

Guidelines for laboratory and field testing of molluscicides for control of schistosomiasis

I. Ayi, Fabrice Chandre, P.M.Z. Coelho, A.K. El-Harawy, M.A. Elemam, K. Gachuhi, D. Jian-Rong, C. Kariuki, H. Madsen, Hélène Moné, et al.

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GUIDELINES
FOR LABORATORY AND FIELD TESTING OF MOLLUSCICIDES FOR CONTROL OF SCHISTOSOMIASIS



World Health Organization

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Abbreviations

AI	active ingredient
EC	emulsifiable concentrate
FAO	Food and Agriculture Organization of the United Nations
LC_{50}	concentration that results in 50% mortality
LC_{95}	concentration that results in 95% mortality
SC	suspension concentrate
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WP	wettable powder



1. Introduction

The purpose of these guidelines is to provide specific, standardized procedures and criteria for efficacy testing and evaluation of molluscicides for schistosomiasis control. The aim is to harmonize the testing procedures carried out in different laboratories and institutions in order to generate comparable efficacy trial data required for registering and labelling such products by the national regulatory authorities.

Schistosomiasis, also known as bilharziasis, is caused by trematode worms of the genus *Schistosoma*. The lifecycle of the parasites involves mammalian hosts, including humans, and freshwater snails as intermediate hosts.

Molluscicides are chemicals, of synthetic or biological origin, used primarily to kill various species of molluscs, including intermediate host snails involved in the lifecycle of schistosomes. In the past, numerous molluscicides have been used in schistosomiasis control programmes (WHO, 1992). However, during the past 35 years niclosamide has been the most widely used compound. Nevertheless, there are certain limitations with the use of formulations based on niclosamide including restrictions on their potable water usage immediately after application and temporary high toxicity to non-target aquatic organisms including fish, amphibians and invertebrates (USEPA, 1999; WHO, 2002).

There is a need for new and safer compounds for use as molluscicides, especially with the renewed interest in control and elimination of schistosomiasis as recommended by resolution WHA65.21 adopted by the Sixty-fifth World Health Assembly in 2012. Although currently there is little evidence for chemical resistance in snail populations, it is necessary to search for new chemicals to replace those that may become less effective after several generations of applications in the field, or there could also be development of new active ingredients (AIs) that are more specific to snails hosting schistosomes.

In 1961, WHO published guidance on laboratory screening and field efficacy testing of molluscicides (WHO, 1961), which was updated in a memorandum drafted during the WHO Informal Meeting of Investigators on Molluscicide Screening and Evaluation in 1964 (Anon., 1965). Thereafter, WHO published a systematic review on snail control in the control of schistosomiasis together with detailed methodologies for studying all aspects related to the intermediate hosts including practical details on molluscicide application (WHO, 1965). Further recommendations were made and research needs listed in 1970 (WHO, 1971). In 1992, a practical manual on the use of molluscicides in schistosomiasis control was published (WHO, 1992). An operational manual on the field use of molluscicides for programme managers of schistosomiasis control programmes has recently been published by WHO (WHO, 2017).

This document provides up-to-date guidance on laboratory studies as well as small-scale (semi-field) and large-scale field trials to assess the efficacy and determine field application rates of new molluscicide products for control of schistosomiasis. *Table 1* summarizes the phases of evaluation and the aims of these studies.

Prior to commencing testing of new candidate molluscicides, the manufacturers must make ecotoxicology profiles available for hazard and human exposure assessments. WHO specifications are required to be developed according to the FAO/WHO specifications manual (FAO/WHO, 2016). WHO is required to assess human risk relating to the use of new molluscicide formulations according to the WHO generic risk assessment model (WHO, 2018). The procedures outlined in this guideline document provide some additional qualitative information on the safety and toxicity of molluscicides for non-target organisms.

Table 1. Aims of various stages of evaluation of molluscicides

Phase	Type of study	Aim
I	Laboratory studies	• Establish dose–response curve (determine LC_{50} and LC_{95})
		• Bioefficacy against eggs and adult snails (determine effective concentration and residual activity)
		• Discriminating concentration[1] for resistance monitoring
		• Assessment of cross-resistance
		• Human risk assessment[2]
II	Small-scale field trials	• Initial and residual efficacy against snails under semi-field conditions and in limited ecological settings
		• Determine optimal method and rate of application
III	Large-scale field trials	• Initial and residual efficacy of optimal application rates against snails in large areas with natural water bodies
		• Observe operational and community acceptance
		• Record the effects on non-target organisms

1. Discriminating concentration is determined as twice the minimum concentration that yields 99.9% or 100% mortality in laboratory bioassays by testing incremental dosage of molluscicide with 24 h exposure.

2. Human risk assessment of only the reference formulated product should be conducted according to a WHO generic risk assessment model (available: https://apps.who.int/iris/bitstream/handle/10665/276706/9789241515047-eng.pdf) and is outside the scope of this document.

