Memoranda

MOLLUSCICIDE SCREENING AND EVALUATION *

Considerable advances have been made in the field of molluscicide research since the WHO Expert Committee on Bilharziasis discussed molluscicides in 1960, and its survey of molluscicides and their properties [1] is no longer up to date. Table 1 presents the data on the molluscicides at present available in a somewhat different and more simplified form, and incorporates information derived from many sources and data provided on request from chemical companies.

The main features distinguishing the table from the WHO Expert Committee's survey are as follows. In addition to the well-established molluscicides Bayluscide, sodium pentachlorophenate (NaPCP), and copper sulfate, four important new substances or groups are included: the new molluscicide WL 8008, which is of considerable promise, the herbicides/molluscicides Gramoxone and Reglone, the " insoluble " copper compounds, and the organo-tins. Two other groups, the dinitrophenol compounds—such as DNCHP—and ICI 24223 are not commercially available, but are shown in the table because of their proved molluscicidal value. The inclusion of other chemicals such as barium chloride and arsenic compounds is no longer justified.

Advantages and limitations of currently available molluscicides

A concise presentation of the advantages and disadvantages of molluscicides is troublesome to make and somewhat unsatisfactory, because the objectives of the users, local conditions, and methods of use vary and affect any judgement of the suitability of a compound. For example, under some conditions solubility in water is an advantage, under others a disadvantage. In addition, the efficacy of molluscicides in general is liable to be influenced adversely by special combinations of salts in natural water in the presence of sunlight.

Availability is also an important factor. Some compounds such as Bayluscide, copper sulfate, and NaPCP have proved effective in several environments and are obtainable in the market. One or two other compounds of considerable promise are not yet commercially available in sufficient quantities for large-scale control. On the other hand, some compounds, such as the " insoluble " coppers and the organo-tins, are readily available because of their many other uses in industry, but still await conclusive field trials as molluscicides.

Four compounds, each with its own advantages and disadvantages, illustrate the range of properties that need consideration (Table 2).

At present Bayluscide is clearly the best molluscicide commercially available and the one to consider first in any control programme. Its only known important defects include the problem of its formulation and its decreased activity in certain environmental situations.

In spite of several undesirable qualities, NaPCP is the next best molluscicide available because of its effectiveness and availability and the fact that it has been thoroughly tested.

Since Bayluscide and NaPCP kill snail eggs, a competitor that fails to do this may be at a disadvantage. However, it has become obvious that molluscicides and formulations of different qualities will need to be used to cope with the different conditions found in snail habitats, the objectives of the application, and the epidemiology of transmission in the area concerned. At present the compounds and formulations that have been tested tend to complement each other.

In the past, consideration has been given mainly to obtaining molluscicide formulations that either depend on water carriage after being introduced at strategic application points in irrigation systems or can be applied by spraying. For water carriage, roads usually allow relatively easy access and the transportation of dispensers and the molluscicide is not an insurmountable problem. In rural Africa south of the Sahara, in Brazil, and in other endemic

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Requests for reprints should be addressed to: Parasitic Diseases, Division of Communicable Diseases, World Health Organization, Palais des Nations, Geneva, Switzerland.

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1961, **214**, 44.

areas water carriage cannot be depended upon to give adequate coverage in natural streams. In addition, isolated pools, pans, ponds, etc. have to be found and treated. Most of these habitats have to be covered by men who are on foot or at best able only to use a bicycle to help transport the molluscicide and a dispenser. For this reason it is essential to have formulations that are easy to apply and contain a very high percentage of active ingredient.

Under such conditions the routine application of the molluscicide will be in the hands of trained but illiterate labourers. For this reason the material must be safe to handle. It will usually also be impracticable to carry out chemical analyses of the water to determine the concentration of the molluscicide. Formulations that can be used under these conditions must be provided.

It is important to remember that flexibility is needed in the use of various molluscicides and formulations, so that unusual combinations of characteristics will be recognized and appreciated. For example, in some situations a well-timed single application of a fast-acting molluscicide may prove to be the most practical for interrupting seasonal transmission, even though the compound may not be ovicidal. In other situations the prolonged application of low concentrations of such a compound may be effective in eliminating the young as they emerge from the eggs, as well as the resident population of young and adults. This type of application could conceivably be more efficient than 4-8-hour applications because it would allow a longer time for the compound to diffuse sufficiently to reach snails in protected pools and reduce the chance of snails completely avoiding contact with treated water as a result of changes in the water level and consequent periodic stranding.

The need for continued screening for more selective and efficient molluscicides

Laboratory and field experience since the 1960 meeting of the WHO Expert Committee on Bilharziasis confirms its view that several molluscicide formulations would be required to meet the variety of terrain to be treated and to deal with resistance if it should be encountered. Significant resistance to molluscicides has not yet occurred, but it must be anticipated.

There is still a need for:

(1) a highly active general molluscicide, of low mammalian toxicity and relatively cheap;

(2) a combined herbicide/molluscicide; and

(3) a compound highly specific for snails.

Other desirable attributes in a molluscicide are:

(1) stability in its formulation under different environmental conditions and in storage before use;

(2) safety to the operator and ease of application; and

(3) low cost of treatment.

Because none of the molluscicides currently available meets all these requirements, the search for more effective molluscicides must be continued.

There are at present only a few molluscicides of accepted effectiveness and these tend to be generally biocidal, affecting many of the plants or animals (or both) in the snail habitats. The cost of molluscicides and their application is a matter of concern and a factor in the prevention of more extensive snail control programmes. It has undoubtedly been uppermost in the minds of public health administrators, who are faced with the problem of finding funds for control programmes. It does not seem very likely, however, that highly active organic compounds costing very much less than Bayluscide or trityl morpholine will be discovered, although a compound more specifically toxic to snails is a possibility.

Formulations, including baits, according to field requirements

A widening of the range of available auxiliary surface active agents (emulsifiers, spreaders, stickers, wetters, dispersants), naturally occurring and synthetic fillers, stabilizers (e.g., anti-oxidants and water scavengers), and solvents, and the accumulation of considerable knowledge about their use have made possible considerable advances in the formulation of pesticides for agricultural purposes and for the control of insect vectors of disease.

It is therefore generally possible, though not necessarily easy, to formulate active ingredients in such a way that the resulting formulations are stable and have an acceptable storage life in the tropics.

