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Results of Laboratory and Field Trials with the Molluscicide Bayer 73 *

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This paper summarizes the results of laboratory and field trials carried out in a number of countries with the molluscicide Bayer 73. The minimum lethal concentrations for snails transmitting Schistosoma *and* Fasciola *(and for the snail ova) were found in different laboratories to be between 0.2 p.p.m. and 0.5 p.p.m., a close measure of agreement. Data are also presented on the influence of exposure time, temperature, water composition and pH, light and mud on the efficacy of this molluscicide.*

Laboratory tests have shown miracidia and cercariae to be highly sensitive to Bayer 73. Its toxicity for fish is very similar to that for snails; warm-blooded animals, however, appear to be highly resistant to this compound, as are plants.

Field trials have furnished information on application methods, optimum concentrations and a variety of factors bearing on the efficacy of Bayer 73 in practice. They show that a concentration of 1 p.p.m. of this compound suffices to destroy snails in both standing and flowing water. In different experiments, such water remained free of snails for periods varying from several weeks to several months.

Comparative trials with other molluscicides are also reviewed. These indicate that against snails and their eggs Bayer 73 is some 10 times superior to sodium pentachlorophenate.

Among the more than 20 000 chemical compounds tested for molluscicidal efficacy by the present author during the course of the past decade, a compound synthesized by Schraufstätter proved to be of special interest. On the occasion of the Sixth International Congresses on Tropical Medicine and Malaria Gönnert & Schraufstätter (1959) reported on this substance, which was then called Bayer 73. Since then several other investigators have had an opportunity of acquiring experience with Bayer 73 in laboratory and field trials. The new molluscicide has proved to be highly effective, and a low phytotoxicity and other advantages have been confirmed. Although the biological activity of any molluscicide is influenced by many factors, the results obtained with Bayer 73 under varying conditions are in excellent agreement. As a molluscicide, Bayer 73 has proved superior to copper sulfate, sodium pentachlorophenate, dinitro-*o*-cyclohexylphenol (DCHP) and Rhodiacid.

This paper summarizes our own results obtained with Bayer 73 in comparison with the findings of other investigators. In preparing this paper I have considered and quoted not only reports already published on this subject but also a number of personal communications and several papers which are being published simultaneously with this one on other pages of this issue of the *Bulletin of the World Health Organization.*[2] However, it was impossible to get data or even preliminary results from all those who had asked for and received Bayer 73 for trial purposes. As only a comparison with other molluscicides can give a clear picture of the value of a new one, data obtained from comparative trials with other molluscicides are also presented.

* Revised version of a paper submitted to the WHO Expert Committee on Bilharziasis, September 1960.

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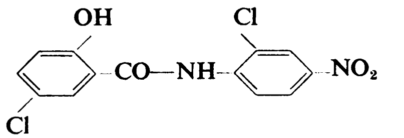
[2] To avoid overburdening the text with footnotes and parenthetical intercalations, personal communications and cross-references to articles appearing simultaneously are indicated only the first time each correspondent's or author's name is mentioned.

PROPERTIES, APPLICATION AND ESTIMATION OF BAYER 73

Chemical data

Chemically Bayer 73 is the ethanolamine salt of the active ingredient 5,2′-dichloro-4′-nitro-salicylic-anilide (formerly called 2-hydroxy-5,2′-dichloro-4′-nitro-benzanilide), with the formula: $C_{13}H_8O_4N_2Cl_2$=



The active component is a yellowish-grey powder, odourless and almost tasteless, with a molecular weight of 327.1 and a melting point of 222°-224°C. The technical product has a purity of 93%-99%.

The ethanolamine salt made from the active ingredient is a powder of an intense yellow colour. It contains 16% ethanolamine, 2% water and has, as a technical product, a purity of 77%-84%, referring to the active compound.

The solubility of the active compound in water is low and depends on the mineral content of the water. The ethanolamine salt has a higher degree of solubility (Table 1).

TABLE 1
SOLUBILITY OF BAYER 73

Water	Temperature (°C)	Active component (mg/l)	Ethanolamine (mg/l)
Distilled water	20	5-8	230 ± 52
Tap water [a]	20	2-6	145 ± 33
Tap water [a]	50	24	372 ± 86

[a] The tap water had an average salt content of approximately 500 mg/l, i.e., corresponding to 15° German hardness or 18.8° British hardness.

Both the active ingredient and the ethanolamine salt dissolve better in organic solvents. In dimethyl-sulfoxide, for instance, up to 35% of the ethanolamine salt may be dissolved at 20°C. Other polar organic solvents too, such as dimethylformamide or the lower ketones and alcohols, are also suited as solvents for the active ingredient and its ethanol-amine salt, especially when small quantities of alkali are added.

Methods of application

As the solubility of the ethanolamine salt turned out to be insufficient for application under field conditions, we began looking for a formulation more suitable for this purpose. First, a 10% wettable powder was prepared and used in the original field trials carried out in Germany by W. Neuhaus [1] and H. G. Keyl.[1] However, the need was already felt for a wettable powder of a higher percentage. The first batch of 70% wettable powder met the standards for laboratory trials of the time, but, when stored over periods, it tended to decompose, discharging ammonium hydroxide, and to form lumps. It was therefore withdrawn immediately. Some early field trials carried out with this batch in Africa by Halawani [2] and by Pitchford [3] yielded unsatisfactory results, as was to be expected.

Efforts to find a formulation which was satisfactory in every way were terminated in 1958 with the production of a 70% wettable powder of Bayer 73 (= 4780) which complied with all WHO requirements for DDT water-dispersible powder (World Health Organization, 1956). Field trials and some laboratory trials have been carried out with this formulation, while the active ingredient and the ethanolamine salt have been used for other laboratory tests.

Early investigators expressed their desire to receive Bayer 73 as briquettes, as granules, in liquid form or as a floating powder. However, to avoid additional complications and in order to simplify the evaluation of field trials, great stress was laid on the fact that the wettable powder only should be employed. Moreover, efforts to develop other formulations had as yet not been completely successful.

Methods of estimation

To calculate the volume of natural waters is often difficult and the results may be incorrect. It is therefore also very difficult to determine the exact dose for a molluscicide. There is an urgent need for a method of estimation which will make it possible to determine even small concentrations of the molluscicide under field conditions and with a small margin of error. Strufe reports in a separate paper on two methods of estimation under laboratory

[1] Personal communication, 1956.

[2] Personal communication, 1958.

[3] Personal communication, 1959.

and field conditions.[1] Both methods allow concentrations as low as 0.2 p.p.m. to be determined.

Relation between constitution and efficacy

After the first salicylic anilide effective against snails had been found, numerous compounds of this or similar groups were synthesized and tested for their biological activity. The molluscicidal efficacy depends on the chemical constitution, as has been reported elsewhere by Schraufstätter, Meiser & Gönnert (1961). Their research allows of the conclusion that maximum efficacy requires, apart from the free hydroxy group, two or three chlorine atoms and a nitro group within the salicylic anilide molecule. The carbamide group may be substituted by a thioamide group without causing essential changes in the efficacy.

LABORATORY TRIALS

Laboratory trials were carried out by a number of investigators and Bayer 73 was tested against different types of water snails and snail ova, and against the miracidia and cercariae of *Schistosoma mansoni*, against freshwater fish and against other water animals and plants. Experience has shown that the efficacy of molluscicides does not depend alone on the concentration of the active ingredient and the time of exposure, but also on other factors, and the laboratory tests therefore included studies of the effect of temperature, of the absorption of the effective ingredient through water plants and mud and of the salt content of the water (Gönnert & Strufe, 1961). The stability of the molluscicide under the influence of light was also tested, as was its toxicity for warm-blooded animals and plants.

Trial methods

The method of procedure used by different investigators was essentially the same in all tests. A quantity of effective material (active ingredient, ethanolamine salt or the wettable powder 4780) weighed on an analytical balance is dissolved in a test-tube or finely suspended by means of Tween 80. Other solvents such as alcohol, acetone, glycolmonomethylether or dimethyl sulfoxide may also be used. Next, the dilution is continued in two or three steps until the desired concentration has been reached. The test concentration is generally obtained by adding tap water from the place where the snails came from (Gillet & Bruaux [2]) or distilled water (Foster, Teesdale & Poulton, 1960; Webbe [3]).

In no test was the water volume per snail too small, as we were later able to calculate through findings which Duhm and his collaborators (1961) obtained with the help of ^{14}C-labelled ethanolamine salt. This applies even to the tests of Gillet & Bruaux, who used only 10 ml of water containing active agent for each snail. Most workers, however, when they gave an indication of the amount of water, were found to prefer a larger quantity. Thus the present author uses at least 20 ml per snail, and Shiff [4] uses as much as 100 ml.

