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THE MOLLUSCICIDAL PROPERTIES OF SOME BAYLUSCIDE HOMOLOGUES

H. A. SHOEB and M. A. EL-EMAM
Laboratory of Bilharziasis, National Research Centre, Dokki, Cairo

Abstract. The molluscicidal properties of 5-nitro- and 3-nitro2′,4′-dichloro salicylanilides and their mixture 5.5 : 1 were investigated against the intermediate hosts of schistosomes *Bulinus truncatus* and *Biomphalaria alexandrina*. Unlike Bayluscide, the activity of the new molluscicides is not affected by strong sun radiation or by acidic pH. Their activity is resistant to storage, river-bed mud and lower temperature.

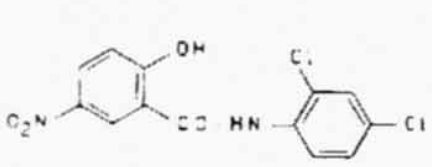
The control of schistosomiasis via eradication of snails encouraged the search for effective molluscicides (Shoeb 1975). Investigations in this field yielded Bayluscide (Farbenfabriken Bayer 1959) as the present molluscicide of choice. The compound is effective at 1 ppm (Jeney and Zsolnai 1967, Duhm et al. 1961) and inhibits snail oxygen uptake at 0.2 ppm (Gonnert and Schraufstaetter 1959). As Bayluscide has the disadvantage of being affected by strong solar radiation (Pawar and Thirumalchar 1965), by acidic pH (Fox et al. 1963) and is toxic to fishes (Kosianowski 1964) further work in this area of research is still in need. The present investigation comprises studies on the molluscicidal properties of some Bayluscide homologues, namely: I — 5-Nitro - 2′, 4′ - dichloro salicylanilide, II — 3-Nitro-2′, 4′-dichloro salicylanilide, III—A mixture of I and II (5.5 : 1) as obtained from the initial mixture.

MATERIAL AND METHODS

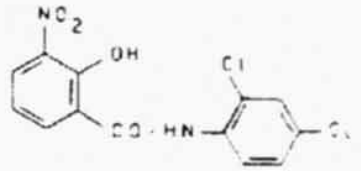
Two common snail intermediate hosts of schistosomes in Egypt, *Bulinus truncatus* and *Biomphalaria alexandrina*, were used in the present study. The snails were collected from irrigation canals located in Giza Governorate that were not treated with molluscicides. The snails were allowed to adapt to laboratory conditions for a period of at least three months before being used in the toxicity tests.

The 5-nitro- "I" and the 3-nitro- "II" salicylanilides were synthesized from the corresponding nitrosalicylic acids. Compound III which is a mixture of I and II, 5.5 : 1 was synthetically obtained from a mixture of the parent nitroacids as obtained by nitration of salicylic acid.

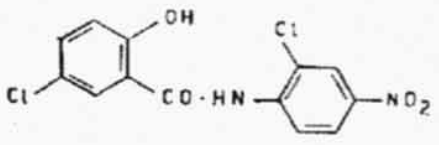
Stock solutions of 100 ppm were freshly prepared on the basis of weight/volume in distilled water. Series of concentrations that permit the computation of LC_{50} values were prepared. Standard test



I



II



Bayluscide

procedures (WHO 1953, 1965) were followed throughout this study. The effect of the three molluscicides compared with Bayluscide has been expressed in terms of LC_{50} and LC_{90} (Litchfield and Wilcoxon 1949).

