would prevent his inhalation of insecticide so that his excretion would represent dermal absorption only.) In themselves, the results of this study have no clinical significance, because the highest rate of absorption involved (0.14 mg of *p*-nitrophenol per hour) would not be expected to produce any clinical effect or a marked inhibition of cholinesterase.[c] To put it another way, the highest measured dose was 2.35 mg of parathion, distributed over a period of 15.5 hours, whereas Edson[d] found that daily oral doses of 3 mg were tolerated by volunteers without cholinesterase depression or clinical effect. The results do offer additional evidence that men applying liquid sprays under the conditions in the Wenatchee, Wash., area are at risk of absorbing a relatively large amount of parathion through the skin. Therefore, in addition to taking precautions to avoid respiratory absorption of this pesticide, the sprayman should also give attention to covering his skin.

Conditions of exposure to pesticides vary greatly, and the relative importance of respiratory and dermal absorption may vary accordingly.[e] It is hoped that the Smyth technique will be further used, for quantitative data obtained in this way would certainly be useful in defining the variation and thus permitting selection of appropriate protective measures.

[c] Arterberry, J. D., Durham, W. F., Elliot, J. W. & Wolfe, H. R. (1962) *Arch. environ. Hlth*, **3**, 476.

[d] Edson, E. F. (1957) *The effects of prolonged administration of small daily doses of parathion in the rat, pig and man* (unpublished report from Fisons Pest Control Ltd.).

[e] Goldblatt, M. W. (1950) *Pharm. J.*, **164**, 229.

Molluscicidal Qualities of Bayluscide (Bayer 73) Revealed by 6-hour and 24-hour Exposures against Representative Stages and Sizes of *Australorbis glabratus* *, †

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As one phase of the molluscicidal evaluation programme of the World Health Organization, we have tested systematically a series of compounds against representative stages and sizes of *Australorbis glabratus*, using both 6-hour and 24-hour exposures. Appropriate dilution series were used that would permit computation of LC_{50} and LC_{90} values and arbitrary determination of 100% mortality endpoints.

The first of a series of molluscicides that was tested according to the above plan is Bayluscide (Bayer 73), which has already received favourable reports from certain other laboratory and field evaluations.[a, b, c, d, e, f, g] The current study has included a more complete stage-size array of the snail than has been used previously. Our intensive comparison of 6-hour and 24-hour exposures appears to have shown that Bayluscide and certain other compounds may be more efficiently utilized in 24-hour applications, while other molluscicides may be used efficiently in the shorter period. When exposure of mature snails to Bayluscide was reduced from 24 to 6 hours, considerably more Bayluscide

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† This note has been reviewed and approved for publication by the Office of the Surgeon General, US Department of the Army, but this review does not imply any endorsement of the opinions advanced or any recommendation of such products as may be named.

[a] Gönnert, R. (1961) *Bull. Wld Hlth Org.*, **25**, 483.

[b] Gillet, J. & Bruaux, P. (1961) *Bull. Wld Hlth Org.*, **25**, 509.

[c] Bruaux, P. & Gillet, J. (1961) *Bull. Wld Hlth Org.*, **25**, 519.

[d] Webbe, G. (1961) *Bull. Wld Hlth Org.*, **25**, 525.

[e] Schiff, C. J. (1961) *Bull. Wld. Hlth Org.*, **25**, 533.

[f] Paulini, E., Chaia, G. & Freitas, J. R. de (1961) *Bull. Wld Hlth Org.*, **25**, 706.

[g] Meyling, A. H., Schutte, C. H. J. & Pitchford, R. J. (1962) *Bull. Wld Hlth Org.*, **27**, 95.

was required than the commensurate fourfold increase. That a disproportionate chemical demand may occur when the exposure time is so reduced does not appear to have been recognized previously.

Materials and methods

The test snail. Only an inbred, laboratory-reared strain of Puerto Rican *Australorbis glabratus* designated " TRML-BLS " was used in this study.[h] All testing was done with the following stages and sizes: (*a*) eggs, with prior incubation periods of 1-6 hours, 24-30 hours, and 4-5 days; (*b*) newly hatched snails; (*c*) 3-5 mm juveniles; (*d*) 8-10 mm adolescents; (*e*) 13-15 mm mature snails; and (*f*) mature forms larger than 15 mm, when available.