The physical properties of the active molluscicide tend to dictate the kind of formulation that can be made. It is generally not possible to formulate liquid active ingredients as water-dispersible powders with a high content of active ingredient; and it is virtually impossible to prepare liquid concentrates

TABLE 1. MOLLUSCICIDES AND THEIR PROPERTIES [a]

Molluscicide	Physical form of technical material	Active ingredient	Solubility in water	Toxicity of technical material [b]				Stability of technical material affected by:					Handling qualities		Formulations	Field dosage		Range of application	Cost	Manufacturers
				Snail LC_{90} ppm/hr	Snail eggs LC_{90}	Herbicidal activity	Mammalian toxicity, acute oral LD_{50}, rats, mg/kg	Ultra-violet light	Mud, turbidity	pH	Algae, plants	Storage	Safe to handle	Easy to handle		Aquatic snails; (ppm/hr)	Amphibious snails on moist soil			
				1	2	3	4	1	2	3	4	5	1	2						
Aqualin	Liquid	Acrolein	22 % w/w 20°C	30-75	15-24	Yes [f]	30-40	?	Yes	Yes	Yes	Yes	No	No	86 % acrolein volatile liquid	75-100 ppm		In flowing water where control of submerged weeds also necessary	$0.75/lb	Shell [c]
Bayluscide	Crystalline solid	Ethanolamine salt of 5,2'-dichloro-4'-nitro-salicylic anilide	230 ppm	5-8	2-4	No	5000	Yes [g]	Normal	Optimum 6-8	No	No	Yes	Yes	Wettable powder containing 70 % active ingredient	4-8	0.2 g/m²	Flowing and static water	$1.65/lb	Bayer
Carbamates	Amorphous solid	e.g., zinc dimethyl-dithio-carbamate	65 ppm	50	50-100	No	1400	?	No	?	No	No	Yes	Yes	(1) Granules 50 % + 50 % $CaCO_3$ (2) Micronized powder (90 % active ingredient)	10 g/m² 5 [o]		Where fish kill is undesirable		Velsicol Rhône Poulenc Bayer (Ziram) Compañía Química Rhodia Brasileira (Rhodiacid) Dow (Carbamates)
Copper (1) soluble compounds	Crystalline solid	e.g., copper sulfate	32 %	20-100	50-100	Yes [h]	?	No	Yes	Yes	Yes	No	Yes	Yes	Crystals of $CuSO_4, 5H_2O$	20-30 [j]		Static and flowing water	$0.10/lb	Various
(2) insoluble compounds	Amorphous solid	e.g., cuprous oxide	Insoluble	7-100	50-100	No	2000	No	Yes	Yes	Yes	In part	Yes	Yes	Powder	60 [j]	([l])	Static water where fish kill is undesirable	$0.25-0.35/lb	Grace & Co. Fisons, Sandoz, UCLAF
Dinitro-phenols	Crystalline solid	e.g., DNCHP 2-cyclohexyl-phenol	0.072 %	30-60	20-40	Yes	60	?	No	?	No	No	Varies	No	40 % wettable powder	30-55	0.1-5 g/m²	Various	?	Dow [e]
Gramoxone (Paraquat)	Crystalline solid	1,1-dimethyl-4,4'-dipyridylium dichloride	Highly soluble	60-100	6-10	Yes	200	No	Yes	No	Yes	No	Yes	Yes	Liquid: 2 lb Paraquat per UKgal (200 g/l)	5-10 [o]		Not yet defined	$7.00/lb	ICI Plant Protection
Molucid (ICI 24223)	Crystalline solid	Isobutyl-triphenyl-methylamine	1 ppm	5-10	24	No	250	Yes	No	?	No	No	Yes	Yes	(1) 94 % wettable powder (2) 40 % xylene solution (3) 35 % emulsifiable concentrate	6-12 16 12	2-3 g/m²	Not defined	?	ICI [d]
Organo-tins	Liquids and crystalline solids	e.g., triphenyl tin acetate, tri-*n*-butyl tin acetate	Varies from <1 ppm to about 50 ppm	1-10 [m]	1-2	No [n]	150	Yes	?	Yes	No	No	Yes	Yes	20 % wettable powder	7-14	([m])	Not defined	$1.50/lb	Pure Chemicals, M & T Pfizer UCLAF Hoechst
Reglone (Diquat)		1,1'-ethylene-2,2'-d-pyridylium dibromide	Highly soluble	60-100	6-10	Yes	200	No	Yes	?	Yes	No	Yes	Yes	Liquid: 2 lb Diquat per UKgal (200 g/l)	5-10 [o]		Not defined	$7.00/lb	ICI Plant Protection
Sodium pentachlorophenate	Crystalline solid	NaPCP	33 %	20-80	3-30	No	40-250	Yes	No	No	No	No	Varies	Yes	(1) Flake form 75 % (2) Pellets 80 % (3) Briquettes 80 %	50-80	0.4-10 g/m²	Flowing and static water	$0.25/lb	Monsanto Mitsui Kagaku Co., Tokyo Various
Trityl morpholine (WL 8008)	Crystalline solid	*N*-trityl morpholine	Insoluble	1-2	240	No	1400	?	?	Yes	No	No	Yes	Yes	(1) 16.5 % w/v emulsifiable concentrate (2) Granular bait	1-2 0.2 [o] 150 lb/acre		Flowing and static water	?	Shell [e]

[a] This table aims at presenting a general summary of molluscicides and their properties in a form designed for easy assimilation and comparison. In order to keep the table within reasonable limits, detail has had to be sacrificed. It would be impossible to include the entire range of experience reported by different workers, or to refer to the exact species of snails tested, the maturity of the snails used, the exact formulation used in all cases, or the precise conditions of the laboratory and field tests. Too much should not be read into properties described under group headings such as 'Carbamates' and 'Organo-tins'. The table is thus a general introductory guide and aide-mémoire. Further details on any particular aspect can be provided on request.

[b] 'Toxicity' to snails. Please note that the standard of comparison—ppm/hr—is normally calculated on the basis of a 24 hours exposure period. As appreciable differences in CT-values (i.e. Concentration x Time) may exist over the range of times and concentrations used, these figures may prove unrealistic if applied to short exposure periods of 1-2 hours.

[c] Not available outside USA.

[d] Not available commercially.

[e] Not readily available commercially in quantity.

[f] For submerged weeds only.

[g] Depending on salt content of water.

[h] Algae only.

[j] For 24 hours or more.

[k] 5 ppm for 12 hours.

[l] Not effective against amphibious snails.

[m] No figures obtainable for *Oncomelania* because of low molluscicide activity to amphibious snails.

[n] Phytotoxic to certain irrigated crops.

[o] Static water.

TABLE 2
ADVANTAGES AND DISADVANTAGES OF SELECTED MOLLUSCICIDES

Molluscicide	Advantages	Disadvantages
Bayluscide	Highly toxic to snails and eggs. Safe to handle and use. Probably the cheapest chemical per unit volume of water treated. Does not upset the biota as much as some compounds.	Difficult to formulate. In certain types of habitat available formulations do not disperse effectively.
Copper sulfate (and other copper compounds)	Kills both snails and their eggs. Active at a low pH. Somewhat less toxic to fish than other molluscicides. Safe to handle. (It may be possible to exploit insoluble copper compounds for use under some conditions.)	Absorbed by soil and organic material. Ineffective at a high pH. Corrosive to equipment. Of variable toxicity to snails under field conditions.
NaPCP	Kills both snails and their eggs. Excellent penetration downstream has been achieved. Widely used for other purposes and so not dependent on a molluscicide "market".	Irritating and potentially dangerous to the handler. Doses required relatively high compared with new molluscicides. Activity may be reduced by bright sunlight.
Tritylmorpholine	Highly toxic to snails. Toxic action against some species very rapid. Apparently safe to handle and use. Initial reports indicate no serious effects on biota. Several types of formulation possible.	Does not kill snail eggs. Removal by absorption may be a serious problem. Inactivated at low pH.

of active ingredients that are sparingly soluble in organic solvents.