Table 1. Comparative susceptibility of *B. alexandrina* and *B. truncatus* snails to molluscidides. I, II, III and Bayluscide

Molluscicide	Exposure periods hrs.	*B. alexandrina*		*B. truncatus*	
		LC_{50}ppm	LC_{90}ppm	LC_{90}ppm	LC_{90}ppm
Compound I	24	0.34 (0.28—0.40)	0.52	0.27 (0.21—0.34)	0.44
Compound II	24	0.64 (0.55—0.74)	0.88	0.31 (0.24—0.39)	0.53
	48	0.32 (0.26—0.39)	0.47	0.165 (0.13—0.21)	0.265
Compound III	24	0.44 (0.33—0.58)	0.85	0.32 (0.244—0.419)	0.58
Bayluscide	24	0.088 (0.068—0.108)	0.32	0.087 (0.067—0.107)	0.255
	48	0.255 (0.21—0.303)	0.31	0.145 (0.115—0.183)	0.21

*) 95% confidence limits in parentheses.

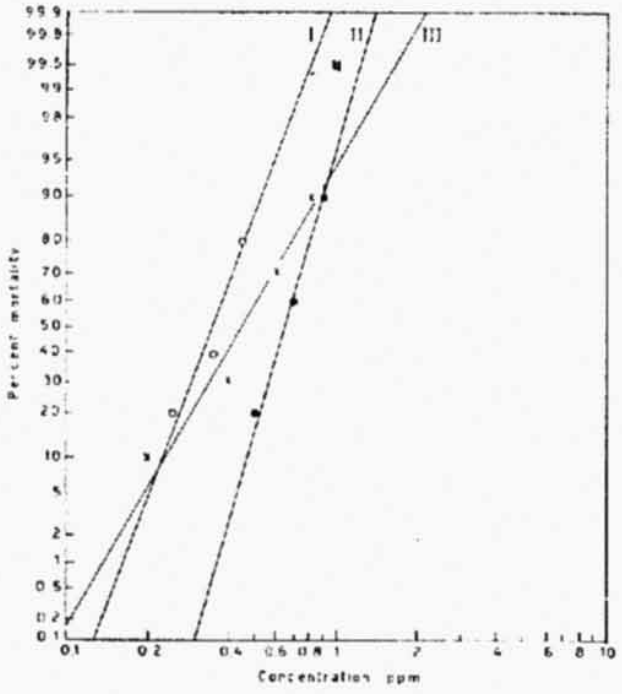


Fig. 1. Mortality dosage for adult *B. alexandrina* exposed to compounds I, II and III.

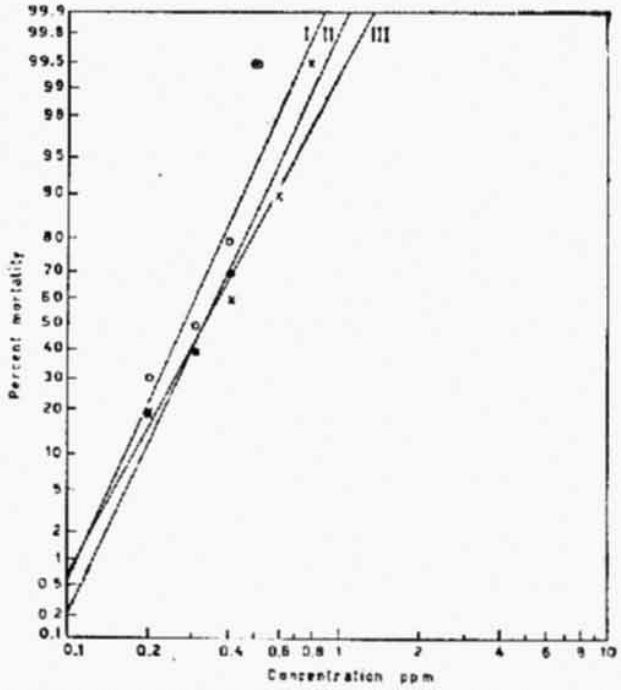


Fig. 2. Mortality dosage for adult *B. truncatus* exposed to compounds I, II and III.

