4. The molluscicide was applied both across the entire surface of the pond and along its marshy margins. The population of snails at time of treatment was 30 per m<sup>2</sup>. Readings were initially made after 24 and 48 hours and later made monthly.

A drip applicator was set up at a breeding site of *B. glabrata* having a very slow rate of flow and a large number of snails. A 30 ppm. concentration of technical grade MC-688 was maintained for 2 hours. Readings were made after 4 days and after 3 months.

Six sites in Itapemerim, State of Espírito Santo, with natural populations of *Biomphalaria tenagophila*, were selected for molluscicide testing because of their accessibility, small size, and non-use by the resident human population (Table I). Sites 3-6 are areas within the same waterbody which at time of treatment were physically separate due to a drought. One breeding place (6) was left as control and pond 5 was treated with Baylusicide.

A bait formulation containing 25% Abbott MC-688 was applied manually to sites 1,2, and 3 at dosages of 2,1 and 0.5 ppm., respectively. Concurrently, quantities of Abbott MC-688 12.5% emulsifiable concentrate (EC) and Baylusicide 70% wettable powder (WP) were formulated to yield end

TABLE I  
Site characteristics and application data

Site	Type	Volume (m <sup>3</sup> )	Snail population	Vegetation	Molluscicide dose
1	Cemented tank	12	31/m <sup>2</sup>	absent	MC-688, 25% bait, 2 ppm.
2	Abandoned well	21	2/m <sup>2</sup>	present	MC-688, 25% bait, 1 ppm.
3	Small pond	20.4	150/m <sup>2</sup>	absent	MC-688, 25% bait, 0.5 ppm.
4	Small pond	32	50/m <sup>2</sup>	abundant	MC-688, 12.5%, EC, 0.5 ppm.
5	Small pond	1000	50/m <sup>2</sup>	abundant	Bayluscide, 70%, WP, 0.5 ppm.
6	Small pond	5500	100/m <sup>2</sup>	abundant	none

concentrations of 0.5 ppm. in sites 4 and 5, respectively, when applied with a Hatsuta Blowmic AM-8 portable mistblower operated at full throttle with the volume control set at one half. Initial results were recorded after 24 hours. A test of the residual action was conducted by placing nylon sacks containing 10 snails into each of the sites 24, 48, 72, 96 and 120 hours after molluscicide application. After exposure for 24 hours, the snails were rinsed in tap water and placed in 5 liters of clean water for a 48-hour recovery period.

#### RESULTS

The activity of MC-688 and PCF on adult snails are shown in Fig. 2. As can be seen, the values for LC<sub>50</sub> and LC<sub>90</sub> were 0.13 and 0.21 ppm. for MC-688 and 0.21 and 0.46 ppm. for PCF, respectively. Thus, the activity was about double that of PCF. MC-688 was found to be extremely active on young snails and especially on egg masses (embryos one-day old). In the latter case (Fig. 3) the values of LC<sub>50</sub> and LC<sub>90</sub> were 0.0035 and 0.0060 ppm. for MC-688 and 0.17 and 0.30 ppm. for PCF. It can be concluded that on egg masses the activity of MC-688 is about 50 times that of PCF. The action on the embryos of *B. glabrata* is slow, begin-

ing 48 hours after exposure. The presence of malformations was a common finding.

The molluscicidal activity of MC-688 in the presence of mud and calcium carbonate was not altered. However, a suspension of yeast produced a 45% decrease in activity.

In the range of 0.1 to 0.2 ppm. MC-688 was lethal for *Lebistes reticulatus*.

In the field trial against *B. tenagophila*, the single application of 1 gram/m<sup>2</sup> achieved 100% control within 24 hours. Attempts to find live snails after 48 hours and in readings after 1, 2, 3, and 4 months have been unsuccessful. Fish mortality was 100% within 48 hours, but initial repopulation of some fish species was observed after 1 month.

In the trial against *B. glabrata*, only dead snails were found in the first mile of stream below the application site in the reading made 4 days after treatment. Three months later, the breeding site had repopulated to pretreatment levels.

In the work conducted at Itapemerim, all three concentrations of Abbott MC-688 25% bait produced 100% snail mortality within 24 hours. The 0.5 ppm. formulations of Abbott MC-688 25% bait, Abbott 12.5% EC and the Bayluscide 70% WP also achieved 100% reduction in the snail population within 24 hours. Both Abbott MC-688 and Bayluscide killed practically all fish at the concentrations used.