In snail control the variety of conditions in which the molluscicide has to be applied is greater than in any other form of pest, disease, or weed control. A number of different formulations to meet these conditions is required.

Experience with insecticides has shown that biological performance can vary with different methods of application and also with the particle size of the active material in water-dispersible powder formulations. This also occurs with molluscicides. For example, with trityl morpholine (WL 8008) the kill of adult *Australorbis glabratus* exposed for 24 hours to 0.06 ppm suspensions of the toxicant, ground to different particle sizes, was as follows:

Particle size (in microns)	1-2	2-6	6-15	10-25
% kill	100	70	40	0

Differences have also been observed in the performance of diluted emulsions prepared from emulsifiable oils based on different solvents and emulsifiers.

It is, therefore, very necessary that all the known factors be taken into account and assessed during the development of any formulation for field use.

In addition to work on water-dispersible powders and emulsifiable oils, experimental work has been undertaken on granules (either floating or sinking), slow-release slabs, capsules, resin formulations, oil-bound solids, pastes, solutions with high spreading characteristics, electrolytically released copper, baits, molluscicidal soaps, and micronized powders that can spread over the surface of the water and then slowly become dispersed throughout the water. This range of formulations has arisen so as to cope with particular circumstances encountered in field operations.

Granules can be applied from the air or thrown mechanically or manually from the bank of a stream or a boat. They appear—particularly in the floating form—to be more successful with water-soluble than with water-insoluble compounds.

Oil-bound solids and pastes, as alternatives, do not seem to have any advantages over water-dispersible powders. Resin formulations, painted on wood or metal plates that can then be lowered into the infested water, depend on the random wandering of the snail for their efficacy and have not been successful in tank experiments.

High spreading solutions and floating micronized dusts appear to offer advantages under conditions similar to those in which floating granules can be used. The final choice would probably depend upon cost, which in turn would be influenced by the price

of the auxiliary chemicals (emulsifiers, stabilizers) and the ease or difficulty of producing the formulation.

Although the idea of *baits* as residual molluscicides is promising and the laboratory work encouraging, difficulties have arisen in the field which need to be studied in greater detail before a final decision about their potential value can be made. The difficulties are microbial decomposition of the bait, silting over with mud, and consumption by fish. The toxicity of some molluscicides to fish, however, is far less as a stomach than as a contact poison.

Molluscicidal soap, which permits the continuous application of low doses of molluscicide without any labour costs, constitutes a novel approach to the snail control problem. It might have some application in very limited areas.

The incorporation of water-soluble molluscicides in porous *slabs* from which the molluscicide would be leached by water movement could be effective and economical in conditions in which the prolonged application of very low dosages provided effective snail control. It would seem that the solubility of the molluscicide in water is a limiting factor and that the technique described below for continuous low dosages would prove the more effective in use.

Capsules. Recent field experiments in a non-endemic area on the natural distribution of small quantities of concentrated formulations added to water as single doses at regular intervals suggest that this method is adequate for snail control. If this is confirmed and snail control achieved, the concentrated molluscicide formulation could be encapsulated and the capsules placed in water at appropriate intervals. Alternatively, measured " shots " could be applied by a syringe or suitable measure.

LABORATORY SCREENING OF MOLLUSCICIDES

Preliminary screening

Molluscicidal activity in a compound may be detected by empirical test or as the result of basic research. It is considered desirable, however, that the activity detected should be reported to WHO in terms of a standard test, the results of which can be compared with those obtained by other laboratories and with other samples.

Definitive screening

Standard methods for testing molluscicides at the definitive stage of laboratory screening are desirable in order to provide a common basis for the comparison of results obtained by workers in various parts of the world against different species of intermediate hosts and under various conditions. The standard tests at present have been revised in the light of more recent experience and are given in Annex 1. The tests for amphibious snails are given in the same Annex.

Comprehensive laboratory evaluation

In the light of the experience acquired since 1961, information has been collected that will serve as a valuable guide to workers on the test procedures available for more detailed laboratory evaluation of molluscicides. The procedures are given in Annexes 2 and 3, and where applicable the immersion test should be used and modified as indicated in Annex 1. There are still some methods of evaluation that need to be standardized, and recommendations have been made in Annex 3.

Characterization of molluscicidal activity

This subject is dealt with in Annex 2. The relevant tests are as follows:

1. Time-concentration relationships
2. Chemical stability of working dilutions
3. Residual potentials
4. Protective behaviour of snail against molluscicides
5. Stage-size array susceptibilities
6. Low prolonged concentrations against infected and uninfected snails
7. Physiological and pathological effects of molluscicides
8. Genetic resistance

Probably only the first two of these tests are essential before field screening is started.

Bioassay of the inactivating effects of physicochemical factors

Field conditions may indicate the need for carrying out such additional tests as determination of the pH, chemical analysis of the water, and investigation of the effects of the salt content, sunlight, the turbidity of the water, the behaviour of the molluscicide in both still and running water, the combination of ultra-violet light and of certain salts in the water in relation to the action of the molluscicide, and the differences in snail response between laboratory and field snails. Laboratory workers should maintain contact with field workers in order to observe any

differences in the performance of molluscicides. Special research studies should also be carried out in those exceptional circumstances in which molluscicides fail owing to unusual combinations of physicochemical factors.

Correlation between laboratory and field results

In general, good correlation has been observed between the action of molluscicides in the laboratory and in the field. Satisfactory correlation has been observed between results obtained in the laboratory using *Australorbis* and those obtained in the field using *Biomphalaria* and *Bulinus*. However, there have been instances where the performance of the molluscicide in the field has either excelled or fallen short of the performance expected from laboratory testing. In a few cases the discrepancy between laboratory and field results has been sufficient to justify some modifications to the existing laboratory methods. Additional laboratory tests, however, need to be carried out only when field performance falls short of the expected results.

With regard to modifications or additions to methods of procedure, the adoption of the sequential testing procedure is desirable, to enable special laboratory tests as suggested by observations of results from field trials to be carried out.

There should also be a reference molluscicide for comparing the performances of other existing or new molluscicides. NaPCP could be used for this purpose.

With regard to relating the performance of molluscicides to LC dosages, the determination of LC_{99} or LC_{100} by extrapolation is not acceptable. In initial field trials in both still and flowing waters the doses should be LC_{90} and twice and four times its value. In preliminary field trials on the effect of molluscicides on *Oncomelania*, starting dosage rates of 1, 5, and 10 g/m^2 of moist soil are useful.