The number of snails used for each test also differs—from 10 (Azevedo & Pequito, 1961) to 150 (Paulini, Chaia & de Freitas [5]). Except for screening tests, the present author generally uses 25 or 30 snails for each dose. Some authors, in order to confirm results, conduct parallel trials. Paulini, Chaia & de Freitas, for instance, use 5 groups of 10 snails each. Foster, Teesdale & Poulton (1960) even kept each snail in a separate glass container. Where no parallel tests have been reported it may be assumed that the results were confirmed by repeated tests.

The time of exposure, too, unfortunately, has not been chosen uniformly, with the exception of those cases in which the shortest lethal time of exposure had to be determined in relation to the concentration of the active agent. Shiff prefers an exposure time of 5 hours, W. M. Alves [6] and G. O. Unrau & F. F. Ferguson [7] a time of 6 hours, L. J. Olivier,[8] Webbe and the present author a time of 24 hours. At the end of the exposure the snails are washed and put into clean water to be observed for a further 24-72 hours. All authors seem to have changed the water once more if the time of recovery was more than 24 hours. During this period Shiff, Webbe and Gillet & Bruaux feed the snails with fresh or boiled leaves of lettuce. However, the author's own experience suggests that it is not necessary to feed the snails during this recovery period. Since snails kept in clean water for purposes of control throughout the entire trial never show any damage

[1] See the article on page 503 of this issue.

[2] See the article on page 509 of this issue.

[3] See the article on page 525 of this issue.

[4] See the article on page 533 of this issue.

[5] See the note on page 706 of this issue.

[6] Personal communications, 1958, 1959 and 1960.

[7] Personal communication, 1960.

[8] Personal communication, 1959.

and retain their full activity, all snails which at the end of the recovery period remain completely within their shells and show no signs of life are considered dead. Breaking the shells for purposes of control (which is very important to ensure that *Oncomelania* are dead) was apparently not done during the tests in question. The present author, working with *Australorbis glabratus*, observed as a typical sign of death the effusion of a red liquid (blood ?) under the influence of Bayer 73 and other compounds.

Snails tested

Up to now Bayer 73 has mainly been tested against intermediate hosts of *S. mansoni* and *S. haematobium*, as well as *Fasciola*. For other snails, including *Oncomelania*, only a few results have been obtained for general information purposes. Table 2 shows the types of snails tested and the investigators, and Tables 3-7 summarize the results.

Efficacy of Bayer 73 against intermediate hosts of Schistosoma mansoni

By means of numerous tests with *Australorbis glabratus*, the present author found the minimum lethal concentration (LC_{100}) to be 0.3 p.p.m. ($10^{-6.5}$). Evaluating these tests, which were carried out over a period of some years, a survival rate of less than 1% (24 hours of exposure; temperature, 22°-26°C) could be calculated for this concentration in tap water. Under these test conditions the greater number of the snails tested died during the first six hours. There was no difference in results whether Bayer 73 was tested as active ingredient, as ethanolamine salt or as wettable powder 4780, nor did the efficacy seem to depend in any way upon the diluent used.

The results obtained by other authors are largely similar. Olivier stated that all snails were killed after 24 hours' exposure to 0.5 p.p.m. of Bayer 73, but that they survived at 0.1 p.p.m. De Freitas & Paulini and Paulini, Chaia & de Freitas found a death-rate of 100% with 24 hours' exposure at 0.4 p.p.m. and of 93% with 48 hours at 0.2 p.p.m. For shorter exposure times (8 hours), however, higher concentrations were required. De Azevedo & Pequito (1961) obtained very good results: namely, 100% mortality after 24 hours of exposure at 0.2 p.p.m. and 93% after 18 hours at 0.4 p.p.m. Unrau & Ferguson made only one test (with 6 hours' exposure) and obtained 100% mortality at 1 p.p.m.

TABLE 2

SNAILS TESTED FOR SUSCEPTIBILITY TO BAYER 73

Snail	Investigator
Taphius (Australorbis) glabratus	Buttner;[a] de Azevedo & Pequito (1961); Gönnert; Paulini, Chaia & de Freitas;[b] Olivier;[a] Unrau & Ferguson [a]
Biomphalaria pfeifferi nairobiensis	Foster, Teesdale & Poulton (1960)
	Webbe [c]
B. pfeifferi bridouxiana	Webbe [c]
B. sudanica sudanica	Blair & Alves;[a] Gillet & Bruaux;[d]
Biomphalaria sp.	Bruaux & Gillet [e]
Bulinus (Bulinus) contortus	Buttner [a]
B. (B.) coulboisi	Webbe [c]
B. (B.) forskalii	Webbe [c]
B. (B.) tropicus	Foster, Teesdale & Poulton (1960); Shiff [f]
Bulinus (B.) sp.	Blair & Alves [a]
Bulinus (Physopsis) globosus	Shiff [f]
Bulinus (P.) nasutus	Webbe [c]
Bulinus (P.) sp.	Blair & Alves;[a] Gillet & Bruaux [d]
Lymnaea natalensis	Foster, Teesdale & Poulton (1960); Shiff;[f] Webbe [c]
L. palustris	Enigk; [a] Gönnert
L. stagnalis	Buttner [a]
L. ovata	Gönnert; Webbe [a]
Lymnaea sp.	Blair & Alves;[a] Gillet & Bruaux [d]
Galba truncatula	Behrenz;[a] Enigk;[a] Gönnert
Oncomelania hupensis	Gönnert
O. quadrasi	Alves [a]
Bithynia tentaculata	Buttner [a]
Physa sp.	Foster, Teesdale & Poulton (1960)
Planorbis complanatus	Buttner [a]
P. corneus	Buttner [a]
P. vortex	Buttner [a]
Planorbis sp.	Enigk [a]

[a] Personal communication.
[b] See the note on page 706 of this issue.
[c] See the article on page 525 of this issue.
[d] See the article on page 509 of this issue.
[e] See the article on page 519 of this issue.
[f] See the article on page 533 of this issue.

TABLE 3
EFFICACY OF BAYER 73 AGAINST INTERMEDIATE HOSTS OF *SCHISTOSOMA MANSONI*

Snail	Concentration (p.p.m.)	Mortality (%)	Time (hours)		Water used	Remarks	Investigator
			Exposure	Recovery			
Australorbis glabratus	0.4	100	24	—	—	0.2 p.p.m.; 93 %; 48 hours	Paulini et al.[a]
„	0.2	100	24	—	—	—	de Azevedo & Pequito (1961)
„	0.3	100	24	24	Tap water	—	Gönnert
„	0.5	100	24	—	—	Survival at 0.1 p.p.m.	Olivier[b]
„	1.0	100	6	—	—	0.5 p.p.m.; 80 %; 6 hours	Unrau & Ferguson[b]
Biomphalaria pfeifferi bridouxiana	0.2	100	24	48	Lake Victoria	Lake Victoria	Webbe[c]
Biomphalaria pfeifferi nairobiensis	1.0	100	12 24	24	Distilled water	0.5 p.p.m.; 60 %; 24 hours	Foster et al. (1960)
Biomphalaria pfeifferi subsp.	0.25	100	24?	—	—	Orientating trial	Blair & Alves[b]
„	1.0	100	24	24	Lake Kivu	—	Gillet & Bruaux[d]
„	0.25	100	24	24	Pond	—	Gillet & Bruaux[d]
„	0.25	100	5	70	Tap water	—	Shiff[e]
Biomphalaria sudanica sudanica	0.2	100	24	48	Lake Victoria	—	Webbe[c]

[a] See the note on page 706 of this issue.
[b] Personal communication.
[c] See the article on page 525 of this issue.
[d] See the article on page 509 of this issue.
[e] See the article on page 533 of this issue.

and 80% at 0.5 p.p.m. Buttner[1] stated that snails which appeared to be dead after 3 hours' exposure at 0.3 p.p.m. recovered after being brought into clean water again. The present author's experience and that of Paulini, Chaia & de Freitas indicate that this time of exposure is too short.

In order to obtain the same effects as with Bayer 73 at 0.3 p.p.m. and under identical conditions the present author found that at least 3 p.p.m. of pentachlorophenol, sodium pentachlorophenate, copper sulfate or DCHP were required. Olivier as well as Paulini, Chaia & de Freitas also came to the conclusion that Bayer 73 is 10 times more effective against *Australorbis glabratus* than NaPCP. Comparative trials with Rhodiacid, carried out by Paulini, Chaia & de Freitas, in concentrations of 5-20 p.p.m. did not reliably kill all snails, whereas Bayer 73 at 0.3 p.p.m. did.

Trials discussed in the foregoing paragraphs are summarized in Table 3.

[1] Personal communication, 1959.