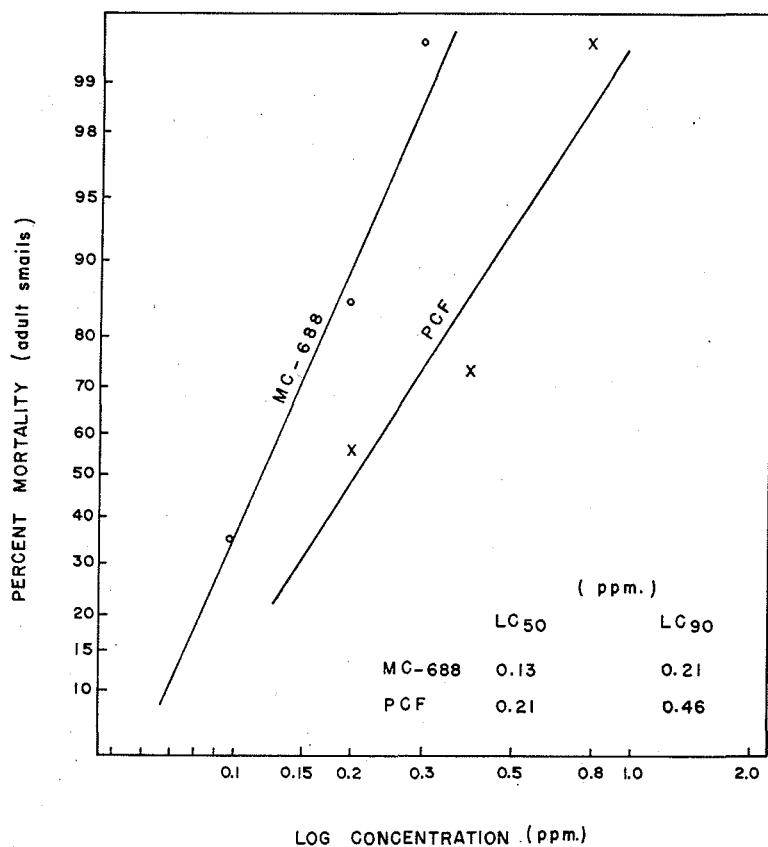


Fig. 2 — Molluscicidal activity of MC-688 on adult *Biomphalaria glabrata*. Note that the activity was about the double when compared with PCF.

The results of the test for residual action are presented in Table II. All products demonstrated effective residual activity 24 hours after application, although the bait formulation of Abbott MC-688 resulted in only 60% reduction. The residual activity of Abbott MC-688 extends to over 96 hours when applied at concentrations of 1 or 2 ppm. The EC formulation of Abbott MC-688 at 0.5 ppm. showed activity 48 hours whereas the Abbott MC-688 bait and the Bayluscide WP at that concentration failed to continue killing snails. Continued readings at sites 3-6 became impossible as rains flooded the pond and unified the sites. One month after initial molluscicide application a population of over 200 *B. tenagophila* per square meter was uniformly distributed throughout the sites 3, 4, 5 and 6.

TABLE II  
Test for residual action: mortality of reintroduced snails

Site	Hours after initial application of molluscicide				
	24	48	72	96	120
1	100%	100%	100%	100%	0%
2	100%	100%	100%	100%	0%
3	60%	0%	a	a	a
4	100%	90%	a	a	a
5	100%	0%	a	a	a
6	20%	0%	a	a	a