In the testing of nearly all molluscicides there is a stage when testing passes from industry into the hands of some other organization for field evaluation. Delays at this important stage might be minimized if the chemical companies were to make members of their technical staff available to assist the field research organizations from time to time when indicated.

FIELD TESTING AND EVALUATION

Definition of the objectives of field trials

Three stages should be recognized in field trials:

(1) Field screening under controlled conditions, in order to:

(*a*) test the effectiveness of candidate molluscicides against various species of medically important snails under field conditions;

(*b*) examine the effect of molluscicides on other aquatic animals and plants; and

(*c*) test the relative effectiveness of various dosage regimes.

(2) Field evaluation under natural conditions, in order to:

(*a*) test the effectiveness of promising molluscicides under a variety of natural conditions;

(*b*) examine the effect of physical and chemical factors on the performance of molluscicides; and

(*c*) investigate handling properties and the effective distance travelled downstream in flowing water.

(3) Transmission control project.

The effect on bilharziasis transmission; evaluations at this stage should include long-term studies on the parasite load within the human population. Also to be considered: exposure of laboratory animals as cercarial detectors.

Studies on the timing of molluscicide applications and long-term studies on the rate of snail reinfestation.

Studies on the long-term effects of the molluscicide on the aquatic fauna.

Areas and habitats for different stages of field testing

Primary field screening can best be carried out under controlled conditions where the habitat may be artificial. There are several known centres where this kind of screening can be carried out.

Field trials under natural conditions can be carried out almost anywhere in the tropics. The aim at this stage of field testing should be to test the molluscicide under as many different conditions as possible.

Transmission control experiments are relatively expensive and time-consuming, calling for the participation of a team of specialists. At the jointly sponsored United-Arab-Republic/UNICEF/WHO Pilot Project Egypt 49, large-scale trials with several molluscicides are being carried out and evaluated from the standpoint of the effect on the transmission of bilharziasis.

Procedures for field testing

In view of the great variation in the kinds of habitat in different areas, it would be unwise to attempt to stipulate any particular sampling tech-

nique for field trials. In Japan, for example, *Oncomelania nosophora* is found chiefly on the soil above the water, whereas in the Philippines *Oncomelania quadrasi* is more commonly found beneath the surface of the water. Because of this difference in the distribution of the two species, techniques used in Japan may have to be modified in the Philippines. With regard to the aquatic snails, the procedures used in field trials in Egypt 49 appear to be applicable only to conditions in Egypt and are therefore unacceptable for general use. For instance, reconnaissance surveys and the co-operation of governmental agencies are unnecessary in Puerto Rico and Arusha Chini (Tanzania), where artificial field screening sites exist.

Nevertheless, despite the lack of uniformity in different places and the many differences in the techniques employed, some generalizations are possible. The data should be amenable to statistical analysis and sufficiently detailed to allow of the calculation of percentage kills within acceptable confidence limits. The collection of dead as well as live snails in both pre-treatment and post-treatment counts should be encouraged. The use of caged snails can often provide useful supplementary data. In places and seasons where the natural snail density is low this may be the only method of assessing the effect of the molluscicide. The cages, without metallic parts, should be designed so as to allow the snails freedom of movement; and natural foods should be present.

In most field trials workers can make only a limited number of chemical estimations. Water samples cannot be stored for examination at a later date. In flowing water the best use of a series of estimations is when the samples are all taken from two or three fixed sampling points. This enables the plotting of time-concentration curves and thus the accurate estimation of dosages in terms of ppm $\times$ hours. Accurate information on downstream losses can be obtained from a series of such curves.

In the past, various anomalies have arisen in field trials carried out in different parts of the world. Considerable variations in results from different areas have been due to the effects of various physico-chemical factors. It is therefore essential that field workers collect and report as much physical and chemical data as time and facilities allow. The following information should always be reported:

(*a*) the nature and size of the water body;
(*b*) the type and amount of vegetation;
(*c*) the temperature of the water; and
(*d*) the pH.

Additional information should include, whenever possible:

(*a*) complete chemical analysis of the water;

(*b*) the duration and intensity of the sunlight (the amount of ultra-violet light might be investigated in special cases where breakdown of the molluscicide is suspected);

(*c*) the turbidity; and

(*d*) the characteristics of the bottom stratum (e.g., the particle size of the mud and the organic content).

Mode of action and the formulation of the molluscicide

The optimum concentration-exposure time should be determined for each molluscicide. The product of the two variables is not necessarily a constant; if the exposure time is doubled, for example, the effective concentration is not necessarily halved. In this connexion, the exposure time may be limited by the adverse effect of such factors as sunlight, silt, and the chemical composition of the water and organic matter.

The method of application is to some extent governed by the type of formulations available. Emulsifiable liquids and soluble chemicals can be conveniently applied to flowing waters by feeding at a more or less constant rate at the head of the water course, and to impounded waters and swamps by spraying. Spreadable oil formulations are particularly useful in the treatment of reservoirs with heavy and dense vegetation. Wettable powders require some means of maintaining the chemical in suspension during the application period.

The use of non-ovicidal molluscicides requires special consideration. For effective snail control the so-called split-dose treatment may be considered. This consists of two applications spaced 2 to 3 weeks apart so that snails hatched after the first application are killed. The molluscicides may also be applied monthly during the transmission season.

A third method of using such molluscicides is by prolonged application (lasting for several weeks) of a very low concentration in irrigation systems where the flow of water is continuous. This method could make the treatment of large areas possible at very low application cost, and could also be considered for single-point application in natural water courses.

Transmission season

Snail population densities are affected by physical and hydrographic changes in the habitat and by

temperature. In warm localities where irrigation is practised all the year round, snail populations may be present at a high density throughout the year and at least two blanket treatments may be necessary. In other localities cold weather, floods, or desiccation by natural or artificial means may cause sufficient destruction of snails to render treatment temporarily unnecessary, and one or two treatments a year may suffice. Low winter temperatures also considerably reduce human contact with snails. Knowledge of when the population peak of infected snails occurs permits a more effective timing of the treatment.

The pattern of chemical dispersal in flowing water and large bodies of still water

It is widely recognized that many environmental factors affect the distribution of molluscicide in natural waters. Examples in flowing water are the vegetation, variation in the shape of the canal bed, and dilution by seepage or tributaries. The dispersal of molluscicide in swamps or marshes may be retarded or even inhibited by a large amount of vegetation. In ponds or lakes stratification of the molluscicide solution applied to the surface may occur. On the other hand, a chemical applied only to the marginal areas of a lake may be dissipated by wave action and underwater currents. Comparatively little is known at the present time about the relative importance of these environmental factors and how to neutralize them.

The use of chemical estimation

Simple reliable colorimetric procedures are available for the rapid field determination of NaPCP, Bayluscide, and copper sulfate,[1] and also for WL 8008. These techniques are used:

(*a*) to check the calculated concentration;

(*b*) to follow the distribution of active material in the water; and

(*c*) to follow the changes of molluscicide concentration in time and under the influence of turbidity, sunlight, vegetation, etc.