In addition to the South American intermediate host of *S. mansoni*, African intermediate snails of this parasite were also tested with Bayer 73. Good results were obtained with *Biomphalaria* spp. of Equatorial Africa and Southern Rhodesia by Webbe (*B. pfeifferi bridouxiana* and *B. sudanica sudanica*), Blair & Alves,[2] Gillet & Bruaux, Bruaux & Gillet[3] as well as Shiff (*B. pfeifferi* subsp.). These authors were able to kill 100% of snails at 0.2-0.25 p.p.m. with an exposure of 24 hours or five hours (Shiff). Gillet & Bruaux used water from Lake Kivu as well as water from a fish pond. To reach the minimal lethal concentration of the active agent they had to increase the concentration from 0.2 p.p.m. for fish-pond water to 1 p.p.m. for Lake Kivu water. These results prove the influence of minerals dissolved in the water upon the efficacy of the molluscicide. The findings of Foster, Teesdale & Poulton (1960) are to be understood in the same way. To kill *B. pfeifferi*

[2] Personal communications (1956, 1957) from D. M. Blair.
[3] See the article on page 519 of this issue.

nairobiensis under laboratory conditions, using distilled water, a concentration of 1 p.p.m. was necessary. Under field conditions—i.e., in less favourable circumstances—better results were obtained with lower concentrations.

Comparative trials with sodium pentachlorophenate (NaPCP) and Bayer 73 were performed by Webbe and by Bruaux & Gillet. To get the same results, Webbe needed 3 p.p.m. NaPCP for *B. pfeifferi bridouxiana* and 5 p.p.m. NaPCP for *B. sudanica* as compared with 0.2 p.p.m. Bayer 73 for both snails. Bruaux & Gillet calculated a ratio of 1: 10 for one hour of exposure in water taken from Lake Kivu, and of as much as 1: 40 for water taken from the fish pond, when comparing the LD_{50} of Bayer 73 and NaPCP in tests made with *Biomphalaria* sp.

Efficacy of Bayer 73 against intermediate hosts of Schistosoma haematobium

Bayer 73 was also tested against intermediate hosts of *S. haematobium* (Table 4). Webbe was able to kill *Bulinus coulboisi* and *B. forskalii* with 0.2 p.p.m., Blair & Alves *Bulinus* sp. and *Physopsis* sp. with 0.25 p.p.m., Webbe *B. (P.) nasutus* and Gillet & Bruaux an undetermined species of *Physopsis* with 0.3 p.p.m. within a 24-hour exposure time. According to Shiff, only five hours of exposure are necessary to kill *B. tropicus* in 0.5 p.p.m.; under the same conditions, 5% of the snails tested would survive if they were *B. (P.) globosus* according to Shiff's data. Buttner tested *B. contortus:* at a concentration of 1.3 p.p.m.—the only one tested—all snails died after an exposure of 5-6 hours. In comparative tests with NaPCP, Bayer 73 proved its superiority against intermediate hosts of *S. haematobium* also. To kill *B. coulboisi* and *B. forskalii* Webbe needed 0.2 p.p.m. Bayer 73 and 0.3 p.p.m. for *B. (P.) nasutus* as against 3 p.p.m. of NaPCP.

Efficacy of Bayer 73 against intermediate hosts of Schistosoma japonicum

There are but few results regarding the efficacy of Bayer 73 against *Oncomelania* (Table 5). Using his own screening method, the present author tested *Oncomelania hupensis* (10 snails per concentration) and found that a concentration of 0.5 p.p.m. sufficed to kill all snails within 24 hours. He observed typical differences in the reaction of *O. hupensis* and *A. glabratus* towards Bayer 73: whereas *A. glabratus*

TABLE 4

EFFICACY OF BAYER 73 AGAINST INTERMEDIATE HOSTS OF *SCHISTOSOMA HAEMATOBIUM*

Snail	Concentration (p.p.m.)	Mortality (%)	Time (hours)		Water used	Remarks	Investigator
			Exposure	Recovery			
Bulinus (Bulinus) contortus	1.3	100	5-6	24	Aerated	Orientating trial	Buttner [a]
Bulinus (B.) coulboisi	0.2	100	24	48	Lake Victoria	—	Webbe [b]
Bulinus (B.) forskalii	0.2	100	24	48	Lake Victoria	—	Webbe [b]
Bulinus (B.) tropicus	1.0	81	24	24	Distilled water	—	Foster et al. (1960)
Bulinus (B.) tropicus	0.5	100	5	24	Tap water	—	Shiff [c]
Bulinus (B.) sp.	0.25	100	24?	—	—	Orientating trial	Blair & Alves [a]
Bulinus (Physopsis) globosus	0.5	95	5	24 70	Tap water	—	Shiff [c]
Bulinus (P.) nasutus	0.3	100	24	48	Lake Victoria	—	Webbe [b]
Bulinus (P.) sp.	0.25	100	24	—	—	Orientating trial	Blair & Alves [a]
Bulinus (P.) sp.	0.3	100	24	24	Pond	—	Gillet & Bruaux

[a] Personal communication.
[b] See the article on page 525 of this issue.
[c] See the article on page 533 of this issue.
[d] See the article on page 509 of this issue.

TABLE 5

EFFICACY OF BAYER 73 AGAINST INTERMEDIATE HOSTS OF *SCHISTOSOMA JAPONICUM*

Snail	Concentration (p.p.m.)	Mortality (%)	Time (hours)		Water used	Remarks	Investigator
			Exposure	Recovery			
Oncomelania quadrasi	1.4	97	6	42	—	Snails submersed?	Alves [a]
Oncomelania hupensis	0.5	100	24	24	Tap water	Orientating trials, snails submersed	Gönnert

[a] Personal communication.

retires into its shell, *O. hupensis* creeps far out of it, exposing itself still more to the influence of the molluscicide. There are also two personal communications from Alves concerning tests with *O. quadrasi.* In the first one (1959) he mentioned that Bayer 73 in the lowest concentration tested (1 p.p.m.) kills *O. hupensis.* The second communication refers to comparative tests between Bayer 73 and NaPCP. These tests demonstrated that Bayer 73 will kill 97% of the snails in a concentration of 1.4 p.p.m., and in higher concentrations up to 100%, provided the snails are exposed to it for six hours. With NaPCP the mortality rate remained at about 85% in the range 1.75-2.82 p.p.m. More tests will be necessary to prove the effectiveness of Bayer 73 in combating amphibious snails.

Efficacy of Bayer 73 against intermediate hosts of Fasciola

Numerous tests have been made with snails transmitting *Fasciola* (Table 6). According to Webbe the lethal dose of Bayer 73 after an exposure of 24 hours is 0.2 p.p.m. for *Lymnaea natalensis,* as against 3 p.p.m. with NaPCP. Shiff, who exposed snails for only five hours, determined the lethal dose at 0.5 p.p.m. When testing Bayer 73 in distilled water (Foster et al., 1960) a higher minimum lethal concentration (1 p.p.m.) was obtained. Blair & Alves and Gillet & Bruaux also tested several *Lymnaea* species; they too, like Webbe, needed a concentration of only 0.25 p.p.m. to kill the snails after an exposure of 24 hours.

TABLE 6

EFFICACY OF BAYER 73 AGAINST INTERMEDIATE HOSTS OF *FASCIOLA*

Snail	Concentration (p.p.m.)	Mortality (%)	Time (hours)		Water used	Remarks	Investigator
			Exposure	Recovery			
Lymnaea natalensis	1.0	100	12 24	24	Distilled water	—	Foster et al. (1960)
,,	0.5	100	5	24	Tap water	—	Shiff [a]
Lymnaea sp.	0.25	100	24?	—	—	Orientating trial	Blair & Alves [b]
,,	0.25	100	24	24	Pond	—	Gillet & Bruaux [c]
Galba truncatula	0.075	100	24	—	Brook water	Orientating trial	Behrenz [b]
,,	1.0	100	1	—	,,	,,	
,,	0.15	100	24	0	Tap water, aerated	,,	Enigk [b]
,,	0.4	100	24	0	Tap water, not aerated	,,	Enigk [b]
,,	0.1	100	24	24	Tap water	,,	Gönnert

[a] See the article on page 533 of this issue. [b] Personal communication. [c] See the article on page 509 of this issue.

TABLE 7

EFFICACY OF BAYER 73 AGAINST SNAILS OF NO MEDICAL IMPORTANCE

Snail	Concentration (p.p.m.)	Mortality (%)	Time (hours)		Water used	Remarks	Investigator
			Exposure	Recovery			
Planorbis complanatus	2.0	100	4 72	24	Tap water, aerated	Orientating trial	Buttner [a]
Planorbis corneus	2.0	100	24 72	24	"	"	Buttner [a]
Planorbis vortex	2.0	100	4 72	24	"	"	Buttner [a]
Planorbis sp.	0.8	100	24	96	Tap water	"	Enigk [a]
Lymnaea (Radix) ovata	0.2	100	24	48	Lake Victoria	—	Webbe [b]
"	1.0	100	24	24	Tap water	Orientating trial	Gönnert
Lymnaea palustris	0.6	100	24	72	"	"	Enigk [a]
"	0.5	100	24	24	"	—	Gönnert
Lymnaea stagnalis	1.0	100	24	24	Tap water, not aerated	Orientating trial	Buttner [a]
"	2.0	100	24	24	Tap water, aerated	"	Buttner [a]
Bithynia tentaculata	1.0	100	48	24	Tap water, not aerated	Orientating trial	Buttner [a]
"	2.0	100	4	24	"	"	Buttner [a]
"	2.0	100	72	24	Tap water, aerated	"	Buttner [a]
Physa sp.	1.0	50	12	24	Distilled water	Only two snails	Foster et al. (1960)

[a] Personal communication.