The test container. A disposable, wax-lined paper container of 1 litre capacity (Lily " Nestrite " 32-SS-N, " Oaken Bucket " design) was used for tests on all stages and sizes of snail. Initially the buckets were discarded after each test, but this was costly and later a very inexpensive plastic bag was used as a liner, thereby permitting re-use of the buckets. Free portions of the bags extending above the bucket may be loosely closed near the water level, if snails tend to crawl out of the molluscicide. This may be done by making a radial slit in the circular paper lid with appropriate trimming along the slit to accommodate the plastic loosely. Control tests against the use and closure of the plastic bags are imperative for each molluscicide.

The dilution series. A dilution series suitable for determination of an LC_{50} and LC_{90}, and a 100% mortality end-point was established for each stage or size of snail for both 6-hour and 24-hour exposures. In establishing the range of the series, and in order to save snails, it was preferable to estimate and test initially a single concentration that might fall within the limits of the LC_{50} and LC_{90} for the stage or size of snail under test, and then extend the series progressively upwards and downwards from this point. The two-tube system of Fox & Garcia-Moll[i] was used in the preparation of the actual series. For Bayluscide this consisted in first preparing a 1% solution, taking into account the fact that the test product contained only 70% active ingredient, and from this a 0.01% or 100 p.p.m. solution. Any desired test concentration was prepared by transferring an appropriate amount of the latter solution to a container and making up to 1 litre with dechlorinated tap water. Complete dechlorination was mandatory for Bayluscide tests since the compound has been shown to be detoxified by residual chlorine (Fox et al.[j]). Generally a twofold dilution series was employed, but on a number of occasions intermediate concentrations were added in order to determine 100% mortality end-points more precisely.

Each concentration in every dilution series was tested a minimum of four times. When inconsistent results occurred in these tests additional replicates were run, up to a total of 8 to 10 in some instances, so as to ensure more reliable mean mortality rates.

Post-treatment recovery period and determination of mortality. After completion of an exposure period, the snails and their containers were thoroughly rinsed with 5 or 6 changes of tap water. After the last rinse 500 ml of dechlorinated water was added to the container and the snails were permitted a 24-hour recovery period, following which the mortality rates were determined.

Determination of the death of snails was based entirely on characteristic changes in the appearance of the shells. In dead snails an initial yellowish translucency of about half of the last whorl becomes progressively whiter and the snail is usually, but not always, withdrawn completely into the shell. Extensive contraction of the snail is not in itself a reliable criterion of death, although it may have some indicatory value immediately after termination of exposure.

Unique features of testing for each stage and size. Egg clutches were collected and tested according to the method of Olivier et al.[k] In the late afternoon plastic sheets were placed in tanks containing young mature snails. Such snails were preferred since the eggs are more easily counted in their medium-sized clutches of 25-40 eggs. Clutches harvested the following morning contained eggs with embryos of from 1 to 8 cells. It has been determined that embryos reach the 8-cell stage in about 6 hours in our air-conditioned laboratory (23-25°C). Clutches were cut out of the plastic sheets and some were tested immediately while others were incubated for

[h] The TRML Basic Laboratory Strain (BLS) of *A. glabratus* was established from a single specimen in 1956 by Captain M. G. Radke, Medical Service Corps, US Army, and had been propagated for more than 30 generations when this project was initiated.

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

[j] Fox, I., Alemán, G., Ritchie, L. S. & Frick, L. P. (1963) *Bull. Wld Hlth Org.*, **28**, 531.

[k] Olivier, L., Haskins, W. T. & Gurian, J. (1962) *Bull. Wld Hlth Org.*, **27**, 87.