RESULTS

COMPARATIVE SUSCEPTIBILITY OF *B. ALEXANDRINA* AND *B. TRUNCATUS* TO THE MOLLUSCICIDAL ACTION OF COMPOUNDS I—III

Table 1 shows that the difference in susceptibility of both snail species to compound I (Figs. 1, 2) and Bayluscide (Fig. 3) is only slight. However, for compounds II and III (Figs. 1, 2) this difference is more detectable. Whereas the LC_{90} of compound II

against *B. alexandrina* dropped from 0.88 ppm after 24 hrs to 0.47 ppm after 48 hrs (Fig. 4), for Bayluscide the corresponding LC_{90} was not changed by increasing the exposure time. Similarly, against *B. truncatus* the LC_{90} of compound II dropped from 0.53 ppm after 24 hrs to 0.26 ppm 48 hrs but for Bayluscide the corresponding LC_{90} 0.255 after 24 hrs was not changed by increasing the exposure time to 48 hrs (Fig.4).

Table 2. Time-concentration relationships of the molluscicidal activity of compounds I, II and III

Concentration ppm	Exposure periods hrs.	Percentage mortality of					
		B. alexandrina			*B. truncatus*		
		I	II	III	I	II	III
3.0	3	100	20	100	90	60	100
2.5	3	100	10	100	90	60	100
2.0	3	100	0	60	80	40	100
2.0	6	100	60	100	100	60	100
1.5	6	100	—	100	80	60	80
1.0	6	100	—	60	40	—	50
0.5	6	20	0	0	20	40	20
1.5	9	—	100	—	—	—	—
1.0	9	—	40	—	—	80	—
0.5	9	100	—	—	100	—	80
0.25	9	0	0	0	20	20	0
0.5	24	—	20	100	—	100	—
0.25	24	0	0	0	70	60	20
0.125	24	0	0	0	0	0	0
0.25	48	0	100	40	—	—	—
0.125	48	0	10	0	40	100	70
0.05	48	0	0	0	0	0	0
control	48	0	0	0	0	0	0

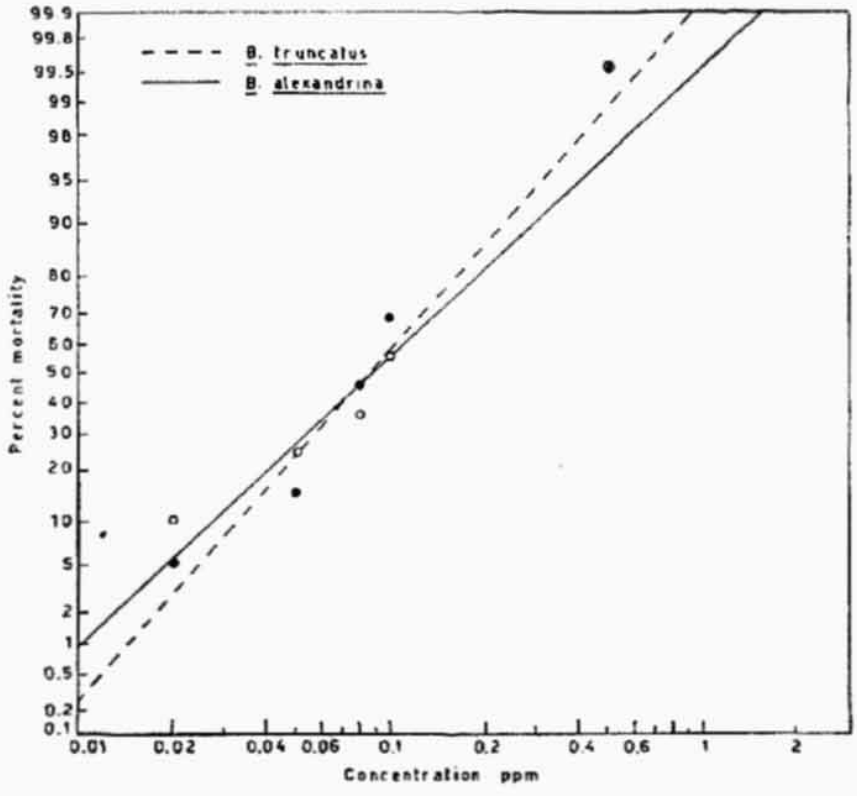


Fig. 3. Mortality dosage for adult snails exposed to Bayluscide.