Field workers should bear in mind that chemical methods of assay supply valid information only when the sampling of water is representative of the total mass treated and the analytical technique is specific for the active ingredient of the product.

[1] World Health Organization (1965) *Snail control in the prevention of bilharziasis*, Geneva (*Monograph Series*, No. 50), p. 151-160.

The use of bioassay

The dispersal of a molluscicide may be followed by biological tests. For this purpose mature snails may be used. Other living aquatic organisms, such as fish, tadpoles, or shrimps, are sensitive and can serve as indicators of the dispersal of a molluscicide. Observations may be based on free-living or caged organisms.

Biological assay is very useful, and necessary not only for testing chemicals that cannot be easily analysed by chemical methods but also when chemical assay can be carried out. The biological test is particularly useful with caged snails to show whether a chemical has penetrated marginal swampy areas or shallow backwaters. It is possible that under such conditions the concentration of the molluscicide might be at or below the limit of chemical estimation but still exert lethal action upon the snail after a prolonged period of contact.

It is good practice in biological testing:

(*a*) to use snails collected in the field;

(*b*) to leave the snails undisturbed in cages for several days before and after the exposure;

(*c*) to avoid transport of the exposed snails to the laboratory; and

(*d*) to maintain a series of control cages under similar conditions.

The use of auxiliary methods, dyes, tracers

The use of fluorescent dyes in studying the flow of underground water and in river estuaries has been successful and seems likely under certain conditions to supply useful information about the dispersal of a molluscicide. However, while the dispersion and attenuation of the tracer dye will give information on the hydrographic characteristics of the habitat, they may not necessarily coincide with those of the molluscicide, and this might lead to misinterpretation of the results. Fluorescent dyes and radioactive tracers might be incorporated in a molluscicide.

Comparative tests have been made with sodium chloride (NaCl) solution as a tracer in combination with a molluscicide, and in most cases the tracer has shown a distribution analogous to that of the molluscicide.

In flowing water the time-concentration curves determined independently for the combinations NaPCP-NaCl and Bayluscide-NaCl at various points downstream were in the same phase and showed

similar proportions. These results suggest that no significant difference may exist between the distribution pattern of the two molluscicides and NaCl.

The areas under the time-concentration curves determined for NaCl at various distances downstream have shown a constant value within the limits of experimental error. It seems likely that in this way the actual loss of a molluscicide might be determined by comparing the time-concentration integral (= areas under the curve) obtained for a given product with those of the tracer compound.

Dispersal according to formulation

Few quantitative field observations have been made regarding the influence of the formulation upon the dispersal of the molluscicide. A spreading oil formulation has been tested on a few occasions and shown promise. Emulsifiable concentrates injected below the surface of the water at regular intervals gave a uniform distribution after 24 hours in a small pond. Sparingly soluble solid molluscicides may get lost in standing water when the bottom is muddy, but give complete solution and good distribution when applied to flowing water in the form of wettable powder of suitable particle size. Emulsion concentrates are more likely to produce uniform concentration in the water during a prolonged period of application, or when very simple equipment without mechanical agitation is being used.

The interpretation of snail reinfestation after treatment

By means of molluscicides it is possible to achieve very high mortalities in populations of aquatic snails, though it is seldom possible to obtain total elimination of snails from the habitat. In the case of amphibious snails (*Oncomelania*), mortalities exceeding 95% can rarely be produced.

The methods of sampling field populations of snails make it difficult to assess the important borderline between high mortality and near or total elimination of snails. The breeding capacity of snails is such that it is only by near elimination that repopulation can be delayed.

A study of the reinfestation of a habitat after the application of a molluscicide is essential in transmission control experiments, and in many cases may prove informative in field trials. Sampling methods for studying reinfestation must be different from those for standard population studies, requiring determination of the extent of reinfestation and a much larger number of samples.

The pattern of reinfestation may be useful in assessing:

(*a*) the effectiveness of the application of molluscicide;

(*b*) the frequency of treatment needed;

(*c*) the possibility of the development of select or acquired resistance to the molluscicide; and

(*d*) the relative merits of different formulations, techniques of application, and dosage regimes.

Resurgence of snail populations may occur after application of a molluscicide. One possible reason for this is high survival rates following the removal of predators and parasites. There might also be a density-dependent factor involved, i.e., an accelerated rate of breeding when the surviving snails are released from conditions of crowding and competition. These very important possibilities require investigation.

In some areas the first surviving snails observed following molluscicide application were consistently found in the same isolated foci. The persistence of these foci despite repeated application of molluscicide must be investigated to determine the reasons for this failure. In the majority of cases the failure may be due to inadequate dispersal of the chemical.

Some possible causes of repopulation are as follows:

(*a*) Inadequate application and dispersal of the molluscicide.

(*b*) The presence of less susceptible stages or individuals in the snail population. An example was cited of differences in the thickness of the gelatinous layers covering individual eggs in egg masses. Penetration of the thicker gelatinous layers by the chemical might be hindered and individual eggs might survive.

(*c*) Snails may show a behaviouristic tendency to move upstream, as has been demonstrated in Brazil and Puerto Rico.

(*d*) Snails may be washed downstream from untreated areas.

(*e*) Snails may be protected from the chemical by light layers of silt or mud or by vegetation.

(*f*) Even aquatic snails may survive on banks of canals or streams above the water line. These snails would be unaffected by the chemical.

(*g*) Some snail species are able to burrow into and aestivate in mud in a drying habitat. These snails will not be exposed to the molluscicide and may

repopulate the habitat when it again fills with water.

(*h*) Snails may be introduced into the habitat by mechanical means, e.g., by man in mud on boots, ploughs, etc., or by animals, birds, and insects. An example is snail egg masses found on bugs of the family Belostomatidae some distance from water.

SUMMARY AND RECOMMENDATIONS

1. The signatories prepared a table on molluscicides and their properties that supplements and brings up to date the one contained in the report of the WHO Expert Committee on Bilharziasis.[1]

2. The signatories recommend Bayluscide as the best molluscicide commercially available and the one to consider first in any control programme. There is, however, a need for molluscicides and formulations with different qualities to deal with all the different conditions of snail habitat and control needs in different countries.

3. Since the range of currently available molluscicides does not meet all requirements, the search for more effective compounds should continue.

4. The two main phases of laboratory investigation on molluscicides are laboratory screening—subdivided into preliminary screening and definitive screening—and comprehensive laboratory evaluation. Special attention is given to the revising of standard methods for definitive screening, and new guide lines are recommended for comprehensive laboratory evaluation, in which there have been particularly marked recent advances in knowledge and experience.

5. In order to achieve a closer correlation between the laboratory findings and the field performance of molluscicides, the adoption of a sequential testing procedure is recommended, with special laboratory tests designed to clarify field observations.

6. In general the efficacy of molluscicides in the field is liable to be influenced adversely by special combinations of salts in natural water in the presence of light. In silt-laden waters molluscicides may, however, be protected from the action of sunlight (though this may be offset by absorption of the chemical by silt particles). Special research studies should therefore be carried out in those exceptional circumstances in which molluscicide failure may be due to particular combinations of physicochemical factors.