a = invalidated because of flooding

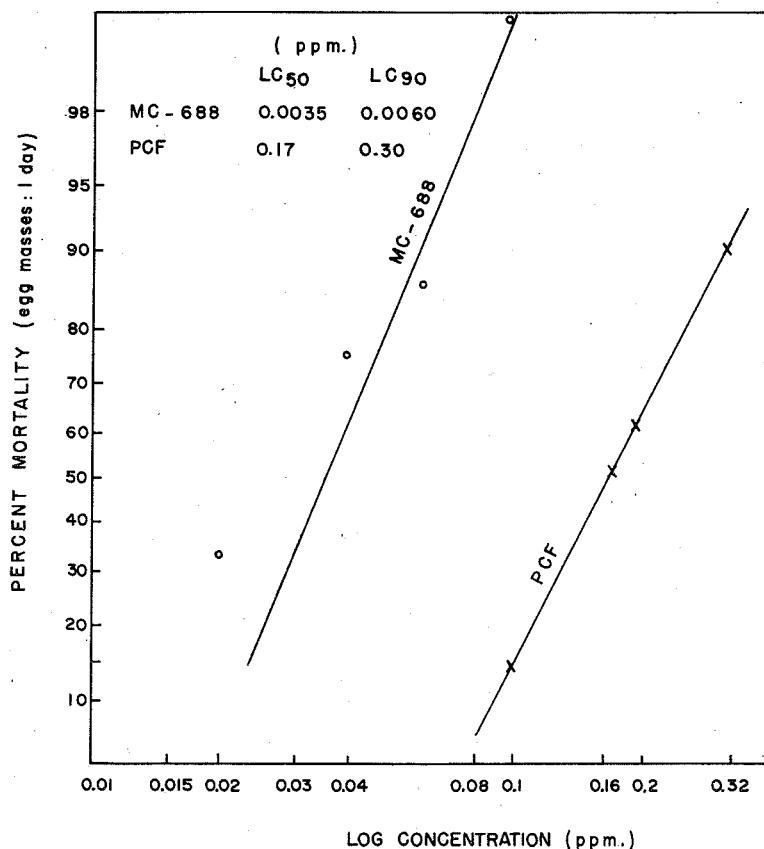


Fig. 3 — Molluscicidal activity of MC-688 on 1-day old embryos of *Biomphalaria glabrata*. Note that the activity was about 50 times when compared with PCF.

#### DISCUSSION

The preliminary molluscicide screening technique here described is similar to those of CROSSLAND<sup>1</sup> and RITCHIE<sup>3</sup> in concentrations used and criteria for further testing. The advantages of the new technique are: 1) the small amount of product necessary; 2) minimal handling of substance; 3) applicability to wide range of substances.

MC-688 is a potent molluscicide. In comparison with PCF, less product is necessary to achieve LC<sub>50</sub> or LC<sub>90</sub> for adult snails. The effectiveness of the compound against egg masses and young snails is remarkable.

Abbott MC-688 applied to yield an end concentration of 0.5 ppm., formulated either as a toxic bait or as an emulsion, provided effective snail control comparable to that of

Bayluscide when applied to small, stationary bodies of water and evaluated after 24 hours. When applied at 1 and 2 ppm., Abbott MC-688 kills reintroduced snails up to 96 hours after application.

MC-688 has two important limitations: a) is extremely allergenic to humans causing irritation upon even minimal exposure; b) it is too toxic to fish.

#### RESUMO

*Atividade moluscocida do composto MC-688 sobre a Biomphalaria glabrata e B. tenagophila*

Um moluscocida muito ativo foi encontrado na triagem de cerca de 2.500 compostos sintetizados nos laboratórios Abbott. A de-

nominação química do novo composto (MC-688) é 2,5 Bis(1-aziridinil)-p-benzoquino-na. O MC-688 apresenta-se como um pó de cor de tijolo, pouco solúvel em água, mas completamente solúvel em Ritchie.

O MC-688 foi testado contra a *B. glabrata*, tendo o pentaclorofenol (PCF) servido como ponto de referência. Para caramujos adultos, os valores de CL<sub>50</sub> e CL<sub>90</sub> foram de 0,13 e 0,21 ppm. para o MC-688 e de 0,21 e 0,46 ppm. para o PCF. O MC-688 mostrou-se extremamente ativo para caramujos jovens e para desovas (embriões com 1 dia de idade). Neste último caso, os valores de CL<sub>50</sub> e CL<sub>90</sub> foram de 0,0035 e 0,0060 ppm. para o MC-688 e de 0,17 e 0,30 ppm. para o PCF.

A atividade moluscocida do MC-688 em presença de barro e de CaCO<sub>3</sub> não se altera. Entretanto, em presença de uma suspensão de levedura sua atividade fica reduzida em 45%. Na concentração de 0,1 a 0,2 ppm. o MC-688 mostrou-se letal para o *Lebistes reticulatus*. Ensaios preliminares de campo com o MC-688, com várias formulações, foram altamente favoráveis em coleções de água parada, com alta densidade de *B. glabrata* e *B. tenagophila*.

#### ACKNOWLEDGEMENT

We are indebt to Drs. W. J. Close and A. O. Geiszler of Abbott Laboratories, No., Chicago, Ill., for providing us with enough technical grade MC-688 to conduct laboratory studies and various formulations of the molluscicide for field tests.

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Recebido para publicação em 5/4/1974.