TIME CONCENTRATION RELATIONSHIP OF THE MOLLUSCICIDAL ACTIVITY OF COMPOUNDS I—III.

In these experiments batches of snails were exposed for 3, 6, 9, 24 and 48 hours to various concentrations of the three molluscicides. Results given in Table 2 reveal that there is an inverse relationship between concentrations and exposure period which is clearly distinguished with compound II. At low concentrations (0.25—0.125 ppm) the difference in susceptibility of both snail species to the three compounds becomes more distinguishable by increasing the exposure time from 9—24—48 hrs.

EFFECT OF pH ON THE MOLLUSCICIDAL POTENCY OF COMPOUNDS I—III

For studying the effect of pH values on the molluscicidal potency of compounds I—III, different concentrations were prepared using standard reference water previously adjusted at pH values of 4.5, 6, 9 and 10. Tests involving 24 hours exposure and 24 hours recovery periods were conducted and the mortality of snails was deter-

Table 3. Effect of pH on the molluscicidal potency of compounds I, II, III

Concentration ppm	Percentage mortality of							
	B. alexandrina exposed for 24 hours to following pH values				*B. truncatus* exposed for 24 hours to following pH values			
	4.5	6	9	10	4.5	6	9	10
								Compound I
0.125	40	0	0	0	100	0	0	0
0.25	100	100	0	0	100	80	0	0
0.5	100	100	60	40	100	100	20	80
1.0	100	100	80	80	100	100	80	80
1.5	100	100	100	80	100	100	100	80
control	0	0	0	0	0	0	0	0
								Compound II
0.125	0	0	0	0	80	0	0	0
0.25	90	0	0	0	100	40	40	0
0.5	100	80	20	0	100	100	80	40
1.0	100	100	80	0	100	100	100	80
1.5	100	100	100	80	100	100	100	80
control	0	0	0	0	0	0	0	0
								Compound III
0.125	0	0	0	0	40	0	0	0
0.25	40	60	0	0	60	80	20	40
0.5	80	80	60	0	80	60	60	30
1.0	100	100	60	20	100	100	80	40
1.5	100	100	100	100	100	100	100	100
control	0	0	0	0	0	0	0	0

*) Recovery period of 24 hours.

mined in each test (Gonnert and Strufe 1962). Results given in Table 3 indicate that the susceptibility of *B. truncatus* to the three compounds increases by increasing the acidity of the molluscicide solution. The activity of the three compounds increased progressively by increasing the acidity to pH 4.5, but decreased by increasing the alkalinity to pH 10.

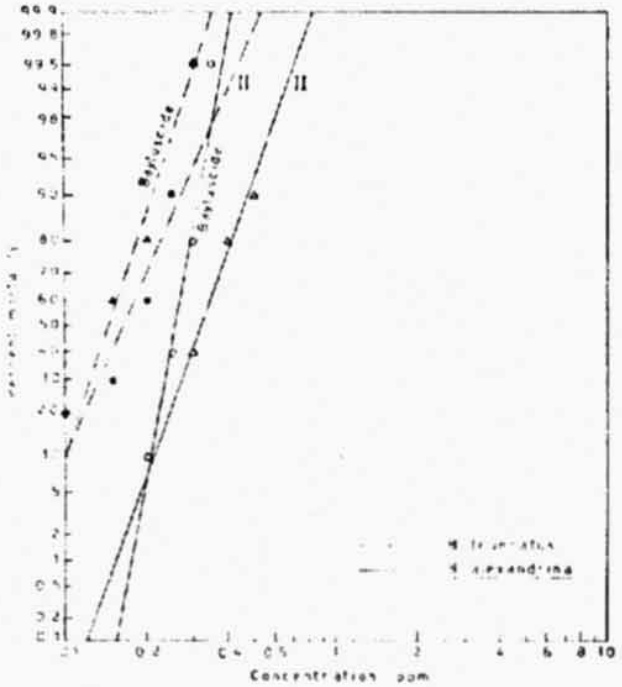


Fig. 4. Mortality dosage for adult snails exposed to Bayluscide and compound II after 48 hours