7. The different phases of field testing need to be clarified. There are three stages: field screening; field evaluation under natural conditions; and transmission control projects.

8. The choice of procedures for field testing is considered, and specific recommendations are made on sampling techniques, the chemical estimation of dosage and the development and use of field test kits, and the collection of ancillary physicochemical data.

9. Some of the possible causes of snail repopulation or resurgence following withdrawal or interruption of molluscicide treatment are listed. One important possibility particularly recommended for investigation is that resurgence might be due to an accelerated rate of breeding by surviving snails released from conditions of crowding and competition. The repopulation liable to occur in some areas in the same isolated foci despite repeated application of molluscicide is also in need of investigation.

10. More rapid exchange of information, ideas, and test data is needed. From this point of view, personal visits to field staff play a great part in stimulating and providing exchange of information, as has been shown by the work of the WHO Bilharziasis Advisory Team.

11. The speed and efficiency of molluscicide evaluation need to be increased. Among other things, it is proposed that chemical companies—apart from those engaged in molluscicide screening—and other bodies able to provide compounds of possible molluscicidal value should be informed of the channels available for getting compounds evaluated in the laboratory and the field by WHO collaborators. The collaborative laboratories in turn should actively seek other sources of molluscicides, so that a greater number of compounds of different chemical structure becomes available for preliminary screening. Manufacturers should furnish full particulars of the conditions under which potency results are obtained and of test methods used; an early indication of the cost range or estimate would also be of assistance. Consideration should also be given to determining criteria by which a candidate molluscicide is declared unworthy of further evaluation.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1961, **214**, 44.

N. O. CROSSLAND, Tropical Pesticides Research Institute, Arusha, Tanganyika

V. de V. CLARKE, Bilharziasis Research Laboratory, Causeway, Salisbury, Southern Rhodesia

R. DESCHIENS, Institut Pasteur, Paris, France

J. DUNCAN, Tropical Pesticides Research Committee, Tropical Products Institute, London, England

R. A. E. GALLEY, Research Director and Manager, Woodstock Agricultural Research Centre, Sittingbourne, Kent, England

R. GÖNNERT, Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany

Y. KOMIYA, Department of Parasitology, National Institute of Health, Tokyo, Japan

D. B. MCMULLEN, Scientific Adviser, Walter Reed Army Institute of Research, Washington, D.C., USA

R. C. MUIRHEAD-THOMSON, Biologist, Parasitic Diseases, Division of Communicable Diseases, World Health Organization, Geneva

E. PAULINI, Chief, Chemical Laboratory, Centro de Pesquisas de Belo Horizonte, Instituto Nacional de Endemias Rurais, Belo Horizonte, Brazil

L. S. RITCHIE, Chief, Parasitology Branch, United States Army Tropical Research Medical Laboratory, San Juan, Puerto Rico

RÉSUMÉ

Dans ce mémoire consacré aux molluscicides utilisés dans la lutte contre la bilharziose et, plus spécialement, aux techniques et méthodes devant servir à sélectionner ces produits et à évaluer leur efficacité en laboratoire et sur le terrain, les signataires font un certain nombre de mises au point et de recommandations. Les caractéristiques et propriétés de douze molluscicides considérés à l'heure actuelle comme les plus prometteurs sont résumées dans le tableau 1 qui constitue une révision de l'Annexe 4 au rapport du Comité OMS d'experts de la Bilharziose réuni en 1960.[1] Chacun de ces produits présente des avantages et des inconvénients particuliers (ce point est illustré au tableau 2) dont les utilisateurs doivent être avertis pour fixer leur choix sur celui qui répondra le mieux aux objectifs des opérations de lutte, aux conditions écologiques locales et aux ressources en matériel et en hommes dont ils disposent.

De l'avis des signataires, le Bayluscide est, pour le moment, le meilleur des molluscicides existant dans le commerce. S'il est recommandé d'envisager son emploi en premier lieu, on devra néanmoins avoir recours à des molluscicides et formulations dont les propriétés différentes conviennent mieux, notamment pour certains types d'habitats de mollusques. Etant donné que la gamme de composés disponibles ne couvre pas tous les besoins, il est nécessaire de poursuivre les travaux de recherche de produits plus efficaces.

L'essai en laboratoire des molluscicides nouveaux s'effectue en deux étapes: la première concerne la sélection proprement dite, elle-même subdivisée en sélection préliminaire et sélection définitive; la deuxième vise l'évaluation complète des produits retenus. Les techniques standard à utiliser plus particulièrement pour la sélection définitive font l'objet d'une révision (Annexe 1), et de nouvelles directives sont proposées pour les épreuves d'évaluation (Annexe 2: caractérisation de l'activité molluscicide; Annexe 3: épreuves biologiques des effets inactivants dus à des facteurs physico-chimiques).

Afin d'assurer une bonne concordance entre les résultats de laboratoire et les essais sur le terrain, il convient également de suivre pour ces derniers une procédure progressive comportant trois étapes: (1) sélection des produits sur le terrain dans des conditions contrôlées; (2) évaluation de leur efficacité sur le terrain dans des conditions naturelles; (3) application dans un projet de lutte visant à interrompre la transmission. Lors des essais préliminaires en eau stagnante aussi bien qu'en eau courante, on utilisera la CL_{90} ainsi que deux et quatre fois cette valeur pour étudier la performance des molluscicides, et non la CL_{99} ou CL_{100} obtenues par extrapolation, qui sont inacceptables. Pour les essais de molluscicides destinés à combattre *Oncomelania*, on choisira pour commencer les doses de 1, 5 et 10 g/m² de sol humide.

Les diverses techniques, et leurs indications propres, sont détaillées (échantillonnage des populations de mollusques, prélèvements d'eau ou de sol pour dosage du molluscicide, emploi de trousses d'épreuves, rassemblement des renseignements sur les facteurs physico-chimiques secondaires, épreuves physiologiques, emploi de colorants fluorescents ou d'autres procédés de marquage, etc.). Enfin, les essais sur le terrain seront complétés, au dernier stade des expériences d'interruption de la transmission, par une étude de la réinfestation des lieux traités. Cette étude est essentielle pour déterminer les causes de réapparition des mollusques. Il y aurait lieu d'examiner tout particulièrement si celle-ci ne serait pas due à un taux de reproduction accéléré parmi les mollusques survivants libérés des contraintes du surpeuplement et de la compétition. Il conviendrait également d'approfondir les raisons de la réapparition des mollusques dans certaines régions de foyers pourtant isolés, en dépit d'applications répétées de molluscicides.