TABLE 1

MOLLUSCICIDAL ACTIVITY OF BAYLUSCIDE (BAYER 73) AGAINST A STAGE-SIZE ARRAY OF PUERTO RICAN *AUSTRALORBIS GLABRATUS* WITH 24-HOUR EXPOSURES

Concentration (p.p.m.)	Percentage mortality among eggs			Percentage mortality among snails [a]			
	1-6 hours [b]	24-30 hours [b]	4 days [b]	Newly hatched	Juveniles 3-5 mm	Adolescents 8-10 mm	Mature 13-15 mm
0.025					(0.0)	(10.0)	
0.05	8.5	7.1	24.4	(0.0) 7.6	(13.0) 98.0	(66.7) 93.3	(0.0) 6.6
0.075				(1.3)	(36.7)	(96.7)	(6.7)
0.1	97.0	34.9	72.4	(1.4) 80.3	(100) 100	(100) 96.6	(96.7) 100
0.2	100	98.3	100	(100) 100	(100) 100	100	(100) 100

[a] The figures in parentheses show mortalities among snails after conditions of culture had been changed.
[b] Period of incubation prior to testing.

prescribed periods. Originally incubation was carried out in Petri dishes; currently, collections of 50 to 100 clutches are incubated in plastic trays, 12 inches × 10 inches × 5 inches, (approximately 30 cm × 25 cm × 12.5 cm), containing 2-3 inches (5-7 cm) of water and provided with constant aeration.

For testing a particular concentration of chemical, sufficient clutches to provide about 100 eggs of each stage were selected and all were exposed simultaneously in the same container. After exposure for 6 or 24 hours the clutches were rinsed thoroughly in tap water, re-identified as to stage and isolated in Petri dishes for a 48-hour recovery period, after which mortality rates were determined.

Newly hatched snails were tested within 24 hours after hatching. One preliminary test indicated that the response to molluscicides of snails between the ages of 1 and 3 days was the same, but we have preferred to stay within the 24-hour age limit. Samples of 20 such snails were exposed at a time. This size of snail is difficult to wash after treatment and to retrieve after the recovery period. The techniques must be learned and our technicians report that they are easier to accomplish if a litre rather than a smaller amount of water is used.

Juvenile (3-5 mm), adolescent (8-10 mm) and mature (13-15 mm) snails were tested in lots of 10 specimens each in litre quantities of solution.

Statistical evaluations. The effectiveness of the molluscicide has been expressed in terms of a 100% mortality end-point, and an LC_{50} and LC_{90}. The first of these was defined as the lowest whole or intermediate concentration within the limits of a twofold dilution series that regularly killed all snails or eggs in a group. This point was determined by inspection and is therefore not a true minimum lethal concentration. The method of Litchfield & Wilcoxon [1] was used for computation of LC_{50} and LC_{90} values, and their 95% confidence limits. If the factor for an LC_{50}, LC_{90} or slope function (S) could not be computed, the parameters themselves were considered to be not computable and were so reported.

Results

24-hour exposures. All stages and sizes of *A. glabratus* were killed by a 0.2 p.p.m. concentration of Bayluscide in 24-hour exposures (Table 1). Using the 100% mortality end-point as the criterion of effectiveness, no stage or size of snail was appreciably more resistant than another, although it is evident from Table 1 that mortality patterns varied markedly within dilution series.

There was also general uniformity among the LC_{50} and LC_{90} values in those instances in which

[1] Litchfield, J. T., jr & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99.

these values could be computed (Table 3). However, there were also a number of instances in which one or both or their confidence limits could not be computed. This was due largely to a trend in all dilution series towards an " all or none " effect by the chemical, with almost complete reactivity occurring between one concentration and the next higher one. Indeed, the general lack of mortalities in the 40-60% range seriously complicated analysis of the data by the Litchfield & Wilcoxon [1] method.

After all tests on Bayluscide had been completed, our snail laboratory was moved to a different building and it became possible to implement certain changes in cultural technique that seemingly have improved conditions for the growth of the snail. Four size-groups of the new lot of snails have been retested with Bayluscide; data from these tests are included in parentheses in Table 1. Younger stages of the snails now seem to be more resistant to Bayluscide. No obvious changes have occurred in 100% mortality end-points, but in 24-hour exposures lower concentrations that previously caused appreciable mortality now have less effect. In the juvenile group, for example, a concentration of 0.05 p.p.m. killed only 13% of the new lot, as compared with 98% of the earlier stock. The difference is even more marked in the newly hatched group: a 0.1 p.p.m. concentration that previously killed about 80% of this group and which still kills up to about 96% of mature snails, now kills only slightly more than 1% of the newly hatched forms.