[b] See the article on page 525 of this issue.

Very much more sensitive towards Bayer 73 than *L. natalensis* is *Galba truncatula*. W. Behrenz,[1] who used over 100 snails for every concentration, determined the LC_{100} to be 0.075 p.p.m. The present author observed that all snails were killed by 0.1 p.p.m., the lowest concentration he tested. K. Enigk [1] reports similarly good data. In his tests the lethal concentration was 0.15 p.p.m., but rose to 0.4 p.p.m. when the test solution was aerated. These data refer to tests with the snails kept submerged. Tests taking into account the amphibious way of life of *G. truncatula* are still wanting. Comparative trials between Bayer 73 and NaPCP would also be of interest.

Efficacy of Bayer 73 against snails of no medical importance

Buttner included in her tests several snails without medical interest (Table 7). Unlike all other authors cited, she added water plants and dead vegetable material to the test solution. She also ran tests in which the test solution was first aerated and then not. In order to kill *Planorbis complanatus* and *P. vortex*, as well as *Bythinia tentaculata*, exposure to a concentration of 2 p.p.m. for four hours was required in a solution that was not aerated and for 72 hours in an aerated one. The latter exposure time also applies to *Planorbis corneus*. For *Lymnaea stagnalis* the lethal concentration was 1 p.p.m. after 24 hours of exposure in an unaerated solution, and 2 p.p.m. in an aerated solution of Bayer 73.

The present author tested *Lymnaea palustris* several times. This snail species is killed by 0.5 p.p.m. Bayer 73 (24 hours' exposure) but only by 3 p.p.m. of NaPCP. Enigk found the same relation for this snail species when comparing Bayer 73 (0.5 p.p.m.) and NaPCP (5 p.p.m.).

Thus the results with snails of no medical importance do not differ from those obtained for snails transmitting *S. mansoni*, *S. haematobium* and

[1] Personal communication, 1957.

Fasciola, not taking into consideration, of course, differences in the techniques of testing.

Efficacy of Bayer 73 against snail ova

Few experimental results on the efficacy of Bayer 73 against snail eggs are available. Most of the research has been done with eggs of *A. glabratus*, *L. natalensis* and *L. palustris*. Tests with eggs of *A. glabratus* by Unrau & Ferguson and by the present author and *L. palustris* (present author) prove that they are just as sensitive to Bayer 73 as are the adult snails, being killed by a concentration of 0.3 p.p.m. after 24 hours' exposure, or by 1 p.p.m. after six hours. Better results have been obtained by de Freitas (1959) against eggs of *A. glabratus* (LC_{100} = 0.1 p.p.m. after 24 hours' exposure), though during these tests only 22.4% of the control eggs survived.

Excellent results were obtained by Gillet & Bruaux against eggs of *L. natalensis*. Comparative trials with Bayer 73 and NaPCP gave the following figures for 100% mortality:

Exposure (hours)	*Bayer 73*	*NaPCP*
1	0.5	40
2	0.5	35
6	0.05	30

These authors point out that there is no fundamental difference between the sensitivity of the eggs of *Biomphalaria*, *Physopsis* and *Lymnaea*, though the eggs of *Lymnaea* are surrounded by a thicker covering of a gelatinous material. These observations are confirmed through the results obtained by the present author with *A. glabratus* and *L. palustris*. Similarly, Gillet & Bruaux and the present author have found that the age of the eggs has no influence on the efficacy of Bayer 73.

According to Alves eggs of *O. quadrasi* are killed by Bayer 73 in concentrations lower than those needed to kill the adult snail. NaPCP, on the other hand, is more efficient against adult *Oncomelania* than against their eggs.

Influence of exposure time on efficacy of Bayer 73

To treat flowing waters it is essential to know the lethal time of exposure for a given concentration of the molluscicide. The importance of the time of exposure has been dealt with several times above. We may now discuss tests dealing particularly with this question, and, in particular, those of Paulini, Chaia & de Freitas and of the present author on *A. glabratus* and those of Gillet & Bruaux on *Biomphalaria*.

As can be seen from Table 8, there is, within a limited range of concentrations, a constant relation between the concentration and the time of exposure. Paulini, Chaia & de Freitas have worked out the relation between the lethal concentration of Bayer 73 and the lethal time of exposure for *A. glabratus* and have obtained a constant factor of 20-25. But from Table 8 it can easily be seen that the constant factor does not depend only upon the species of the snail but also upon the conditions of the test (water from fish pond or from Lake Kivu.

TABLE 8

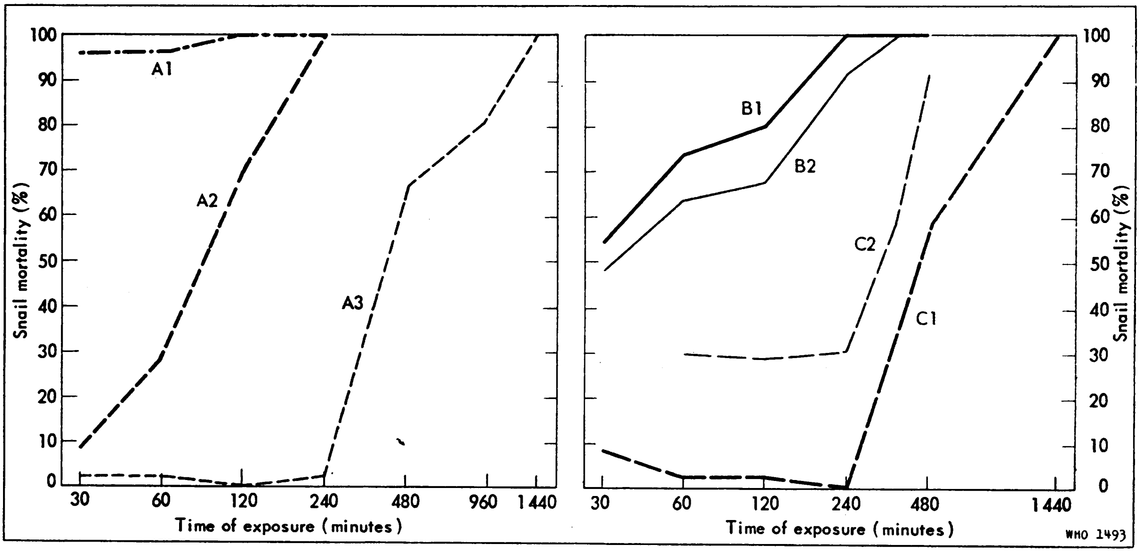
RELATION BETWEEN LETHAL CONCENTRATION OF BAYER 73 AND LETHAL TIME OF EXPOSURE

Concentration of Bayer 73 (p.p.m.)	Lethal time of exposure (hours)			
	Australorbis glabratus		*Biomphalaria* sp.	
	Data from Paulini, Chaia & de Freitas [a]	Data from Gönnert	Data from Gillet & Bruaux[b] for: Fish-pond water	Lake Kivu water
8	—	2	—	1
4	—	4	—	2
3.2	8	—	—	—
2	—	6	—	—
1.6	16	—	—	—
1	—	8	1	—
0.8	24	—	—	—
0.5	—	—	2	24
0.25	—	—	6	—

[a] See the note on page 706 of this issue.
[b] See the article on page 509 of this issue.

The relation between snail mortality and time of exposure has been recorded graphically in the accompanying figure, which is based on the results of various authors with copper sulfate, NaPCP and Bayer 73. Two main conclusions may be drawn from these graphs. First, curves for the same concentration of a molluscicide obtained by different authors run parallel to one another, thus indicating a similarity of action but different test conditions. Secondly, there seems to be a fundamental difference in the mode of action of copper sulfate on the one hand and of NaPCP and Bayer 73 on the other, since with copper sulfate the time of latency is shorter and the curve is flatter.

RELATION BETWEEN MORTALITY OF *AUSTRALORBIS GLABRATUS* AND TIME OF EXPOSURE TO DIFFERENT CONCENTRATIONS OF BAYER 73, COPPER SULFATE AND SODIUM PENTACHLOROPHENATE



A1 = 10 p.p.m. Bayer 73 (Gönnert).
A2 = 1 p.p.m. Bayer 73 (Gönnert).
A3 = 0.8 p.p.m. Bayer 73 (data from the note by Paulini et al. on page 706 of this issue).

B1 = 10 p.p.m. copper sulfate (Gönnert).
B2 = 10 p.p.m. copper sulfate (Neto & de Morais, 1955).
C1 = 10 p.p.m. NaPCP (Gönnert).
C2 = 10 p.p.m. NaPCP (Neto & de Morais, 1955).