Les auteurs soulignent la nécessité d'échanger rapidement tous renseignements, idées et résultats d'essais. Selon eux, l'évaluation des molluscicides devrait être activée. A cet effet, les fabricants de produits chimiques qui ne pratiquent pas les épreuves de sélection des molluscicides, et d'autres organismes en mesure de produire

[1] *Org. mond. Santé Sér. Rapp. techn.*, 1961, **214**, 50.

des composés susceptibles d'avoir une action molluscicide doivent être informés qu'ils peuvent faire tester et évaluer leurs produits en laboratoire et sur le terrain par des instituts, laboratoires ou équipes collaborant avec l'OMS. Ces derniers devront rechercher d'autres sources de molluscicides de façon à élargir l'éventail des composés de structure différente soumis aux épreuves de sélection préliminaire. Les fabricants sont également invités à fournir des renseignements détaillés sur les conditions et les méthodes utilisées par eux pour déterminer l'activité de leurs produits ainsi qu'une indication du coût approximatif de la fabrication.

Annex 1

DEFINITIVE LABORATORY SCREENING—STANDARD METHODS

1. Snail source	*Immersion test—aquatic and amphibious snails* Aquatic snails: laboratory-reared snails, and/or uninfected local vector snails. Details to be given in reports. Amphibious snails: as for plate test.	*Plate test—amphibious snails only* Active, freshly collected (24-48 hrs) field snails.
2. Standard strain	Use of the same strains of snails in all laboratories would be the ideal but is probably not feasible; the strain might cease to be standard in the different conditions of culture and might preclude successful use of a specific molluscicide in certain endemic areas where snails of high susceptibility occur. Each collaborating laboratory should therefore use its own strain and differences in existing strains should be confirmed.	
3. Nature of container	The containers and the composition of the materials used in the containers have various advantages and disadvantages, depending on the compound being tested. Each laboratory should experimentally evaluate the possible effects of the containers, and report the type(s) used. For amphibious snails, as for plate test but with dish 3 cm in depth.	150 cm x 1 cm Petri dish, of glass or disposable plastic.
4. Container size (ml) 5. Volume/snail (ml)	Tests have suggested that the volume per snail probably should not be less than 40 ml for aquatic snails. For amphibious, 10 ml.	
6. Bottom covering		One sheet of fine-grained filter paper.
7. Number of containers	Two containers at each concentration with 10 snails each are deemed a test minimum. The use of 5 snails per test with four containers at each concentration is an acceptable alternative.	(*a*) Two when a single concentration is used. (*b*) One for each concentration if three or more concentrations are used.
8. Number of snails	On the basis of the number of containers, most tests might be expected to require at least 80 snails, which is considered a minimum for computing LC_{50} and LC_{90} values.	10 per container, placed in the centre of the paper.
9. Snail age	Aquatic snails should be young-mature and relatively uniform in age and size. Daily or weekly use of control tests with a reference molluscicide would detect unfavourable changes in susceptibility with increased age. Amphibious snails as for plate test.	Mature snails, uniform in size.
10. Cover	If toxicity is the specific objective of the test rather than protective behaviour (a test reserved for comprehensive evaluation), a cover is indicated. Types of cover have not been adequately tested for aquatic snails, because it has been noted that susceptibilities are altered according to the type of material used. For amphibious snails, vinyl net stretched on wooden frame.	The Petri dish cover.
11. Water	Dechlorinated tap water. Aerated standard reference water (10 % hardness) should be used to assay the influences imposed by local water.	Dechlorinated tap water.
12. Chemical concentration	A 10-fold dilution' (solution, suspension, emulsion, etc.) series of 0.1, 1.0 and 10 ppm appears to be in general use, and in some cases, a twofold series is further used after the critical range is determined.	(*a*) 100 ppm for single concentrations; (*b*) 10, 100, and 1000 ppm for multiple concentrations and a twofold series can be used after the critical range is determined. If not soluble in water, use a volatile solvent if possible. If insoluble, use finely divided material to get a suitable distribution.

Annex 1 (continued)

13. Preparation of solutions	Some laboratories use serial dilutions, others dilutions prepared by micropipette ; experimental evaluation is indicated.	As in immersion test.
14. Volume used		2 ml evenly distributed on filter paper
15. Aeration	It is believed that aeration is not necessary during the exposure or recovery periods.	
16. Pre-exposure handling		Allow solvent to evaporate.
17. Wetting of impregnated paper		3 ml of water at time snails are added.
18. Light	Laboratory lighting with normal diurnal light changes. Experimental evaluation of the effects of laboratory lighting is indicated as this may affect the result.	As in immersion test.
19. Mixing	Mixing during test not considered necessary.	
20. Temperature	26°-28°C.	26°-28°C.
21. Period of exposure	Exposures of 24 hours are thought adequate and a matter of convenience. Other exposure intervals may be used for comprehensive evaluation and in experiments with operculated amphibious snails.	4 days.
22. Observations during exposure	Behaviour that may be protective is important, but specific testing is indicated as a part of comprehensive evaluation.	First day: Observe three times, note reactions, centre snails, add water as required to keep paper well moistened but leave no free water. Second day: Observe once, note reactions, centre snails, and add water as required. Third day: As on previous day. Fourth day: Note reactions and prepare for final examination.
23. Washing	For aquatic snails, rinsing under tap or 3 changes of standard water. For amphibious snails, as for plate test but place snails in a clean Petri dish.	In small strainer. Place washed snails in respective Petri dish lid, add water to cover.
24. Recovery period	The recovery period should be varied according to the action of specific chemicals. The possibility of a carry-over of the chemical on the snail into the recovery water should be borne in mind.	
25. Criterion of death	Death criterion for aquatic snails: by examination (discoloration, heart rate, activity of muscle, etc.) and/or crushing. For amphibious snails, as for plate test.	Crush all snails showing no activity.
26. Food during test	Feeding is not thought to be necessary during exposure or recovery period as snails are able to survive some days without apparent ill-effects.	As in immersion test.
27. Snail controls	1 or 2 containers with 10 snails each is preferred.	5 containers, 10 snails in each
28. Molluscicide control	Trials using a reference compound should be repeated regularly.	Determine LC_{50} with NaPCP at concentrations of 20, 50, and 100 ppm, 2 containers for each concentration, once per month.
29. Determination of LC_{50}	LC_{50} and LC_{90} values should be computed, particularly when the standard test is used in comprehensive evaluations. The use of the probit analysis method appears to be replacing the Litchfield & Wilcoxon statistical method.[a]	As in immersion test.

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

Annex 2

COMPREHENSIVE LABORATORY EVALUATION — TEST PROCEDURES FOR CHARACTERIZATION OF MOLLUSCICIDAL ACTIVITY

Test 1. *Determination of time-concentration relationships*

Exposure times : 24, 6, and 1 hour(s).

Chemical concentrations: dilution series for each exposure time suitable for computing the LC_{50} and LC_{90} values.

Type of snails : young-mature.

Number of snails: 3 or more replicates of 10 snails each.

Manner of testing: except for the above, according to WHO provisional plan.[1]

Test 2. *Chemical stability of candidate molluscicides*

Stability under working dilutions. Litre quantities of water with LC_{90} value for 24-hour exposures are set up and the following intervals are allowed to lapse before snails are added: 6 hours and 1, 2, 4, and 8 days. (Longer intervals if indicated.) Exposure time 24 hours, followed by 24-48-hour recoveries.

