Molluscicidal Qualities of Copper Protoxide (Cu_2O) as Revealed by Tests on Stages of *Australorbis glabratus* *

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Deschiens et al.[a] have reported that "Stabilized Chevreul Salt" (SCS, produced by Pastac & Pastac, France) is an effective molluscicide. The product, which contains 50% cuprous oxide as active ingredient, is prepared in a micronized form that disperses readily in water and settles out slowly. These investigators stated also that SCS is innocuous to fish and a variety of other animals and plants. Berrios-Duran et al.[b] also have shown its minimal toxicity for four species of fish.

The objectives of the current study were to determine the activity of SCS against different stages and sizes of Puerto Rican *Australorbis glabratus* and to investigate its residual qualities, presumably associated with its low solubility in water. It has been recommended [c, d] that the possible benefits of the residual potential of insoluble compounds should be studied, and Barbosa and his colleagues [e, f] have reported interesting results with copper carbonate in field trials.

Materials and methods

A sample of SCS was supplied through the World Health Organization by the investigators who introduced it.[a] None of the common organic solvents dissolved SCS adequately, but because of its dispersibility in water the "two-tube" stock dilutions [g, h] were prepared in dechlorinated tap water. All concentrations referred to hereinafter pertain to Cu_2O, active ingredient. The procedures and methods used were those described previously,[g] except that a 48-hour recovery period was used. However, in addition to 6-hour and 24-hour exposure tests on a stage-size array of *A. glabratus*, other tests were performed; the methods used in these are described below under "Results".

Results

24-hour exposure tests. Eggs that had been incubated 24-30 hours prior to exposure were essentially unaffected by concentrations of active ingredient up to 16 p.p.m. and 32 p.p.m., the mortalities not exceeding 10%. In contrast to this, the compound was quite effective against hatched snails. LC_{50} and LC_{90} values for newly hatched snails were respectively 0.4 p.p.m. and 0.9 p.p.m. (Table 1). Comparable values were obtained for juvenile snails (3-5 mm), the LC_{50} being 0.31 p.p.m. and the LC_{90} 0.74 p.p.m. On the other hand, decidedly higher concentrations were required for mature snails (13-15 mm); LC_{50} and LC_{90} values for this stage were 1.3 p.p.m. and 2.7 p.p.m., respectively, or about three times greater than for young snails.

TABLE 1

LC_{50} AND LC_{90} CONCENTRATIONS OF Cu_2O FOR THREE CLASSES OF *AUSTRALORBIS GLABRATUS* IN 24-HOUR EXPOSURES

Group	LC_{50} in p.p.m. (and 95% confidence limits)	LC_{90} in p.p.m. (and 95% confidence limits)
Eggs	Ineffective	Ineffective
Snails:		
Newly hatched	0.4 (0.339-0.472)	0.9 (0.69-1.26)
3-5 mm (juveniles)	0.31 (0.27-0.36)	0.74 (0.59-0.93)
13-15 mm (mature)	1.3 (1.06-1.6)	2.7 (2.0-3.6)

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[a] Deschiens, R., LeCorroler, Y., Pastac, I. & Pastac, S. (1961) *C.R. Acad. Sci. (Paris)*, **252**, 4221.

[b] Berrios-Duran, L. A., Ritchie, L. S., Frick, L. P. & Fox, I. *Comparative piscidal activity of Stabilized Chevreul Salt (SCS), a candidate molluscicide, and Bayluscide* (to be published).

[c] World Health Organization, Expert Committee on Bilharziasis (1953) *Wld Hlth Org. techn. Rep. Ser.*, **65**.

[d] World Health Organization, Study Group on the Ecology of Intermediate Snail Hosts of Bilharziasis (1957) *Wld Hlth Org. techn. Rep. Ser.*, **120**.

[e] Barbosa, F. S. (1961) *Bull. Wld Hlth Org.*, **25**, 710-711.

[f] Barbosa, F. S., Carneiro, J. C., Morães, J. G. & Carneiro, E. (1956) *Publ. avuls. Inst. Aggeu Magalhães*, **5**, 7.

[g] Ritchie, L. S., Berrios-Duran, L. A., Frick, L. P. & Fox, I. (1963) *Bull. Wld Hlth Org.*, **29**, 281-286.

[h] Fox, I. & Garcia-Moll, I. (1961) *Science*, **133**, 646.

TABLE 2

MOLLUSCICIDAL ACTIVITY OF Cu_2O AGAINST *AUSTRALORBIS GLABRATUS* IN 24-HOUR EXPOSURES WITH AND WITHOUT CHANGE OF TEST CONTAINER FOR RECOVERY PERIOD

Concentration of Cu_2O (p.p.m.)	Container not changed			Container changed for recovery period			
	Mortality (%) in group [a]			Mortality (%) in group [a]			
	3-5 mm (juveniles)	8-10 mm (adolescents)	13-15 mm (mature)	Newly hatched	3-5 mm (juveniles)	8-10 mm (adolescents)	13-15 mm (mature)
0.25		85		26	34	85	
0.5	64	100		51	77	98	7
0.75				83			
1.0	98	100	100	100	96	100	23
2.0	100	100	100		100	100	73
3.0	100	100	100		100	100	100
4.0			100				98
6.0+							100

[a] Three or more replicates with 10 snails each were performed for each concentration in most cases.