Influence of temperature on efficacy of Bayer 73

The tests discussed under the heading " Trial methods " above were made, as far as can be concluded from the data available, at temperatures between +22° and +26°C. That is why the influence of temperature need not be considered when evaluating these tests. But during field trials greater differences in temperature have to be reckoned with, and the present author, in his tests with *A. glabratus* and *O. hupensis*, therefore took the influence of temperature into consideration. For a time of exposure of 24 hours a slight decrease in the efficacy of Bayer 73 was noticeable only when the temperature went down to 13°C or and less. At a concentration of 0.3 p.p.m. only 50% of the snails died after six hours' exposure at a temperature of 11°C, whereas 80% died at room temperature (22°C) or higher. After 24 hours' exposure, only 93% died at 11°C as against 100% at room temperature.

It may therefore be concluded that the range of water temperatures to be expected under field conditions will not decisively influence the efficacy of Bayer 73.

Influence of composition and pH value of water on Bayer 73

Though several workers pointed out the influence that the composition of the water and its pH might have, few experimental results are available.

Gillet & Bruaux made comparative tests to determine the efficacy of Bayer 73 on *Biomphalaria*, using water from fish ponds with an approximately neutral pH value and water from the strongly alkaline Lake Kivu (pH 9). These trials (see Table 3) showed a difference to the disadvantage of the water of Lake Kivu; this might be due either to the strong alkalinity of the lake water or to differences in the composition of the two waters compared.

Foster, Teesdale & Poulton (1960) used nothing but distilled water for their laboratory tests. To kill *Biomphalaria pfeifferi nairobiensis*, *Bulinus tropicus* and *Lymnaea natalensis* they needed higher concentrations than other authors who used " normal " water (see Tables 3, 4 and 6). During comparative tests with distilled and tap water (Lake Victoria) Webbe saw a difference in favour of tap water.[1]

[1] See Table 1 of Webbe on page 526 of this issue.

This difference, of course, is only small, but it did not exist with NaPCP. The present author repeated the tests with *A. glabratus* and came to the same conclusion as Webbe. These tests prove that the slight differences in the results of Foster et al. (1960) are due to the influence of distilled water. They also explain the more favourable results obtained by Foster et al. during field trials as compared with laboratory tests.

The influence of the pH of the water was tested by Gönnert & Strufe (1961) with *A. glabratus* exposed for 24 hours. Solutions buffered with phosphate (1.18%) were used to prepare the test concentrations of Bayer 73. Since no differences could be observed within the pH range of 5-9, it is probable that the reduced effect which the molluscicide exerts in the water of Lake Kivu is not due to the pH value but rather to the composition of the water. From tests on the influence of different mineral salts on the efficacy of Bayer 73, it appears that those salts in particular which give an alkaline reaction in watery solutions (for instance, calcium oxide or certain alkali silicates) can reduce the efficacy of Bayer 73 provided they reach high concentrations.

Influence of light on stability of Bayer 73

If molluscicides are used in limpid, shallow water they are often exposed to strong solar radiation. Recently Meyling et al. (1959) reported that NaPCP is quickly destroyed under such conditions. According to these authors the speed of the decomposition is also dependent upon the degree of hardness of the water.

Gillet & Bruaux tested the ultraviolet stability of Bayer 73. They exposed solutions of 0.5 and 0.25 p.p.m. in a thin layer to the rays of a Philips UV-lamp (30 W) at a distance of 104 cm. The efficacy of the solution was tested biologically after the exposure with planorbids from a pond. After a radiation of 6-12 hours no significant differences were observed in comparison with the controls. After 24 hours a decline in efficacy down to 1/11 (0.5 p.p.m.) and 1/6 (0.25 p.p.m.) was found biologically.

According to Shiff, the activity of dilute solutions in distilled water was significantly reduced after a short period of exposure to sunshine.

In earlier comparative tests Gönnert & Strufe (1961) had also observed a sensitivity of Bayer 73 to ultraviolet radiation. More recently, tests showed a slight superiority of Bayer 73 over NaPCP in this respect. The ultraviolet stability is influenced by the spectral composition and the intensity of radiation of the lamp. Comparing the findings of Meyling et al. (1959) for NaPCP with those for Bayer 73, there seems to exist the same dependency upon the composition of the water and its degree of hardness.

Adsorption on mud and organic matter

Copper sulfate has the great disadvantage that it is rapidly adsorbed on mud and other organic material; NaPCP is adsorbed to a much lesser degree. The adsorption of Bayer 73 has been studied by Gönnert & Strufe (1961) and by Duhm and collaborators (1961). Its adsorption on mud is very much less than that of copper sulfate and corresponds more or less to that of NaPCP in the range of concentrations used in practice (see Gönnert & Schraufstätter, 1959; Fig. 2).

The present author has tested Bayer 73 against *A. glabratus* in beakers containing water plants and in aquaria with rich plant growth. In the minimal effective concentration of 0.3 p.p.m. no biological loss of efficacy was found in the presence of water plants. The findings of Buttner, however, indicate that the efficacy of Bayer 73 may be reduced in the presence of water plants and dead organic matter (see Table 7).

In this connexion the studies of Duhm et al. (1961) with ^{14}C-labelled Bayer 73 are of special interest. According to them, 10 g of water plants (weighed moist) per litre [1] take up approximately 25% of the effective agent in 1½ days and 50% in six days. With 60 g of water plants per litre [2] more of the active agent will, of course, be taken up (80% in 24 hours). The initial concentration for these tests was 1 p.p.m. Representatives of the following genera were available for these tests: *Ambulia*, *Ceratopteris* (two species), *Cryptocoryne*, *Ludwigia*, *Riccia* and *Vallisneria*. How much of the active agent is taken up seems to depend upon the size of surface of the plant; water plants with a larger surface seem to take more of the active agent out of solution than those with a smaller surface.

Influence of aeration of water on Bayer 73

Buttner and Enigk have both reported on the influence of aeration of the water on the efficacy of Bayer 73. Both workers found effectiveness reduced as a consequence of aeration. In contrast to this, Gönnert & Schraufstätter (1959), in their biological tests with aquaria, were unable to find any difference

[1] This corresponds approximately to the plant growth in a well-supplied aquarium.

[2] In this case the entire beaker is densely filled with plants.

between aerated and still water during the first 24 hours after adding the molluscicide. The concentration of the active agent, however, decreases when the tests are extended over longer periods. This is probably due to the fact that the water is to some extent stirred by the aeration, and this in turn causes greater adsorption of the molluscicide on mud.

TOXICITY OF BAYER 73 TO AQUATIC ANIMALS

The toxicity of a molluscicide is of interest not only so far as snails are concerned but also in regard to other animals living in the water; for instance, fish, which in many tropical areas are of considerable importance as human food; plankton organisms, which are essential for the biological purification of the water; and, finally, the medically important larvae of trematodes.

Toxicity to fish

According to laboratory tests by Gönnert & Schraufstätter (1959) 1 p.p.m. is the dose toxic for *Lebistes reticulatus* and *Xiphophorus helleri.* All fish survived at 0.3 p.p.m. A. Müller [1] repeated the tests with *Lebistes reticulatus* and determined the LC_{50} at 1 p.p.m. Paulini, Chaia & de Freitas did much more intensive research regarding fish toxicity, working with *Poecilia januarensis.* In a concentration of 1.6 p.p.m. all fish died after two hours; in 0.8 p.p.m. 14% were still alive after 48 hours, and in 0.4 p.p.m. 99.6% survived after 48 hours. The tests thus show that the minimal dose toxic to fish is 2-3 times higher than that for snails. The same result was obtained by J. Holz [2] for Indonesian fish. Enigk, who tested German cold-water fish, arrived at an even better ratio. In addition he found that even highly affected fish recovered in fresh water. According to tests made by Buttner, who used relatively high concentrations of the active agent for killing snails, the fish *Phoxinus laevis* and *Gasterosteus aculeatus* are at least as sensitive as or even more sensitive than snails. Also, Webbe reports on the dying of fish some hours after being exposed to a concentration of 0.3 p.p.m.

More detailed investigations, of the toxicity to fish were made by H. W. Denzer,[3] who compared Bayer 73 and NaPCP. For this purpose he chose fish of different biotopes—namely, *Petromyzon planeri*, *Cyprinus carpio*, *Phoxinus laevis*, *Rhodeus amarus* and *Salmo shasta* (= *S. irideus*). These tests were not limited to ascertaining the toxic doses, but also included determination of the rate of respiration. As to toxic doses, there was no difference between the two molluscicides, the tolerated dose being approximately 0.2 p.p.m. for fish from cold-water biotopes and 0.5 p.p.m. for warm-water fish. However, there was a difference between the two molluscicides in their effect on the rate of respiration, NaPCP in toxic doses accelerating the rate and Bayer 73 depressing it. Denzer thinks that this might be an indication of a different mechanism of action of the two molluscicides.