EFFECT OF SUNLIGHT ON THE MOLLUSCICIDAL ACTIVITY OF COMPOUDNS I–III

A stock solution of 10 ppm was made up in distilled water and exposed to direct sunlight for 6 hours, then different dilutions were prepared. Tests involving 24 hrs exposure and 24 hrs recovery periods were carried out and the mortality percentage of snails was determined. Results given in Table 4 show that the activity of the three molluscicides is quite resistant to the effect of sun radiation, specially at concentrations above 1 ppm.

Table 4. Effects of sunlight*) on the molluscicidal activity of compounds I, II and III

Concentration ppm	Percentage mortality**) of					
	B. alexandrina			*B. truncatus*		
	I	II	III	I	II	III
0.125	0	0	0	0	0	0
0.25	0	0	0	60	40	10
0.5	100	40	100	90	70	60
1.0	100	80	100	100	90	80
1.5	100	100	100	100	80	100
2.0	100	100	100	100	100	100
Control***)	100 (0.5 ppm)	100 (1 ppm)	100 (1 ppm)	100 (0.5 ppm)	100 (0.5 ppm)	100 (0.8 ppm)

*) Stock solutions were exposed to direct sunlight for 6 hours
**) Exposure periods were 24 hours and recovery periods were 24 hours
***) No exposure to direct sunlight and values in parentheses indicate the lowest concentration of molluscicides to produce 100 % mortality.

EFFECT OF RIVER-BED MUD ON THE MOLLUSCICIDAL ACTIVITY OF COMPOUNDS I–III

Under natural conditions flowing water has sufficient turbulance to cause rapid mixing of particles in the water. In an attempt to stimulate this condition in the laboratory, solutions of compounds I—III were prepared with water containing different concentrations of river-bed mud. A number of *B. alexandrina* and *B. trun-*

Table 5. Comparative effects of different concentrations of river-bed mud on the molluscicidal potencies of compounds I, II and III on *B. alexandrina* and *B. truncatus* for 6 hours exposure and 24 hours recovery periods

Concentration ppm	Percentage mortality of *B. alexandrina* exposed to compounds I, II and III with following concentrations of river-bed mud ppm								
	I			III			II		
	5,000	10,000	0 control	5,000	10,000	0 control	5,000	10,000	0 control
0.5	80	60	100	20	40	80	0	0	40
1.0	100	100	100	80	80	100	60	20	100
1.5	100	100	100	100	100	100	40	60	100
2.0	100	100	100	100	100	100	40	80	100
2.5	100	100	100	100	100	100	80	80	100
3.0	100	100	100	100	100	100	100	100	100
B. truncatus									
0.5	100	100	100	100	100	100	100	100	100
1.0	100	100	100	100	100	100	100	100	100

Table 6. Storage stability on the molluscicidae potency of compounds I, II, III

Concentration	Percentage mortality of					
	B. alexandrina			*B. truncatus*		
	I	II	III	I	II	III
A — Refrigerator						
0.125	0	0	0	0	0	0
0.25	0	0	0	0	40	40
0.5	80	50	60	80	70	60
1.0	100	100	100	90	80	90
1.5	100	100	100	100	100	100
2.0	100	100	100	100	100	100
control*)	100 (0.5 ppm)	100 (1 ppm)	100 (1 ppm)	100 (0.5 ppm)	100 (0.5 ppm)	100 (0.8 ppm)
B — Room temperature						
0.125	0	0	0	0	0	0
0.25	20	0	30	30	40	20
0.5	100	30	70	100	100	60
1.0	100	100	100	100	100	100
1.5	100	100	100	100	100	1000
control*)	100 (0.5 ppm)	100 (1 ppm)	100 (1 ppm)	100 (0.5 ppm)	100 (0.5 ppm)	100 (0.8 ppm)

*) Values in parentheses indicate the lowest concentration of molluscicides to produce 100 % mortality.

catus snails were placed in beakers containing different concentrations of river bed mud and compounds I—III, and the beakers were continuously shaken on an electric shaker for 6 hrs. Results given in Table 5 indicate that whereas compound I is stable

at the different river-bed mud concentrations, compounds II and III are less stable, specially at the low concentrations.