2. Laboratory studies (phase I)

2.1 Objectives

The main objectives of laboratory testing are to evaluate the efficacy and determine the intrinsic toxicity of new technical grade compounds with molluscicidal activity by establishing a dose–response curve.

The major aims and objectives of the Phase I testing are:

- to establish dose–response curve(s) against intermediate host snail species for determining the lethal concentrations (LC) of the molluscicide that produce 50% (LC_{50}) and 95% (LC_{95}) mortality of adult snails;[1]
- to determine the minimum effective concentration that kills 100% of a snail species and residual activity;
- to establish a discriminating concentration for monitoring resistance to the molluscicide in the field populations of intermediate host snails; and
- to assess cross-resistance to other compounds.

When a new molluscicide formulation is to be tested for the first time, the hazard data on its active ingredient and human risk assessment data should be presented by the manufacturer. The product should be tested in Phase II only after clearance of human risk assessment.

2.2 Materials, test species and test conditions

Materials required for testing

The main materials include laboratory wares such as glass beakers of 100, 500 and 1000 mL capacity (no plastic containers should be used to hold treated water); micropipettes, pipettes, disposable tips, droppers, graduated measuring cylinders, gloves, netting pieces, a bucket with dechlorinated water, a timer and data recording forms. The dechlorinated water for testing must be from the same source as the water routinely used for snail rearing.

Test organisms – adult snails

To evaluate the biological activity of a molluscicide, preferably laboratory-reared intermediate host snails are exposed to water treated with the candidate molluscicide at various concentrations within its activity range, and mortality is recorded. For species that are difficult to rear in the laboratory, snails should be collected from sites with minimal human–water contact and in the laboratory they should be checked for patent trematode infections repeatedly over 1–2 weeks using the shedding procedure. Snails with patent trematode infection (any species) should be discarded.

[1] Include 95% confidence intervals around the estimates.

Batches of exposed snails are compared with unexposed batches (i.e. untreated control). Amphibious snails are also tested in the same way by exposing them to treated water.

Laboratory-reared, egg-laying adult snails should be used for the Phase I testing. Ideally, the intermediate host snail species tested should be those of major importance for the region (Figure 2.1). Only certain intermediate snail host species primarily belonging to the genera Bulinus, Biomphalaria, Oncomelania and Neotricula play a role in transmission of schistosomiasis (WHO, 2017). It is important to use homogenous populations of snails of the same developmental stage and of uniform size. Mean shell height (with 95% confidence interval) of Oncomelania spp. and Bulinus spp. or shell diameter of Biomphalaria spp. must be documented. Any inactive snails must be excluded. Depending on the claims of the manufacturer, additional tests may also be conducted against juvenile snails.

Test organisms – eggs of snails

Depending on the claims of the manufacturer, test may be conducted on eggs using the minimum concentration that killed 100% of adult snails. Eggs of pulmonate snails aged 3–5 days should be exposed to test concentration using standard methodology for immersion tests described below (section 2.3.1). Mortality in eggs should be determined through observation of hatching rate until one week after exposure. Ten egg masses should be exposed per replicate, with the same number in the untreated (negative) control. Mortality is calculated by the proportion of eggs in each egg mass that didn't hatch out.

Test conditions

Molluscicidal effects are influenced by several factors such as snail species, snail development stage (eggs and adult snails), and environmental factors such as water hardness, pH and its other chemical characteristics. It is critical to standardize these test variables to ensure reproducibility of results. Information on the speed of activity of the active ingredient is important, as longer holding periods for recording delayed mortality can be justified for slow-acting molluscicides.

For the immersion bioassays, the test conditions are as follows: water temperature of 25 ± 1 °C, water pH of 7.0 ± 0.5, and photoperiodicity 12:12 h (light:dark). Water temperature and pH must be recorded at the beginning and the end of the exposure period. Water aeration devices should not be used during exposure. The containers must be covered by netting pieces with water filled up to the top of the beaker to prevent the snails from escaping the test solution.

Preparation of stock solutions and test concentrations

The technical materials of many organic molluscicide compounds are insoluble in water. These materials have to be first dissolved in appropriate organic solvents such as acetone or ethanol (the manufacturer should be consulted) in order to prepare diluted solutions for laboratory testing. The formulated products are, however, miscible with water. Suspending or mixing these formulations in water requires no special equipment – a homogeneous suspension can be obtained by gentle shaking or stirring. Test solutions should be prepared shortly before testing.