The difference between doses toxic to snails and those toxic to fish is, however, small. This became especially evident during field trials, where fish are regularly lost (Foster et al., 1960; Gillet, Bruaux & Nannan, 1960; Keyl; Schiff; Webbe). The toxic dose—chemically or mathematically estimated—varied between 0.3 p.p.m. and 1.5 p.p.m. The concentration must clearly be a great deal higher at the place of application. The reports on field trials do not indicate whether all fish die or only some. Indications as to whether affected fish recover—an observation made by Enigk—are also missing. So far reports about the death of fish have been obtained for the following species: *Astatoreochromis alluaudi*, *Barbus* spp., *Bambusia*, *Clarus mossambicus*, *Gasterosteus*, *Haplochromis* spp., *Protopterus aethiopicus*, *Tilapia esculenta* and *T. melanopleura.*

Toxicity to other aquatic fauna

Concerning the effect on aquatic animals other than snails and fish, such as Anura, Crustacea, arthropods or oligochaetes, systematic studies have been performed by Shiff & Garnett [4] only; everything else is based on casual observations. These two authors investigated the influence of Bayer 73, NaPCP and copper sulfate on the zooplankton in biologically stable, balanced ponds. They found that all three molluscicides led to an immediate reduction of all plankton organisms. Only the hexapods and their larvae were not noticeably affected by Bayer 73 or NaPCP. The effect of NaPCP and Bayer 73 on plankton organisms proved to be of short duration only. Following application of both molluscicides the biological equilibrium was almost completely restored during the follow-up period of one month. With copper sulfate, however, insect larvae and the

[1] Personal communication, 1958.

[2] Personal communication, 1959.

[3] Personal communication, 1960.

[4] See the article on page 543 of this issue.

Cladocera disappeared completely during the follow-up period. Differences between Bayer 73 and NaPCP can be seen only in the fact that the Cladocera recovered quicker after application of Bayer 73.

Findings concerning the effect of Bayer 73 on water animals can be summarized as follows:

Anura are quite sensitive. Frogs and tadpoles die after a short exposure to concentrations of 1 p.p.m. (Foster, Teesdale & Poulton, 1960; Gillet, Bruaux & Nannan, 1960; Keyl; Shiff & Garnett; Webbe), but they survive in 0.2 p.p.m., which is a concentration that will kill *Triton* larvae (Neuhaus). According to Buttner young frogs and adult *Triton cristatus* were not heavily damaged in aerated aquaria.

Insect imagines and larvae are resistant to Bayer 73 when applied in concentrations of at least up to 2 p.p.m. According to Buttner, Keyl, Neuhaus and Shiff & Garnett the larvae of Agrionidae, Coleoptera, Chironomidae, Diptera, Ephemoptera, Neuroptera, Odonata, the imagines of Dytiscidae, Gerridae, Gyrinidae, Notonecta and the water mites (Acarina) survived.

Crustacea turned out to have different degrees by sensitivity, as can be seen from data presented of Buttner, Keyl, Shiff and the present author, as well as by Shiff & Garnett. According to Shiff & Garnett, Decapoda (*Potamon*) die at 1 p.p.m. On the other hand, Shiff observed, during field trials, that big crustacea destroyed the nylon bags in which test snails were kept. *Asellus aquaticus*, *Cyclops* and *Gammarus* survived in concentrations of 2 p.p.m., though the *Cyclops* population was highly damaged. Cladocera are very sensitive. According to the present author's observations the lethal concentration for these Entomostraca is 0.3 p.p.m.; in 0.1 p.p.m. approximately 50% die. The remaining populations recover very quickly, however.

For oligochaetes, only the findings of Neuhaus and of Gillet and co-workers (1960) are available. In a concentration of 0.2 p.p.m. oligochaetes survived according to Neuhaus; Gillet et al. saw that they died in 1 p.p.m. but did not state the survival rate.

Efficacy against miracidia and cercariae

In combating bilharziasis it is not only the effectiveness of a molluscicide against snails which is of importance, but also the degree to which the free-living larvae of the trematode parasite are destroyed. Testing Bayer 73 against miracidia, the present author found that they were killed within a few minutes by a concentration of 0.3 p.p.m., and that even in concentrations of 0.1 p.p.m. and 0.05 p.p.m. they died within one hour in two of three tests whereas the controls survived almost two hours.

The sensitivity of cercariae has been studied in more detail, especially those of *S. mansoni*. All workers agree (de Azevedo & Pequito, 1961; Buttner; Gillet & Bruaux; Bruaux & Gillet; Webbe; and the present author) that cercariae are very sensitive and die in a few minutes when exposed to concentrations effective to snails. In concentrations between 0.7 p.p.m. and 0.2 p.p.m. they die after 20-25 minutes at the latest. However, according to Gillet & Bruaux and to observations by the present author, they survive in a concentration of 0.1 p.p.m. As might be expected, not all species of cercariae have the same sensitivity. Buttner was able to prove that the cercariae of *Lymnaea stagnalis* died just as quickly as did those of *S. mansoni*, but that the cercariae of *Planorbis corneus* exposed to the same concentrations died only after a time of exposure several times longer.

Summarizing these studies the conclusion can be drawn that concentrations effective against snails will also kill cercariae within a few minutes and that miracidia are even more sensitive than cercariae.

TOXICITY OF BAYER 73 TO WARM-BLOODED ANIMALS

An exact knowledge of the toxicity of molluscicides to warm-blooded animals (pets, farm animals) is of paramount importance, as contamination of such animals and of human beings with water containing molluscicides cannot always be avoided. In tropical countries such water may occasionally even be used for drinking purposes. Taken orally, a molluscicide should not be toxic. It should not be absorbed through the skin or cause irritation of skin and mucous membranes.

Bayer 73 therefore has been thoroughly tested toxicologically by Hecht & Gloxhuber (1962). During the course of these investigations the pure substance as well as its ethanolamine salt turned out to be quite non-toxic. Rats and rabbits tolerated single oral doses of 5 g/kg of the pure compound suspended in tragacanth. Following doses of 0.25 g/kg cats and dogs occasionally vomited but these animals tolerated doses of up to 1 g/kg without showing any undue signs of intoxication. Human beings failed to show symptoms after having taken oral doses of about 0.3 g/kg. The compound's ethanolamine salt proved to be similarly non-toxic; the LD_{50} for a single oral application is 2.5 g/kg in rats, 1 g/kg in mice.

Given parenterally, a mode of application which is not actually practised with molluscicides, the tolerance is somewhat decreased. Intraperitoneally, the LD_{50} for a single dose is 0.75 g/kg in rats, and 0.25 g/kg in mice. The poor tolerance of mice to Bayer 73 applied intraperitoneally, as demonstrated by Gillet, could not be confirmed by Hecht & Gloxhuber (1962), no matter whether the molluscicide was suspended in Tween 80 or tragacanth.

Far more important than the acute toxicity is the chronic toxicity. Here too the exceptional tolerance of warm-blooded animals to Bayer 73 has been confirmed. Rats received an average of 165.13 g orally in 381 days (2.5 g/kg 64 times by stomach tube, followed by the administration of the compound with their food in a ratio of 2.5% for 288 days). In spite of the large amount of the compound administered the animals did not show any changes in appearance or weight, as compared with the controls. The same was true of dogs which received 0.1 g/kg orally for more than 13 months; these animals were apparently not affected in their health. Macroscopic and histological investigation of the organs of these rats and dogs did not reveal any pathological changes caused by the compound. Furthermore, there are no indications that as a result of these long-term studies Bayer 73 had any carcinogenic effect.

K. Kaemmerer [1] tested Bayer 73 in a calf weighing 230 kg. He gave daily doses of 2.3 g of the ethanolamine salt and had good results; no disturbances of growth or appearance were observed.

These findings prove that Bayer 73 is not toxic when applied orally. Equally favourable results were obtained by Hecht & Gloxhuber (1962) when the substance was tested for skin tolerance. Wet substance was applied to the ears of rabbits by means of a cotton sponge for a period of 24 hours. No signs of irritation were seen when the sponge was removed. Minor skin abrasions observed must be attributed to mechanical causes. Trials on human beings also speak in favour of an exceptionally good skin tolerance; out of seven persons to whose forearms the wet substance had been attached securely over a period of 24 hours, only four showed a rather weak and transient erythema. Results with the ethanolamine salt are identical; under corresponding conditions two out of six persons showed a similarly transient erythema.

[1] Personal communication, 1957.

To gain information on the absorption, distribution and excretion of the molluscicide by internal organs of warm-blooded animals, trials with ^{14}C-labelled Bayer 73 were carried out by Duhm et al. (1961).[2] In summary, the results of these studies are as follows:

Provided there is no entero-hepatic circulation, 33% ± 5% of the molluscicide is absorbed from the gastro-intestinal tract and 80% of the absorption is completed within 12 hours.

Some 33% ± 5% of the molluscicide is excreted into the urine, 67% into the faeces. The former excretion is practically completed at the end of 24 hours, the latter at the end of 48 hours.

An application of 50 mg/kg orally for 7 days does not provoke any changes in the absorption and excretion values during the period of observation.