The approximate 100% mortality end-point for newly hatched, juvenile (3-5 mm), and adolescent snails (8-10 mm) was 1.0 p.p.m., whereas 3.0 p.p.m. were required for mature (13-15 mm) snails (Table 2).

In our standard procedure the test container is rinsed following exposure of snails so that it can also be used for the recovery period. The characteristic of Cu_2O to adhere to the surface of the container and then subsequently to be eluted in lethal concentrations during the recovery period was noted in tests with mature snails. Such specimens, when exposed to concentrations that did not kill within 24 hours, remained in a moribund state or died during the recovery period when the same container was used for exposure and recovery. If, however, they were transferred to new containers for the recovery period, survival markedly increased. The result of this was that without change of container for the recovery period, all mature snails died at concentrations of 1.0 p.p.m. or greater, whereas if the containers were changed, the 100% mortality end-point was increased to 3.0 p.p.m. (Table 2). Data for tests in which the container was changed are considered to be more reliable, and in addition permit comparison with molluscicides that do not exhibit the property of adsorption to surfaces. It is also deemed that merely not changing the container is not a suitable method for testing for residual properties.

The situation described above was not evident in tests with 3-5 mm and 8-10 mm snails. Presumably at low concentrations (of 1.0 p.p.m. or less) that were effective against snails of these sizes, the amount of chemical that adhered to the containers was insufficient to exert continued effects; alternatively, all or nearly all of the Cu_2O may have been in solution in dilutions of this order.

6-hour exposure tests. Reproducible data could not be obtained for either juvenile or mature snails in these tests when containers were changed for the recovery period. This was true even when concentrations were increased to disproportionately high levels as compared with the requirement for 24-hour exposures. The higher concentrations used were quite certainly greater than saturated solutions. However, results were reasonably good when containers were not changed for the recovery period; about 97% of 3-5 mm snails were killed with 2-4 p.p.m. Cu_2O, while all 13-15 mm specimens were killed with 10-16 p.p.m. (Table 3).

Relation between solution time and molluscicidal activity of Cu_2O. The inference drawn from results of 6-hour exposure tests was that the activity of Cu_2O might be largely a function of solution rate, i.e., within reasonable limits of original concentration a minimum time is required for lethal amounts of chemical to become dissolved. This requirement presumably was met in the 24-hour exposure but not in the 6-hour tests.

TABLE 3

MOLLUSCICIDAL ACTIVITY OF Cu_2O AGAINST *AUSTRALORBIS GLABRATUS* IN 6-HOUR EXPOSURES, WITH AND WITHOUT CHANGE OF TEST CONTAINER FOR RECOVERY PERIOD

Concentration of Cu_2O (p.p.m.)	Container not changed for recovery period	Container changed for recovery period
	Mortality (%)	Mortality (%)
3-5 mm snails (juveniles)		
1	6	0
2-4	97	0
8-16		19
20-32		26
13-15 mm snails (mature)		
2-8	55	
10-16	100	0
20-24		66
28		55
32		70

Two experiments were performed to clarify this point. In the first the supernatant fraction of a 3.0-p.p.m. suspension was carefully decanted after the suspension had stood for 6 hours, and its activity compared with that of the SCS sediment remaining in the container. Results from three tests showed that the supernate was capable of killing all 13-15 mm snails in a 24-hour exposure period. Furthermore, sufficient Cu_2O was eluted from the sediment when it was resuspended in a litre of water to kill at least 90% of such snails.

In the second experiment the dissolved and undissolved fractions of 3.0-p.p.m. suspensions were separated either by filtration or by centrifugation after the suspension had stood for prescribed periods of time. Clear filtrates and supernates obtained after as short a time as 15 minutes after preparation of the original suspensions regularly killed all mature snails in a 24-hour exposure period. Precipitates and sediments obtained 15 minutes and 6, 12, and 24 hours after preparation of the original suspensions and resuspended in a litre of water also contained sufficient Cu_2O to kill all mature snails in 24-hour exposures. Of more interest was the fact that, in one replicate, filter-papers and their adherent precipitates were dried after being tested. They were capable of killing all snails when tested a second time two weeks later. It is apparent from these tests that lethal concentrations of Cu_2O are reached very quickly in at least 3.0-p.p.m. suspensions, and that in a suspension of such strength there is an actual excess of active ingredient.

Tests on the residual molluscicidal activity of Cu_2O. A 0.8-ml quantity of a 1% Cu_2O suspension (tube 1 of our two-tube stock dilution series [g, h]) was pipetted on to two filter-paper circles (90 mm diameter). This quantity contained 8 mg Cu_2O, which afforded a concentration of 8 p.p.m. in one litre of water, as employed in our tests. The filter-papers were then air-dried free of contact with any object that would cause loss of chemical. After drying, the surfaces of application were brought together and the margins of the circles were bound with waterproof masking tape. A pair of circles was soaked for successive periods of 15 minutes, 1 hour, 6 hours, and 1, 2, 4, and 7 days in a corresponding series of litre quantities of dechlorinated tap water. After each transfer, the preceding quantity of water was tested against 13-15 mm snails with a 24-hour exposure. Four such tests were performed. Lethal concentrations were reached quickly since water in which the filter-papers had soaked for only 15 minutes killed all snails. One-hour and 6-hour soakings gave mean mortalities of 82.5% and 97.5%, respectively, but all subsequent intervals gave 100% mortality (Table 4).