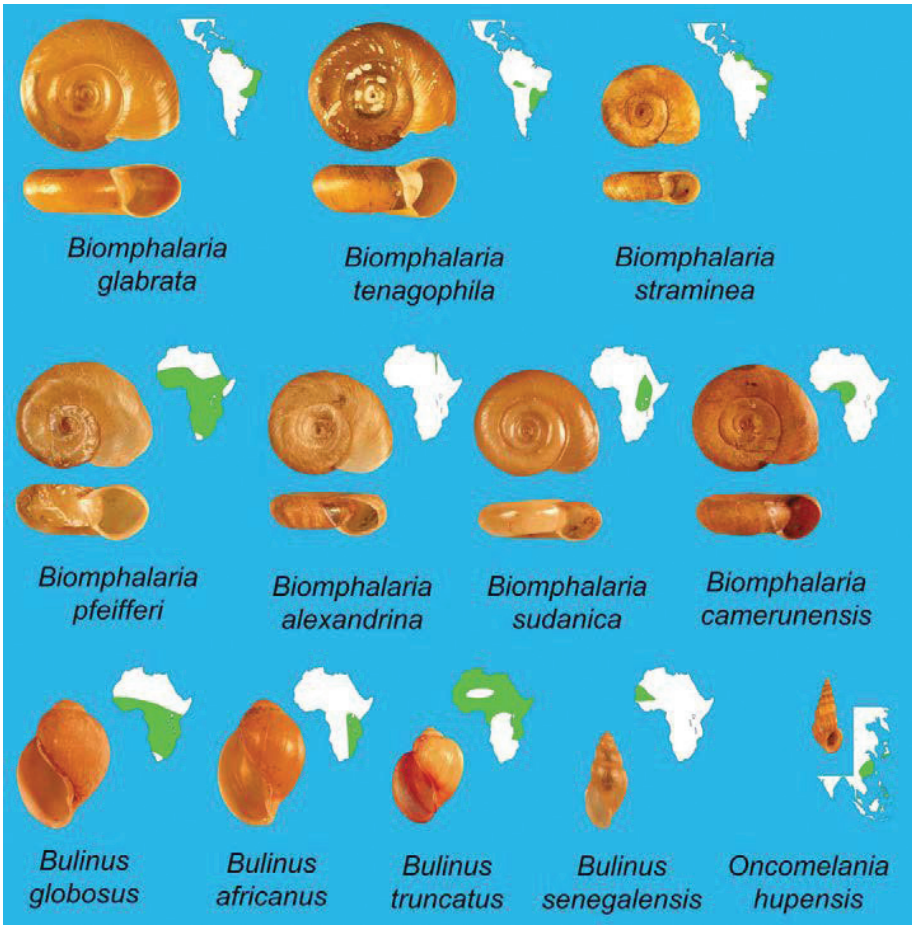


Figure 2.1 Distribution of major intermediate snail host species of major global importance (adapted by Dr H. Madsen from World Health Organization (1989) slide set: The intermediate snail hosts of African schistosomiasis. Taxonomy, ecology and control)

Examples of measurement and conversions are given in *Annex 1* and dilution and concentrations are given in *Annex 2*.

2.3 Determination of biological activity

2.3.1 Intrinsic molluscicidal activity

The objective is to determine the intrinsic toxicity (biopotency) of a technical material (active ingredient) with molluscicidal properties. It is determined by establishing a dose–response curve in immersion bioassays against a target snail species i.e. either aquatic (Biomphalaria and Bulinus spp.) and/or amphibious (Oncomelania spp.) intermediate host snails. Testing of new candidate molluscicides may include a positive control, such as niclosamide, and must always include a negative control of untreated water. A range of serial dilutions of the technical material (active ingredient) should be used to find out the activity range of the candidate molluscicide being tested. Dechlorinated water (as used for routine snail maintenance) is used for dilution and immersion bioassays.

For the initial immersion bioassay, 3–5 replicates of 10 snails each are set up for each serial concentration to be tested (according to the available number of snails, the number of replicates can be reduced from 5 to 3). Snails should be allocated to the treatments or control randomly. The results for each treatment are statistically analysed. See Table 2.1 for an example of a balanced testing plan, with all concentrations tested concurrently to minimize day-to-day test variations. The snails of Oncomelania spp. are exposed in 100 mL water held in 100 mL beakers; Biomphalaria and Bulinus spp. are exposed in 500 mL water in 500 mL beakers, i.e. the beakers must be covered with a netting piece/wire gauze with water filled to the top to prevent snails from escaping the test solutions. The standard exposure time in the laboratory should be 24 h, although monitoring can then take place every 24 h thereafter for a total of five days. No food is provided during the exposure period of 24 h and feeding can resume thereafter.

After a pre-test with a wide range of concentrations, a narrower range of molluscicide concentrations should be tested, with 2–3 concentrations that kill < 50% of snails and 2–3 that kill > 50–100% in order to establish a dose–response curve. Batches of 10 snails each are exposed in glass beakers with treated or untreated water for a period of 24 h.

As suggested earlier, water temperature and pH should be recorded at the beginning and the end of the exposure period; water aeration devices should not be used during the exposure period.