The maximum content of molluscicide or its metabolites in the animal organism (minus gastro-intestinal tract) was obtained 3-5 hours after the application and amounted to about 8% of the molluscicide applied.

Following a single oral application of 50 mg/kg, 0.051 mg of the molluscicide (i.e., 0.6% of the total amount given) or its metabolites was found in the animal body (minus gastro-intestinal tract) 24 hours after the application. Repeated applications (seven) did not increase this value. 48 hours after the application the content of molluscicide in the animal body was less than 0.2% of the amount given.

The tissue concentration in the organs reached its peak about 4 hours after the application and decreased exponentionally with a biological half-value period of about 5 hours. With other words, 24 hours after the application the tissue concentration amounted to only 1/8 of the amount measured 8 hours after the application (mean tissue concentration: 2.5 γ/g tissue related to molluscicide).

It is reasonably safe to assume that cumulation is not to be expected even after repeated application of molluscicide (50 mg/kg daily, orally).

PHYTOTOXICITY OF BAYER 73

Requirements as to the phytotoxicity or lack of phytotoxicity of a molluscicide differ greatly and depend on the kind of water to be treated. To destroy snails in irrigation schemes used to water crops a compound which is exceptionally well

[2] These authors gave one oral dose of 50 mg of ^{14}C-labelled Bayer 73 per kg of body-weight to rats.

tolerated by the plants is required. If the molluscicide is to be applied to waters not used for farming purposes phytotoxicity may even be desirable, as it rids the water of plants and is thus a source of economy.

The phytotoxicity of Bayer 73 may therefore be considered under these two headings. Field trials with a concentration of 1 p.p.m. did not lead to any damage to plants with the exception of some algae. Submersed plants, though, occasionally showed signs of wilting. Foster, Teesdale & Poulton (1960) and Gillet & Bruaux (unpublished), point out that the substance is not toxic to plants. Keyl is in agreement as far as *Batrachium* sp., *Hydrocharis morsus ranae* and *Lemna trisulca* are concerned. Webbe, by contrast, noticed a certain amount of wilting in water plants, which recovered rapidly, however. There is no visible effect on higher forms of plant life (Cyperaceae, Nymphaceae, Naiadaceae, *Eichhornia*, *Potamogeton pusillus*, *Polygonum salicifolium* and emergent grasses such as *Leesia hexandra*). Shiff & Garnett, too, saw no changes in higher forms of plants, but noticed a severe setback of dense beds of *Chara* sp. *Spirogyra* was stimulated to rapid growth after an initial decrease in the population.

No visible damage to water plants was observed by the present author during trials in the aquarium with concentrations of 0.3 p.p.m. Concentrations of 3 p.p.m., however, destroyed most plants after prolonged exposure. The surviving plants started to sprout once the concentration decreased.

L. Eue [1] gives valuable information on the influence of Bayer 73 on crops. Data on one field trial (M. Pugliese [2]) with sugar cane in the Congo are also available. A field of sugar cane, nine months old, was the object of the study and Bayer 73 was added to the water supply in a concentration of 5 p.p.m. over a period of 12 hours. Neither immediately nor during the entire period of observation (35 days) were there any visible signs of damage to the plants, nor was there a noticeable decrease in sugar content.

In the laboratory, too, sugar cane proved to be very resistant to Bayer 73. In another test, slips of sugar cane were watered exclusively for a period of 98 days with a solution of the molluscicide. Other plants were watered with clean water alone until the cane had grown to a height of about 15-20 cm. Then watering with molluscicide solution was started and continued throughout 40 days. Even with the highest concentration of 100 p.p.m. (i.e., a total dose of 253 mg Bayer 73 per plant), no differences between treated plants and the controls were observed. Mustard and oats, too, are indifferent to Bayer 73 even under continued application of 100 p.p.m. In this case it does not matter whether watering with molluscicide solution is begun immediately after sowing the grain and continued for 28 days or started when the plants are young and continued for 21 days. Under similar conditions rice is less resistant. Rice plants, however, watered with a 10-p.p.m. solution over a period of two months showed slight damage only. A solution of 1 p.p.m. turned out to be absolutely harmless even when applied continuously over long periods. In addition to the usual untreated controls, comparative tests were also carried out with NaPCP, which turned out to be quite phytotoxic in concentrations necessary to kill the snails in the field.

[1] Personal communications, 1956, 1957, 1958 and 1959.

[2] Personal communication, 1960.

MECHANISM OF ACTION

It is important to know the spectrum of intensity and of action of a chemical compound which is biologically active. Just as important is the knowledge of its mechanism of action, i.e., the way in which the compound acts on the metabolism of a living organism and the way this organism reacts to the influence of the compound.

Snails placed in a solution of Bayer 73 at first show no typical reaction. Apparently they are not irritated and do not try to escape the solution (Foster, Teesdale & Poulton, 1960). This applies also to field conditions. Unlike NaPCP, Bayer 73 does not provoke an increased excretion of mucus which, in turn, might delay the absorption of the molluscicide. Depending on the concentration of the compound, the snails sooner or later retract into their shells (*A. glabratus*, *L. palustris*) or they try to leave them as far as possible (*O. hupensis*). Once death has occurred, body fluid (blood ?) diffuses into the surrounding water. The present author has observed the striking phenomenon that dead *A. glabratus* often are surrounded by red-coloured water.

The mode of action of Bayer 73 is not yet fully understood. Strufe (unpublished investigations) studied the oxygen intake of snails and isolated snail tissue by means of the Warburg apparatus. The results corresponded to those obtained by Weinbach & Nolan (1956) with NaPCP. Concentrations of Bayer 73 which are lethal to snails strongly inhibit the oxygen uptake. By contrast, lower concentrations stimulate the respiration up to 40%. The respiration is blocked by Bayer 73 about twice as much as by NaPCP (Gönnert & Schraufstätter, 1959, Fig. 3). The blocking of the respiration is irreversible when the snails are transferred into pure water. With NaPCP it is reversible. Further studies, not yet completed, indicate that Bayer 73 may also act on the carbohydrate metabolism.

STUDIES WITH ^{14}C-LABELLED BAYER 73

By relying on the classical methods of investigation only, quite a few problems cannot be solved at all or not without difficulty. Duhm et al. (1961), therefore, looked into these questions with the aid of radioactive Bayer 73 (labelled with ^{14}C) and for the first time obtained exact data on the absorption of a molluscicide by water plants (see page 493 above). Unfortunately, results of similar trials with other molluscicides are not yet available for comparison.

Important data concerning the absorption, excretion and distribution of Bayer 73 in the organism of warm-blooded animals were also gathered, using rats as test animals (see above under the heading " Toxicity of Bayer 73 to warm-blooded animals "). There is proof that the amount of molluscicide absorbed in the intestinal tract is quickly excreted and that there is no cumulation within the organs. Even a daily application of 50 mg/kg for seven days did not result in cumulation.

Pertinent data concerning the absorption by snails (*A. glabratus*) were also collected with the aid of ^{14}C-labelled Bayer 73. A solution of the molluscicide containing 1 p.p.m. of active substance was used. Snails 1 cm in diameter and weighing about 250 mg absorbed 2%-3% of the active compound to which they were exposed for seven hours, and 4% with a 24-hour exposure. In soft tissues a higher concentration of active substance was detected than in the shell. With an exposure time of seven hours, the factor of concentration [1] is 4-6, and 6-10 for 24 hours' exposure. The shell, mechanically cleaned of soft tissues, has a factor of 1.5-3.

Also for the first time, the amount of a molluscicide needed to kill a snail can be calculated. A single snail (*A. glabratus*) 1 cm in diameter takes up about 1 γ of active substance when exposed to a 1-p.p.m. solution over a period of 24 hours. Under these conditions, the snail is killed or irreparably damaged within 4-6 hours. The minimal lethal dose for snails of the described size must therefore be considerably less than 1 γ. For smaller snails possessing a sensitivity similar to that of *A. glabratus*, the absolute lethal dose should be even less.

Another conclusion may be drawn from these investigations: the size of a snail population does not exert a decisive influence on the effect of Bayer 73. For laboratory trials small amounts of molluscicide solutions therefore suffice. The active compound obtained in 10 ml of a 1-p.p.m. solution of Bayer 73 will, theoretically, kill more than 2.5 g of snails (*A. glabratus*). It would certainly be desirable to obtain information on similar trials with other molluscicides.

FIELD TRIALS

The previous sections have dealt mainly with experimentally gathered data on the properties of Bayer 73. The value of a molluscicide has to be proved not only by laboratory investigations but above all by field trials. During these trials and in practical application a large number of factors hitherto unknown exert an effect on the highly diluted molluscicide solution. These factors may definitely change the properties of the substance.