Other pairs of filter-papers, similarly prepared, were soaked for 7 days in each of a series of litre quantities of water. Even in the 11th litre of the first test 90% of the snails were killed, but mortality was

TABLE 4

MORTALITY IN GROUPS OF 10 MATURE (13-15 mm) *AUSTRALORBIS GLABRATUS* AFTER 24-HOURS' EXPOSURE IN LITRE SAMPLES OF WATER IN WHICH Cu_2O-TREATED FILTER-PAPER HAD SOAKED IN SEQUENCE FOR PERIOD INDICATED

Period of soaking	Mortality (%) by replicate, and mean				
	1	2	3	4	Mean
15 min.	100	100	100	100	100
1 hr	90	100	80	60	83
6 hrs	90	100	100	100	97
24 hrs	100	100	100	100	100
2 days	100	100	100	100	100
4 days	100	100	100	100	100
7 days	100	100			100

TABLE 5

PERSISTENCE OF TOXIC LEVELS OF Cu_2O IN FILTER-PAPER THAT HAD BEEN IMPREGNATED, DRIED AND THEN TRANSFERRED AT WEEKLY INTERVALS TO FRESH QUANTITIES OF WATER

Test No.	Mortality (%) after indicated weekly changes of water: [a]							
	1	2	4	6	8	10	12	14
1	100	80	50	80	100	100	0	
2	50	100	100	100	100	90	70	90 [b]
3	50	100	100	90	100	80	70	90 [b]

[a] Percentages for odd-numbered weeks were comparable and therefore omitted.
[b] Test discontinued.

zero thereafter. In two subsequent replicates, sufficient chemical was eluted in the 14th litre, after three months of testing, to kill 90% of the snails (Table 5).

In another test, 8-mg quantities of Cu_2O in a small amount of water were dispersed over the inner surfaces of wax-coated, 1-litre, paper buckets and allowed to dry. The containers were filled and the water was changed repeatedly at varying intervals. Two buckets thus tested still contained enough Cu_2O to kill 80% of the snails in the 11th litre, with 14 days of dissolution, and completing 106 days of testing.

Discussion

Although Cu_2O was ineffective against egg clutches, its residual activity under field conditions might be lethal to newly hatched snails emerging from the clutches. The compound is active at relatively low concentrations against newly hatched snails, and equally so against 3-5 mm and 8-10 mm specimens. Certainly, destruction of egg clutches is desirable, but molluscicidal evaluations have not progressed to the point at which an active compound should be excluded from comprehensive evaluation and field trial because of ineffectiveness against eggs, particularly if it has good residual quality.

A possible explanation for the ineffectiveness of Cu_2O with 6-hour exposures was that the chemical might have a slow dissolution rate. However, this can be excluded, because maximum mortalities occurred even after the chemical had been in the water for only 15 minutes before removal of undissolved chemical by filtration and centrifugation. Another inference is that Cu_2O imposes its lethal effect slowly and that prolonged contact is necessary. This is consistent with the fact that the snails remain in a moribund state longer after a lethal exposure than with some other chemicals. As a result, 48-hour recovery periods were needed to include all mortalities resulting from Cu_2O, particularly at lower concentrations in the dilution series.

Although 3 p.p.m. of Cu_2O were necessary to kill all mature snails in a 24-hour exposure period, the undissolved residue remaining after test was still sufficient to kill all snails in two subsequent trials. Thus it is apparent that the activity of Cu_2O is greater than the dilution series indicated. When 8.0 mg of Cu_2O were carried through a series of litre quantities of water, there was considerable activity shown in the 14th change of water. It may be concluded, then, that this copper compound actually has a mortality end-point at approximately 0.5 p.p.m. Copper sulfate is also effective in our laboratory tests between 0.5 p.p.m. and 1.0 p.p.m. If the copper ion *per se*, rather than the Cu_2O molecule, is the active ingredient, then this compound may have the same disadvantage under field conditions as copper sulfate, for which the requirements are excessive in comparison with laboratory results. Whether low solubility and the related residual quality will make for better field performance by Cu_2O should be determined. Barbosa [e] has emphasized the importance of considering compounds of low solubility for molluscicidal purposes, and he used copper carbonate in a field trial with favourable results.

Cuprous oxide as contained in Stabilized Chevreul Salt is considered worthy of further evaluation as a molluscicide for the following reasons: (1) its basic toxicity is relatively favourable; (2) in conjunction with low solubility it is characterized by a striking residual activity in tap water under laboratory conditions; (3) it has a favourable threshold of safety for fish; [b] (4) it is relatively more toxic for newly hatched and young snails; and (5) the micronized formulation of SCS gives to it a water-dispersible quality.