Table 2. A balanced testing plan, assuming six test concentrations, two controls and three test replicates of each on three different days. For each replicate, all the test/control concentrations are tested concurrently on the same day to minimize day-to-day variation in test and snail conditions.

Test/control concentrations (mg/mL)	Test replicates*		
	R1	R2	R3
Concentration 1	Day 1	Day 2	Day 3
Concentration 2	Day 1	Day 2	Day 3
Concentration 3	Day 1	Day 2	Day 3
Concentration 4	Day 1	Day 2	Day 3
Concentration 5	Day 1	Day 2	Day 3
Concentration 6	Day 1	Day 2	Day 3
Untreated control	Day 1	Day 2	Day 3
Positive control (e.g. niclosamide)	Day 1	Day 2	Day 3

* Each replicate includes 10 snails.

Mortality observations

At the end of the 24 h exposure period, snails are removed from treated or control water, rinsed and transferred to containers with standard snail water; after a 24h recovery period, mortality is recorded. Snails that remain completely within their shells and show no movement are suspected to be dead. Snails that are alive at 24 h should be placed in freshwater with food and monitored for a further 48 h (72 h in total) or possibly longer if relevant. Suspected dead snails should be transferred to separate containers because if they are dead, the decomposition could affect mortality among surviving snails.

At the end of the recovery period, death of suspected dead snails should be confirmed. For confirmation of death, the soft tissue of *Bulinus* and *Biomphalaria* spp. is stimulated with a needle to determine if there is a contractile response. For *Oncomelania* spp., place the snail between two thick glass slides and gently crush the shell before assessing contractile response (Chinese Ministry of Health, 2000). If no contractile response occurs, the snail is considered as dead.

If there is > 10% mortality in untreated control, the test replicate should be discarded, and the replicate is repeated. For any mortality in untreated control, the results should be corrected using the Abbott's formula given below (Abbott, 1987).

$$\text{Corrected mortality (\%)} = \frac{X - Y}{100 - Y} \times 100$$

Where,

X = percentage mortality in the treatment

Y = percentage mortality in the untreated (negative) control

A dose–response curve is needed to determine LC50 and LC95 values. The results are recorded in a standard data recording form. The relationship between dose and mortality is analyzed using log-probit (Finney, 1971) or logit regression.

Special formulations of molluscicide

Bait and slow-release formulations require a special methodology for testing; although in principle a dose-response curve could also be worked out, the exact methodology depends on the molluscicide used, the matrix into which it is embedded and the rate of release (for slow-release) and the snails' ingestion rate (bait formulations).

In principle, a bait formulation is composed of an ingestible matrix into which chemicals have been incorporated, i.e. an attractant that slowly will be released into the water to attract snails and a toxin that upon ingestion by a snail will act as a systemic poison. For toxicity testing, different concentrations of toxin could be tested in the same manner as for direct exposure, but there would be need to measure the amount of chemicals taken up by the group of snails on average (or test snails individually). More controls need to be included to check that mortality is not just caused by the toxin leaching into the water, whether the toxin has a repellant effect on snails and how much weight loss is due to leaching from the formulation.

Slow-release formulations consist of an inert matrix (e.g. glass or rubber) from where a molluscicide is released slowly into the water. For dose–response studies, formulations with different release rates are tested over a long period of time under conditions resembling routine maintenance of snails and possibly water should be replaced regularly and snails should be fed during the trials. Indicators of molluscicidal effects would be comparing egg laying and growth and survival rates with control snails not exposed to the molluscicide, but to the matrix without molluscicide.

2.3.2 Efficacy and residual activity

For the new candidate molluscicide compound (technical grade active ingredient) and for new end-use formulations of existing molluscicide compounds, an optimum effective dose should be determined based on the dose–response curve. The manufacturer's label claim may also be considered to decide the optimum dose to be tested. The efficacy (mortality recorded at the end of 24 h exposure or longer for slow-acting compounds) of exposure in immersion bioassay and residual activity at this effective dose is then determined according to the immersion bioassay described in section 2.3.1. The efficacy cut-off is the 80% mortality and the effective residual action is the duration until when the mortality in treatments remains ≥ 80%.

2.4 Determination of discriminating concentration

The discriminating concentration of a molluscicide is used to detect the presence of resistance to the molluscicide in a wild snail population. Details of bioassay test procedure are given in section 2.3.1. Based on the results, a discriminating concentration will be determined. The discriminating concentration is expected to kill 100% of molluscicide-susceptible individuals in a field population after 24 h of exposure. For safety margin and to prevent an overestimation of resistance in field populations, the discriminating concentration is taken as double the minimum concentration that yields 100% mortality in laboratory bioassays.

2.5 Cross-resistance assessment

New, candidate molluscicide compounds are tested simultaneously against snail species that have shown resistance to available classes of molluscicides, according to the immersion bioassay procedure outlined earlier. The cross-resistance studies may include niclosamide, which has been widely used in the field for snail control, although at present there is no evidence on resistance of snails to this compound.