EFFECT OF STORAGE ON THE MOLLUSCICIDAL ACTIVITY OF COMPOUNDS I—III

Different dilutions of compounds I—III were either stored at room temperature 27—28° C or kept in the refrigerator at 10 °C for seven days. The activity of the stored solutions was tested on the used snails through 24 hrs exposure and 24 hrs recovery periods. Results given in Table 6 indicate that the molluscicidal activity of the three compounds is not affected by storage at concentrations above 1 ppm.

EFFECT OF 8° C ON THE MOLLUSCICIDAL ACTIVITY OF COMPOUNDS I–III

The tested snails were exposed to different dilutions of compounds I—III at 8 °C for 24 hrs exposure followed by a similar recovery period. Results given in Table 7 indicate that the activity of the three compounds is not affected by low temperature (8 °C) at concentrations starting from 1 ppm upwards. Lower concentrations are slightly affected.

Table 7. Effect of 8 °C on the molluscicidal activity of compounds I, II, III

Concentration ppm	Percentage mortality of					
	B. alexandrina			*B. truncatus*		
	I	II	III	I	II	III
0.125	0	0	0	0	0	0
0.25	0	0	0	20	40	20
0.5	80	20	20	20	60	20
1.0	100	10	80	100	100	80
1.5	100	40	100	100	100	80
2.0	100	80	100	100	100	100
2.5	100	100	100	100	100	100
control	0	0	0	0	0	0

DISCUSSION

In continuation to a research programme aiming at the finding of efficient molluscicides, the three molluscicides under investigation have been selected from a large number of newly synthesized nitrosalicylanilides that were tested for molluscicidal activity.

Concerning compound I, a 100 % mortality of both snail species was attained within 9 hrs at 0.5 ppm and could not be observed by increasing the exposure time at lower concentrations. This behaviour indicates that: a — Compound I is a quick acting molluscicide, b — Its maximum activity is mainly dependent on its concentration, c — More than 9 hrs, the time factor is of no value for the activity of compound I.

On the other hand, the characteristic features concerned with compound II are: a — It is slow acting molluscicide, i. e. its action reaches a peak after 48 hrs exposure period at a minimum dose of 0.25 ppm for *B. alexandrina* and 0.125 ppm for *B. truncatus*. This fact indicates that its activity is mainly dependent on time factor. b — It showed distinct specificity to *B. truncatus*.

Compound III which is a mixture of I and II and which is obtained as it is for economic purposes, has characteristic features in common and its maximum activity to a large extent depends on time factor.

Both nitrosalicylanilides I and II are comparable to Bayluscide in activity and have the advantages of being resistant to acidic pH and sun radiation and may be of greater economic value.

МОЛЛЮСКОЦИДНЫЕ СВОЙСТВА НЕКОТОРЫХ ГОМОЛОГОВ ПРЕПАРАТА BAYLUSCIDE

Г. А. Шоеб и М. А. Эл-Емам

Резюме. Моллюскоцидные свойства некоторых гомологов препарата Bayluscide изучались в воздействии на промежуточных хозяев шистосом *Bulinus truncatus* и *Biomphalaria alexandrina*. В отличие от препарата Bayluscide, на действие этих моллюскоцидов не влияет солнечное сияние или кислая среда pH. Действие их устойчиво к хранению, илистой почве речного русла и низкой температуре.

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H. A. S., Laboratory of Bilharziasis,
National Research Centre Dokki,
Cairo, A. R. E.