Stability in storage. If a chemical shows promise through the time-concentration test, samples should be set aside in covered but unsealed containers, and allowed to stand for testing after 6 months and 1, 2, and 3 years. Storage stability tests should not be carried out until formulations have been evaluated in field tests.

Test 3. *Residual potentials of a molluscicide*

The concentration of molluscicide equivalent to five to ten times the 24-hour LC_{90} value of a compound required for 1 litre of water is pipetted from a 1 % solution on to 2 filter-paper circles (90 mm diameter). The papers are then air-dried away from contact with any object that might cause loss of chemical. After drying, the surfaces of application are brought together and the margins of the circles bound together with waterproof masking tape. The papers can be used in various ways to simulate still water, flowing water, etc. This may give data indicative of how the material may behave under field conditions.

Test 4. *Protective behaviour of snails against molluscicides*

This test is concerned with the tendency of snails to crawl out of the test container, the speed with which they contract into their shells, any tendency to become distended or narcotized, any " surfacing behaviour," and any other behaviour that fosters their survival or makes them more vulnerable.

Test 5. *Stage-size array susceptibilities*

Stages and sizes to be tested :[2]

Eggs — newly laid
— incubation nearly complete
Snails — newly hatched (1-24 hours old)
— juveniles (3-5 mm diam.)
— adolescents (8-10 mm diam.)
— mature (13-15 mm diam.)

Exposure times

The choice of exposure time must depend on results obtained from time-concentration relationships. The shortest interval that gives nearly maximum efficiencies is indicated. Possibly a second interval should also be used. For compounds like ICI 24223, 1- and 6-hour exposures might be chosen, while for NaPCP 6- and 24-hour exposures are indicated.

Concentrations :

Dilution series for each stage or size.

Number of test organisms :

Eggs: For amphibious snails at least 4 replicates of at least 10 eggs for each stage and each concentration. For aquatic snails at least 4 replicates at each concentration using egg clutches containing at least 10 eggs.

Newly hatched: at least 4 replicates of 20 specimens exposed in 50 or 100 ml of test medium.

Older snails: at least 4 replicates of 10 snails each.

Manner of testing : except for above, according to WHO provisional plan and Ritchie et al. (1963).[3]

Test 6. *Effects of low, prolonged molluscicidal concentrations against the snail and its schistosome infection*

Possible tests : to be developed.

Test 7. *Physiological and pathological effects of molluscicides on snails*

The need for studies on the physiological action of molluscicides has been previously stated.

Test 8. *Genetic resistance of snails to molluscicides*

It is doubtful whether laboratory tests should be pursued, at least until there is more evidence for the need. Evidence of resistance among field populations should be sought.

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[1] *Wld Hlth Org. techn. Rep. Ser.*, 1961, **214**.

[2] Most of the work on stage array susceptibility has been done with *A. glabratus*. The size-arrays given refer to this snail; if tests are made on other species, snails of comparable age should be used.

[3] *Bull. Wld Hlth Org.*, 1963, **29**, 281.

Annex 3

COMPREHENSIVE LABORATORY EVALUATION — TEST PROCEDURES FOR BIOASSAY OF THE INACTIVATING EFFECTS OF PHYSICOCHEMICAL FACTORS

Test 1. *Inactivating effects of sunlight*

(*a*) *Test procedure using artificial irradiation in the laboratory.* Lamps are becoming available which can be used to produce an intensity and quality of ultra-violet light approximating to those found under natural conditions. Reproducible results should be possible using this kind of lamp.

Care must be taken to use solutions of standardized depths because ultra-violet light is readily absorbed by water more than a few centimetres deep. For initial tests it would probably be best to expose the molluscicide to ultra-violet light in shallow enamel trays.

(*b*) *Complementary tests using sunlight.* These tests should be carried out to determine the effects of ultra-violet irradiation under natural conditions in the endemic area.

Intervals of exposure to sunlight before introducing snails: 1, 2, and 4 hours.

Control tests. Bayluscide and NaPCP are always used as standards.

Bayluscide: 0- and 4-hour exposures to sunlight.

NaPCP: 0-, 1-, and 2-hour exposures to sunlight.

With candidate molluscicides there should be parallel tests under sunlight and under laboratory light with snails introduced at intervals of 0, 1, 2, and 4 hours after molluscicide exposure to those conditions.

Chemical concentrations

LC_{90} values obtained in time-concentration tests. Standards: Bayluscide — 0.1 ppm, NaPCP — 2 ppm.

Exposure time : 24 hours.

Recovery time : 24 hours or 48 hours.

No. of replicates : 2 for each test and control category.

Water temperature: 27°-28°C, these temperatures being maintained by setting containers exposed to sun in flowing water.

Light readings : Weston Meter (Model 745). Incidence readings of 500-700.

Test 2. *Inactivating effects of physicochemical absorption*

Although less than 1 ppm of copper sulfate will kill mature *Australorbis* (13-15 mm) after 24-hour exposures in a laboratory test, 20-30 ppm are commonly required under field conditions. This is explained as being due to " adsorption " by mud or organic materials, but the physical or chemical factors apparently involved are not well understood. Standardized tests for detecting such factors are needed.

Several materials have been used, including rat faeces, bentonite, powdered charcoal, kaolin, refined proteins (e.g., powdered liver), and cholesterol crystals (as used in serological tests). It is proposed at present that the faeces of some laboratory animal be used, e.g., of rats that are on a uniform diet. These should contain various substances commonly occurring as contaminants in natural waters. A standard concentration (ppm) must be determined—50 ppm appears to be a good concentration to start with. Faeces might bring about physical adsorption, chemical binding, or bacterial breakdown of the molluscicide.

An initial test with charcoal powder indicates excessive adsorption activity, as Bayluscide (LC_{90}) was completely inactivated with a 1-hour " waiting period " and 24-hour exposure. Bentonite is currently under test and appears to have possibilities. Cholesterol-lecithin crystals probably are not sufficiently adsorptive, and refined proteins may not provide a broad enough spectrum of activity. The effects of green plants, including algae, should be assayed.

Test 3. *Inactivating effects of minerals in natural waters*

Minerals of various types, particularly those causing water-hardness, should be tested separately and in combinations of varying concentrations. Initial tests with separate compounds have not revealed much detoxifying activity.

Test 4. *Inactivating effects of pH on molluscicides*

There appears to be general agreement regarding the importance of the pH on molluscicidal activity. Hitherto tests have been developed in one or another particular laboratory and it seems desirable, before trying to give specific details, for more collaborative research to be undertaken.

Test 5. *The effect of temperature on molluscicidal action*

It has been shown clearly that temperature affects molluscicidal action, but field control efforts may be largely within a temperature range where this effect is of no significance. For this reason temperature may be of less importance than most physicochemical factors; however, tests may be indicated.