3. Small-scale field trials (phase II)

3.1 Objectives

Candidate molluscicides or those showing promise in laboratory studies (Phase I) may be subjected to small-scale testing (Phase II). In Phase II, field trials of formulated molluscicide products are performed on a small scale against target snail species, preferably in representative natural breeding sites, or, where such trials are not feasible, under simulated field conditions. The candidate molluscicide formulation is tested using a range of concentrations according to the manufacturer's label claim or efficacy determination in Phase I. Treatment concentrations are calculated on the basis of the amount of active ingredient per volume of water (if known or measurable) or surface area of the habitat to be treated.

The objectives of small-scale trials are:

- to determine efficacy against different snail species in different aquatic and ecological settings; and
- to determine the optimum method and rate of application.

3.2 Methodology

In Phase II trials, the formulated products are tested on a small scale against target snails in man-made plots/ponds or under simulated field conditions.

Equipment

The WHO operational guidelines provide suggestions for essential equipment, including personal protective equipment, required for field trials (WHO, 2017).

Test plots

Tests may be conducted under controlled conditions in man-made semi-field experimental ponds or canals for pulmonate snails, and in marshland for amphibious Oncomelania spp. (in areas free from molluscicide use or use of any other biocides). The man-made discrete ponds are constructed with concrete or dug in the earth and lined with plastic sheeting, with a surface area of approximately 1 m x 1 m and a depth of 1 m. A 0.1 m thick layer of local soil should be added before transferring water and vegetation from natural habitats and local snail species seeded into the ponds. The simulated habitats should be constructed well in advance of chemical testing and water should be transferred after a coarse filtration from natural habitats.

For testing against amphibious snail species, natural small-size ponds, pools or marshland plots measuring 1 m x 1 m and protected by wire fencing can also be used for testing. The fence is needed to minimize the risk of children encountering a drowning hazard. The fine wire mesh should be tightly sealed in a base of concrete to prevent disturbance of test plots by animals. There should be a distance of about 2–3 m between the experimental plots to avoid cross-contamination.

Each of the confined man-made or natural habitat can be considered as a discrete plot or replicate. Sufficient replicates of treatments, untreated (negative) control and positive control[1] plots are included in the trial.

Application of molluscicide

A range of concentrations of the candidate molluscicide and positive control should be applied to the artificial/semi-field ponds according to the product label recommendation. The test products should be applied according to the method recommended by the manufacturer. Normally for a small-scale testing, the application of the test dosage is strictly controlled such as by using a hand-held sprayer or better to dilute the exact amount of molluscicide formulation according to the volume of water and then applied by a hand-held sprayer. Depending on the artificial conditions in the simulated habitats, food is added before molluscicide application and should be the same as used routinely for snail rearing.

Release of snails and mortality counts

The snails should be released in each experimental plot/pond. The number of ponds and snails per pond should be determined on the basis of a sample size calculation in consultation with a statistician.

Adult snails from laboratory-reared stocks (provided that local strains are used and there is no risk of introducing new snail strains) or wild populations are used. Shell size, i.e. diameter for *Biomphalaria* spp. or shell height for *Bulinus* and *Oncomelania* spp., should be measured and recorded as a proxy of snail age. The snails are placed in a polyethylene netting cage (30 cm x 30 cm x 30 cm) and immersed in the experimental/natural test pond 20 cm above the bottom of the pond. The cages should be removed after 24 h and mortality in snails recorded using the method described in section 2.1.

3.3 Efficacy and residual activity counts

The small-scale field testing is particularly useful with caged snails to show efficacy of molluscicides. This includes observations on caged snails to determine:

- the mortality after 24 h (or longer for slow acting compounds) of exposure; and
- the residual efficacy when new batches of caged snails are required to be exposed daily or at different intervals in the same treated ponds and mortality is recorded 72 h or longer post-exposure until the period mortality counts decline below the 80% cut-off level.

[1] Examples of standard application rates of niclosamide 70 WP, or an EC formulation, as positive control are: 1 ppm niclosamide (i.e. 1 mg AI/L) for aquatic application for control of pulmonate or amphibious snails (McCullough, 1992; Kariuki, Madsen et al., 2013); and 1 g AI of niclosamide/m^2 for marshland treatments against amphibious snails (Yang, Li et al., 2010). A positive control is included to ensure that test procedures have been undertaken correctly, with the expectation that the positive control provides reasonable control of adult snails (e.g. ≥ 80% mortality).

3.4 Data collection and analysis

All field data are collected in standard forms (Annex 3).