6-hour exposures. With a 6-hour period of exposure the concentrations of Bayluscide required to yield

TABLE 2

MOLLUSCICIDAL ACTIVITY OF BAYLUSCIDE (BAYER 73) AGAINST A STAGE-SIZE ARRAY OF PUERTO RICAN *AUSTRALORBIS GLABRATUS* WITH 6-HOUR EXPOSURES

Concentration (p.p.m.)	Percentage mortality among eggs			Percentage mortality among snails				
	1-6 hours[a]	24-30 hours[a]	4 days[a]	Newly hatched	Juveniles 3-5 mm	Adolescents 8-10 mm	Mature 13-15 mm	Mature 18-20 mm
0.05	7.6	0.0			30.0			
0.1	62.7	11.9	12.1	60.6	65.0			
0.2	100	58.8	47.4	92.2	72.9			53.3
0.3	100	92.3	93.4	94.9	93.3	23.3		53.3
0.4		99.5	92.0	99.6	96.0	26.0	63.3	82.0
0.5		98.8	95.8		100	53.3		78.0
0.6		100	100			55.3	62.0	70.0
0.7						96.4		60.0
0.8						100	63.3	82.0
0.9							78.0	98.3
1.0							90.8	
1.1								
1.2							100	

[a] Period of incubation prior to testing.

100% mortalities ranged from 0.3 p.p.m. for newly laid eggs to 1.2 p.p.m. for 13-15 mm mature snails, instead of one concentration being uniformly effective against all stages as in the 24-hour exposures (Table 2). Each of the two main groups (eggs and snails) tended to respond independently, and within each group there was a progressive increase in chemical demand to exert complete kill that was roughly proportional to the stage of development or size. Newly laid eggs, for example, required only a half to a third as much Bayluscide for total kill as did 1- and 4-day-old eggs. The chemical was about equally effective against newly hatched snails and 3-5 mm juveniles, a concentration of 0.4 to 0.5 p.p.m. killing all such forms; while for 8-10 mm adolescents and 13-15 mm mature snails the concentration had to be increased to 0.8 and 1.2 p.p.m., respectively. A generally similar pattern was also evident in the LC_{50} and LC_{90} values (Table 3).

Discussion

The susceptibility of the various stages and sizes of *A. glabratus* to Bayluscide was strikingly similar when applications were for 24 hours. In contrast, the 6-hour exposures showed a marked gradient of declining susceptibility in correlation with increasing size or age of hatched snails. Newly laid eggs were more susceptible with both exposure times than eggs that had been incubated for 24-30 hours or 4 days, and the variations related to the age of eggs seemed to be independent of those related to the size or age of hatched snails.

Against mature (13-15 mm) and adolescent (8-10 mm) snails, Bayluscide appears to be more efficient when exposures are for 24 hours than when they are for 6 hours. On the basis of the LC_{90} values for mature snails for each of these exposure intervals, the time-concentration factors are 2.4

TABLE 3

TOXICITY OF BAYLUSCIDE (BAYER 73) TO A STAGE-SIZE ARRAY OF PUERTO RICAN *AUSTRALORBIS GLABRATUS* IN 6-HOUR AND 24-HOUR EXPOSURES

Stage or size of snail	LC_{50} in p.p.m. (95 % confidence limits)		LC_{90} in p.p.m. (95 % confidence limits)	
	6-hour exposure	24-hour exposure	6-hour exposure	24-hour exposure
Eggs:				
1-6 hours [a]	0.086 (0.08 to 0.09)	0.06 (0.02 to 0.2)	0.14 (0.13 to 0.15)	0.13 (Not computable)
24-30 hours [a]	0.18 (0.14 to 0.21)	0.09 (0.02 to 0.4)	0.29 (0.22 to 0.38)	0.17 (Not computable)
4 days [a]	0.2 (0.15 to 0.26)	0.06 (0.03 to 0.12)	0.4 (0.27 to 0.58)	0.16 (0.05 to 0.46)
Snails:				
Newly-hatched	0.15 (0.13 to 0.17)	0.08 (0.04 to 0.17)	0.23 (0.19 to 0.28)	0.17 (0.06 to 0.47)
Juveniles 3-5 mm	0.09 (0.05 to 0.14)	Not computable	0.25 (0.1 to 0.63)	Not computable
Adolescents 8-10 mm	0.45 (0.38 to 0.52)	0.04 (0.03 to 0.05)	0.66 (0.53 to 0.83)	0.07 (0.05 to 0.09)
Mature 13-15 mm	0.37 (0.27 to 0.5)	Not computable	1.1 (0.42 to 2.9)	Not computable

[a] Incubation period.