Keeping this fact in mind, the present author carried out laboratory tests which were not limited to small glass containers. Trials were initiated early in aquaria in which fish had been kept and snails had been raised for months. These aquaria, 60 litres in volume, had acquired a rich plant life (*Ludwigia*, *Vallisneria*, etc.). Their bottoms were covered with a 2-cm layer of garden soil on top of which was a layer about 1 cm deep of decomposed food remains, plant detritus and fish and snail faeces. They had been continuously aerated, and were biologically balanced with a large population of *Australorbis glabratus*. Under these conditions, unfavourable to molluscicides as compared with those in glass beakers, all snails were killed with concentrations up to 0.3 p.p.m. Bayer 73. In spite of the mud, the rich plant growth and the large snail population, the concentration of molluscicide decreased only slowly. A water sample taken from an aquarium 19 days after treatment with 3 p.p.m. killed all specimens of *A. glabratus* tested.

First field trials were carried out by Neuhaus and by Keyl in Germany in 1956 and by Alves in Southern Rhodesia in 1957. Neuhaus applied a concentration of about 0.2 p.p.m. only (calculated and

[1] Factor of concentration = activity in 1 g soft tissue: activity in 1 g initial solution.

chemically determined). A small pool with a population of *Galba truncatula* was used (0.22 m^3 in volume). In spite of the low concentration the snail population was greatly reduced.

Keyl tested the molluscicide in small, well-limited sectors of irrigation ditches with typical eutrophic fauna, applying concentrations of 1 and 2 p.p.m. Although heavy and continuous rains set in a few hours after the trials had been started, the results were positive. No living specimens of *Lymnaea stagnalis* could be found a week later. The population of *Radix ovata* had been reduced to less than 5%. As compared with the situation prior to the trial and obtaining in adjoining, untreated sectors of the ditches, the remaining species of snails were reduced by 50%-90%. *Aplexa hypnorum*, *Bythinia tentaculata*, *Galba truncatula*, *Planorbis corneus*, *Radix* sp., *Spiralina vortex* and *Tropidiscus planorbis* were represented in these trials.

Alves did not limit his trials to Bayer 73 alone. He ran a parallel test with NaPCP. Two adjoining creeks of similar character with a strong plant life and large snail populations in certain localities were used for purposes of comparison. The concentration of Bayer 73 was 1/20th that of NaPCP. Beginning at the spring the molluscicide solutions were applied by means of a portable stirrup-pump over a long-distance downstream. Both molluscicides succeeded in temporarily clearing the creeks of snails. In the creek treated with Bayer 73 the snails reappeared three months after application of the molluscicide, which is a few weeks later than in the creek treated with NaPCP.

The Bayer 73 used in these first field trials was a technically unsatisfactory preparation; this became especially evident when extensive trials were begun. At that time, Bayer 73 was available only as a 70% wettable powder which tended to form clots and which was poorly suspendable. This powder was soon withdrawn. Trials run with this batch by Gillet,[1] Halawani[1] and Pitchford yielded unsatisfactory results. Definite conclusions may only be drawn from trials in which the new wettable powder (4780), introduced during the summer of 1958, was used. These field trials were carried out by Foster, Teesdale & Poulton (1960) and Foster & Crossland [2] in Kenya; Gillet, Bruaux & Nannan (1960) in the Lake Kivu area; Paulini, Chaia & de Freitas in Brazil; Shiff and Shiff & Garnett in Southern Rhodesia, and Webbe in Tanganyika. A short note is also available from Alves in the Philippines.

The field trials were carried out under varying conditions. Factors to be reckoned with were: the quality of waters treated, the density of plant growth in these waters, their content of ground mud and their degree of natural turbidity. The size of the snail population and the species of snails represented also varied greatly. Methods applied to control the snail population before, during and after treatment were not uniform either. The same is true for the concentrations of Bayer 73 actually applied and the methods of estimation of the effective amount of molluscicide contained in certain sections of the water treated. In addition, different methods of application were used. As most of the field trials have been reported in detail, only the most important results need be summarized here.

1. Bayer 73 proved to be effective even in low concentrations. Only Paulini, Chaia & de Freitas had poor results. These authors treated creeks with dense plant growth by suspending cloth bags containing the wettable powder in the water.

2. Methods of treating flowing water which stood the test are: the dispenser developed by Foster & Poulton (1960), the continuous pouring of a concentrated molluscicide suspension into the water as practised by Gillet et al. (1960), or the continuous drip-feed system of Shiff. To treat standing waters a suspension of Bayer 73 was sprayed with the stirrup-pump (Shiff; Webbe) or applied by means of a high-pressure boom (Shiff).

3. The lowest effective concentration of Bayer 73 was 1 p.p.m. or even less. Webbe succeeded in killing all snails in pools with 0.4 p.p.m. Foster et al. (1960) determined the effective dose for flowing waters containing mud in places and relatively dense plant life to be 0.75-0.8 p.p.m., applied over a period of eight hours.

4. Flowing waters may be kept free of snails over a distance of three to seven miles below the point of application by one treatment of Bayer 73 with an initial concentration of 1 p.p.m. (Foster et al., 1960; Gillet et al., 1960; Webbe).

5. The reappearance of snails in waters successfully treated occurred one to three months after treatment at the earliest. In flowing waters this is probably caused by migration of snails from untreated sections. In standing waters it may be caused by flooding or other means (Webbe later found

[1] Personal communication.

[2] Miscellaneous report No. 273 from the Colonial Pesticides Research Unit, Arusha, Tanganyika (unpublished).

species of snails which had not been present prior to the application).

6. The effectiveness of Bayer 73 apparently may be reduced by the presence of water plants and ground mud, by the turbidity of the water and by intensive solar radiation. However, the concentration seems to decrease slowly. The effectiveness of Bayer 73, therefore, is not decisively diminished during the first hours following treatment. Very dense plant life may hinder the circulation of water which is necessary for a uniform distribution of the molluscicide. Thus, the effectiveness may be further decreased.

7. In effective concentrations Bayer 73 is toxic to fish and certain other animals living in the water, but not to arthropods and their larvae. There are no data available to show whether the application of the molluscicide entirely destroys fish populations or only reduces them.

8. Higher plant life is not visibly damaged by Bayer 73. Submersed plants may show slight signs of wilting but recover rapidly. The same is true of algae, which sometimes show a greater degree of damage.

9. The biological balance in pools is severely disturbed by the application of Bayer 73, and by NaPCP and copper sulfate. One month after application, however, it is restored when Bayer 73 or NaPCP has been applied (Shiff & Garnett).

10. Field trials have proved that Bayer 73 is effective against the snails transmitting *S. mansoni*, *S. haematobium* and *Fasciola* (and against snails which have no medical interest) and their eggs. The doses to be applied are considerably lower than those which are, according to the literature, necessary for NaPCP and copper sulfate.

11. Not the slightest ill-effect has been observed in persons handling Bayer 73. The molluscicide may therefore be considered harmless to human beings.

12. There are no reports available concerning the effect of Bayer 73 on snails transmitting *S. japonicum*, but a brief personal communication by Alves states that good results were obtained by applying dry dusting powder or liquid preparations.

RÉSUMÉ

Des quelque 20 000 composés chimiques dont l'auteur a mis à l'épreuve le pouvoir molluscicide, le Bayer 73 (dichloro-5,2′ nitro-4′ salicylanilide) est le plus intéressant, en raison de son efficacité à faible concentration sur les adultes, cercaires, miracidies et œufs de mollusques, sa toxicité relativement faible pour la flore aquatique et son innocuité pour l'homme. L'auteur rend compte de ses propres expériences en laboratoire et sur le terrain, et constate une concordance remarquable de ses résultats et de ceux d'autres chercheurs.

La concentration létale minimum du produit en laboratoire, pour les mollusques adultes vecteurs de *Schistosoma mansoni* et *haematobium* est de 0,2-0,5 p.p.m. Sur le terrain, une concentration de 1 p.p.m. a permis de débarrasser un cours d'eau de ses mollusques sur une distance de 5-8 km en aval du point d'application. Les mollusques n'ont reparu qu'après 1-3 mois après traitement, par migration à partir de secteurs non traités des cours d'eau, ou d'inondations, dans les eaux stagnantes.

L'efficacité du Bayer 73 peut cependant être réduite par l'abondance de la végétation aquatique qui ralentit la circulation de l'eau ou empêche la répartition régulière du molluscicide, par la boue qui l'adsorbe partiellement ou l'insolation qui l'altère.

Aux concentrations efficaces, le Bayer 73 est toxique pour les poissons, mais non pour les arthropodes et leurs larves. On ne peut dire encore si les poissons sont détruits ou leur nombre seulement diminué. Les plantes submergées et les algues, quoique paraissant affectées peu après le traitement, y résistent. Dans les étangs, l'équilibre biologique est profondément perturbé, que ce soit par le Bayer 73, le pentachlorophénate de sodium ou le sulfate de cuivre. Mais un mois après l'application de l'un ou l'autre des deux premiers molluscicides, il se rétablit.

Le Bayer 73 est également actif contre les œufs des mollusques vecteurs, à des concentrations 10 fois inférieures à celles du pentachlorophénate de sodium.

Aucun effet toxique n'a été observé sur les personnes maniant ce produit. On peut le considérer comme inoffensif pour l'homme.

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