The reduction in snail counts is estimated for each replicate of each treatment using Mulla's formula (Mulla and Darwazeh, 1975), as given below.

$$\% \text{ Reduction} = 100 - [(C1/T1) \times (T2/C2)] \times 100$$

Where,

C1 = pre-treatment measure of snail abundance in unsprayed (negative) control
C2 = post-treatment measure of snail abundance in unsprayed control
T1 = pre-treatment measure of snail abundance in sprayed area
T2 = post-treatment measure of snail abundance in sprayed area

The difference between treatments can be compared by two-way analysis of variance (ANOVA) with treatment and number of sampling points as independent factors. The ANOVA should be carried out after transforming the percentage reduction to arcsine values. The post-treatment day up to which 80% reduction in snail counts is observed for each treatment or dosage will then be compared to determine the residual effect and optimum application dosage. Statistical comparison should be made with the positive control.

3.5 Selection of optimum field application dosage

From the dosages tested against a target species in the small-scale or simulated field trials, the minimum efficacious dosage (mortality and residual effect) achieved should be selected as the optimum field application dosage for each type of habitat. The frequency of molluscicidal treatment is determined based on the length of residual action.

3.6 Efficacy criteria for Phase II studies

The WHO cut-off for the efficacy of a molluscicide formulation against adult snails is ≥ 80% mortality in 24 h (or up to 72 h for slow-acting compounds) post-exposure. The residual efficacy of the molluscicide is calculated as the duration (days) when mortality in snails remains ≥ 80%.

A candidate molluscicide does not necessarily need to be equivalent or superior to the positive control since its efficacy will be determined based on its own performance according to the WHO criteria above.

4. Large-scale field trials (phase II)

The efficacy of molluscicides found to be suitable in small-scale field trials (Phase II) should be validated in large-scale field trials against natural snail populations in their natural breeding habitats.

4.1 Objectives

The objectives of small-scale trials are:

- to determine the efficacy of the molluscicide at the selected field application rate against the target snail population in natural habitats when applied on a large-scale;
- to determine residual efficacy in natural treated habitats and application intervals;
- to make observations on the ease of application of the test product;
- to observe community acceptance;
- to record any perceived side-effects on operators; and
- to observe the effects of the treatment on non-target organisms in natural habitats.

4.2 Methodology

For field trials, regulatory and institutional ethical clearance as well as communal consent should be obtained according to national or local requirements.

Selection of habitats

The characteristics of trial sites selected will depend on the local species of intermediate host snail and its preferred habitats. Care should be taken so that the representative habitats of the target intermediate host snail are included in the trial. Smaller habitats such as small ponds may be considered as a single plot, and multiple ponds may be required. Just as for the small-scale trials, each confined habitat can be considered as an independent replicate. In large confined habitats such as ponds, pools, lakes, irrigated fields and marshlands, discrete sampling plots are made provided there is sufficient distance between plots, say about 2–3 m, to prevent contamination from treatments. In other habitats such as irrigation canals and streams, discrete sampling plots are distantly located to avoid cross-contamination).

The number of plots of each type of habitat is determined through sample size calculations. Usually, a minimum of 25–30 replicates or plots of each type of habitat of the target snail species should be selected for control and treatments.

Where amphibious snail species are the target, marshland plots measuring 100 m² (10 m x 10 m) should be marked out. Each plot should be surrounded by stakes and wire mesh to prevent the escape of the snails from the test plots.

Sufficient replicates of treatments, negative control and positive control[1] plots are included in the trial. The plots are assigned randomly to treatments or control.

Some examples of natural habitats used successfully in the past for large-scale field testing of molluscicides are given in *Annex 5*.

Assessment of pre-treatment density

The population density of both immature and mature snails should be determined prior to the molluscicidal treatment at least once on the same day and snails should be returned to the same sampling point after sampling but before treatment. The sampling method must be appropriate to the type of breeding habitat and whether the target snail species is aquatic or amphibious.

Aquatic snails

For freshwater pulmonate snails such as *Biomphalaria* spp. and *Bulinus* spp., purpose-built dip-net[2] scoops and/or hand-held sieves should be used for cross-sectional sampling. Sampling should be done along a specified area of the aquatic habitat being surveyed by engaging two field technicians for 15 min each. During this time a series of scoops should be taken by passing the sieve along the bottom through water and any vegetation present. In large and deep-water bodies, sampling can be done along the perimeter of the waterbody up to about 5 m, or up to 20 m from the shore using a boat). Snails are most often found on vegetation, especially on decaying leaves, sticks, water lilies and discarded plastics. They should be transferred to a bowl and handled using plastic or rounded forceps for species identification. For consistency, sampling should be done by the same field technicians each time in the control and treatment plots.

Amphibious snails

A square or round metal frame should be used for sampling snails at each collection site where the soil or mud surface is readily accessible for visual inspection (the same frame must be used throughout sampling). A total of 20–50 frames should be sampled at regular intervals along the cross-section of the habitat being surveyed. All snails inside the frame should be collected with forceps, placed in sample tubes and counted as dead or alive. For consistency, sampling should be done by the same field technicians each time in the control and treated plots.