(24 hours × 0.1 p.p.m.[m]) and 6.6 (6 hours × 1.1 p.p.m.), respectively.[n] This disproportionately greater chemical demand for the shorter interval did not prevail for 3-5 mm and younger stages, including eggs; that is, the increased chemical demand did not exceed the expected fourfold increase commensurate with reduction in exposure time. The newly laid eggs were unique in that they were killed in 6 hours by the same molluscicidal concentration required for 24-hour exposures. Whether this relationship between exposure time and molluscicidal efficiency will be apparent in field evaluations remains to be seen. Thus far field tests have not been set up in a way that would reveal such a molluscicidal characteristic.

[m] An approximate value, as the LC_{90} for mature snails was not computable; actually the LC_{90} was less than 0.1 p.p.m.

[n] These results, which were obtained during the period August-December 1961, were confirmed in March-April 1963 by means of parallel 6-hour and 24-hour tests on the same stock of *Australorbis*. In the recent tests LC_{90} computations were possible, yielding values of 0.7 and 0.073 p.p.m. for 6-hour and 24-hour exposures, respectively. Although the current stock of snails appears to be more susceptible to Bayluscide, the ratio of chemical demand is consistent with the original results.

Egg Clutches as against Individual Eggs of *Australorbis glabratus* as Test Units in Molluscicide Evaluation *

by LYMAN P. FRICK *and* WILMA Q. DE JIMENEZ, *US Army Tropical Research Medical Laboratory, Fort Brooke, San Juan, Puerto Rico*

Australorbis glabratus eggs that have been incubated for four days are used as the representative egg group in molluscicidal evaluation at this laboratory. In tests as usually performed, individual eggs are the test unit since mortality rates are based on the proportion of eggs that are killed at each concentration of molluscicide. Thus, in effect, individual eggs can be compared with individual snails in a complete evaluation.

The examination of each egg in a number of clutches to determine deaths or survivals and the recording of these data are tedious and time-consuming operations. Therefore we have been prompted to modify the test procedure so that an entire clutch is considered as an individual and thus becomes the test unit. With this change it is necessary to stipulate that a dead clutch is one in which all eggs have been killed; conversely, if at least one living egg is found in a clutch, that clutch is recorded as a survivor. This in itself greatly simplifies the assembly of results.

The modified procedure has been tested with tri-n-propyl tin oxide [a] and tri-n-butyl tin acetate [a] according to general, standardized methods developed in this laboratory.[b] Parallel tests were performed; in one series groups of 10 egg clutches were exposed to serial dilutions of chemical, while in the other single clutches were similarly exposed and handled in the usual manner as controls. Both 6- and 24-hour exposure periods were used, and all tests were replicated three or four times. LC_{50} and LC_{90} values were computed by the method of Litchfield & Wilcoxon.[c]

Results of these tests, which are summarized in the table, show that LC_{50} and LC_{90} values for clutches as test units were almost invariably higher than the corresponding figures for individual eggs as test units. Differences between respective LC_{50} or LC_{90} values for clutches and for eggs were tested statistically by the method given by Litchfield & Wilcoxon.[c] The concentration of chemical required to yield either an LC_{50} or LC_{90} for clutches was significantly greater ($P = 0.05$) than that for eggs in the case of propyl tin in the 24-hour exposure and butyl tin in the 6-hour exposure. Only in the latter instance, however, was the difference numeri-

* This investigation was supported in part by a contract between the World Health Organization and the University of Puerto Rico.

[a] These chemicals were furnished by the courtesy of Pure Chemicals Ltd., England.

[b] See the note by L. S. Ritchie, L. A. Berrios-Duran, L. P. Frick & I. Fox on page 281 of this issue.

[c] Litchfield, J. T. & Wilcoxon, F. (1949) *J. Pharmacol. exp. Ther.*, **96**, 99.