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[1] Examples of standard application rates of niclosamide 70 WP, or an EC formulation, as positive control are: 1 ppm niclosamide (i.e. 1 mg AI/L) for aquatic application for control of pulmonate or amphibious snails; and 1 g AI of niclosamide/m² for marshland treatments against amphibious snails. A positive control is included to ensure that test procedures have been undertaken correctly, with the expectation that the positive control provides reasonable control of adult snails (e.g. ≥ 80% mortality).

[2] Dip-nets can easily be made using a metal sieve, with holes of approximately 2–3 mm in diameter, attached to a wooden handle of about 1–2 m in length.



Figure 4.1 Sampling of aquatic snail species in a pond (above); and quadrat sampling of amphibious snail species using a metal frame (below – photo courtesy of Dr RM Oxborough)

Application of molluscicide

The molluscicides are applied into the natural habitats of the target snail population at the optimum application rate as determined in the small-scale field trials or based on the manufacturer's label recommendations. Molluscicides should be applied using appropriate application and personal protective equipment, depending on the formulations used and the habitat being treated. The optimum rate determined for the major or most important habitat of the target species in the area can be used for all the habitats being treated. Positive and negative control habitats are included in the trial. A detailed description of the molluscicide application method and calculations related to application rate must be documented.

Assessment of post-treatment density

The impact of molluscicide treatment on the target snail population is evaluated by sampling snails, using the same procedure as used during the pre-treatment surveys, 72 h after molluscicide application. Further monitoring for residual activity may also

be done at day 7, 15, and 30 after application in different locations of the same treated habitats. The residual efficacy may also be assessed in treated water bodies by conducting cage bioassays using laboratory-reared snails, or taking water from the treated and control habitats and doing bioassays in the laboratory at regular intervals based on the label claim. Data are recorded in standard forms.

Water condition

Temperature, pH, salinity and conductivity of the water should be measured and recorded for the test site on all sampling days. If possible, the GPS location of the test sites should be recorded.

Operational and community acceptability

The purpose of monitoring operational acceptability is to find out the ease of storage, handling and application of the molluscicide formulation on the field sites, and of the effects of the molluscicide formulation on the proper functioning of application equipment such as nozzle tips and gaskets, and disposal of waste containers to prevent environmental contamination.

A questionnaire-based assessment of the qualitative observations is made by recording community perceptions of the molluscicide treatments by discussion with local leaders and molluscicide applicators at the end of the trial.

Effects on non-target organisms

During the large-scale trial, qualitative observations of mortality of non-target aquatic local fauna such as fish, frogs, crabs and insect species should also be reported. The purpose is not to assess ecotoxicity of molluscicides but to gather information for control operators and for better communication with communities living around the treated areas.

4.3 Data collection and analysis

The mean number of snails collected per dip-net scoop/quadrat for each replicate of each treatment and control is calculated for each sampling point. The data may be analysed using generalized linear mixed models (GLMMs) or another statistical approach that accounts for repeated measures.

4.4 Efficacy criteria for Phase III studies

The bioefficacy of a molluscicide formulation is determined by the mortality and relative reduction in density of free-living snails in their natural habitats. The WHO cut-off for the efficacy of a molluscicide formulation against adult snails is ≥ 80% mortality in 24 h post-exposure. The residual efficacy of the molluscicide is calculated as the duration (days) when mortality in snails remains ≥ 80%.

A candidate molluscicide does not necessarily need to be equivalent or superior to the positive control since its efficacy will be determined based on its own performance according to the WHO criteria above.

5. Regulatory and ethical considerations

For testing of commercially available chemical molluscicides, regulatory approval from the national pesticide registration board/authority should be obtained in advance. The institution responsible for conducting field trials should also obtain national or institutional ethical clearance and provide an information sheet to the spray operators about the test products and safety precautions to be used for application. If the trial is conducted in privately owned areas, written informed consent should be obtained from the owners of the land where the molluscicide is to be applied. An informed consent form is suggested in Annex 4, which should be adapted and translated into the local language.

The trial institutions should provide appropriate personal protective equipment to molluscicide applicators and training on suitable application methods according to the manufacturer's recommendation. A provision should be made for prompt and free of charge medical care in case of acute poisoning associated with the handling and application of the trial product. Applicators and local communities exposed to molluscicide treated water should also be advised to seek medical care at the nearest health facility if they observe any sign or symptom of adverse reactions.

6. Community consent

As part of the trial planning, consent of local community leaders should be obtained to ensure compliance with the operations. They should be informed of the purpose, methodology to be used, expected outcomes and their relevance to them. When possible, local notices can be placed on community noticeboards close to treatment sites or community oral announcements can be made. Farmers or other people living in close vicinity should be advised to restrict use of treated habitats for a day or more after molluscicide treatment (refer to WHO, 2017 for an example of a community notice). At the completion of the trial, they should be informed of the trial results as an exit strategy.

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Annex 1. Measurements and conversions

Volume
1 L = 1000 mL
1 mL = 1000 µL
1 cubic foot = 7.5 gallons = 28 L
1 gallon = 4 quarts = 8 pints = 128 ounces = 3785 mL

Surface
1 ha = 10 000 m^2 = 2.2 acres
1 acre = 43 560 square feet
1 square foot = 0.111 square yard = 0.105 m^2

Length
1 km = 0.62 miles = 1093 yards
1 m = 39.7 inches
1 inch = 2.54 cm = 0.0254 m
1 foot = 0.333 yards = 0.3048 m
1 yard = 91.44 cm = 0.9144 m
1 mile (statute) = 1760 yards = 5280 ft = 1609.3 m

Weight
1 pound = 0.454 kg
1 kg = 2.2 pounds
1 g = 0.035 ounces

Conversion factors
Square inches to square centimetres, multiply by 6.5.
Square yards to square metres, multiply by 0.8.
Square feet to square metres, multiply by 0.09.
Acres to hectares, multiply by 0.4.
Square miles to square kilometres, multiply by 2.6.

Annex 2. Dilutions and concentrations

Aliquots of various strength solutions added to 99 mL water to yield final concentration

Initial solution		Aliquot (mL) for 100 mL water[a]	Final concentration (PPM) in 100 mL[a]
%	PPM		
1	10000	1	100
		0.5	50
		0.1	10
0.1	1000	1	10
		0.5	5
		0.1	1
0.01	100	1	1
		0.5	0.5
		0.1	0.1
0.001	10	1	0.1
		0.5	0.05
		0.1	0.01
0.0001	1	1	0.01
		0.5	0.005
		0.1	0.001
0.00001	0.1	1	0.001
		0.5	0.0005
		0.1	0.0001

PPM, parts per million

a. For 200 mL volume, double the volume of aliquots.

Annex 3. Data recording form

Phase I (laboratory evaluation): individual test record sheet

Experiment no.: Investigation: Date (dd/mm/yy):

Formulation: Species: Age: Food (Y/N):

Water: (tap/distilled/natural): Volume of water (ml): Temp (C°): Lighting:

Test concentration:

Replicate (choose 3–5)	No. of dead snails post-exposure*									Remarks
	Control			Positive control			Treatment			
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	
1										
2										
3										
4										
5										
Total										
Average										
% mortality										

a. Mortality is usually recorded 24 h post-exposure and is extended to a total of 72 h for slow-acting molluscicides.

Phase II/III data recording form

Investigator:
Study site (region/district/village):
Date (dd/mm/yy):
Habitat type and size sampling plot:
Snail spp. present:
GPS Coordinates):
Negative or positive control/treatment:
Formulation and application rate (or treatment):
Hour of treatment (hh/mm):
Equipment used:
Water temp (C°):
Remarks

Habitat no./code	Hour of collection	Density and mortality of snails												
		Scoop* # 1		Scoop # 2		Scoop # 3		Scoop # 4		Scoop # 5		Scoop # 6		Average mortality rate %
		No. collecte	No. dead	No. collecte	No. dead	No. collecte	No. dead	No. collecte	No. dead	No. collecte	No. dead	No. collecte	No. dead	
1														
2														
3														
4														
5														
6														
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13														
14														
15														
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25														

* A scoop using a dip-net or a hand-held sieve

Annex 4. Guidelines for development of informed consent form

Notes: This sheet is a suggestion/example that can be modified according to the national rules and guidelines. Separate informed consent should be obtained from the spray operators and the community leaders from treatment areas. It should be printed in the local language.

For: [name the group of individuals for whom this consent is written]

Name of principal investigator:
Name of organization:
Name of sponsor:
Name of study/project:

PART I: Information sheet

1. Introduction
Briefly state who you are, explain that you are inviting them to participate in research that you are carrying out, and inform which ethical committee has cleared the study.

2. Purpose of the research
Explain in lay (non-technical) terms why you are doing the research.

3. Type of research intervention – information on the molluscicide formulation [name of the formulation]
Briefly state the type of intervention that will be undertaken. Explain to the participant why you are testing the molluscicide formulation. Provide as much information as is appropriate and understandable about formulation, such as its manufacturer or location of manufacture, and the reason for its development. Explain the known experience with this formulation. Explain comprehensively, if any, all of the known side-effects/toxicity of this formulation.

4. Description of the process, procedures and protocol
Describe or explain to the participant the exact procedures that will be followed, on a step-by-step basis, and the tests that will be done.

5. Duration
Include a statement about the time commitments of the research for the participant, including both the duration of the research and follow-up.

6. Participant selection
State why this participant has been chosen for this research, what recruitment process has been used ensuring that equal opportunities have been provided to every potential participant, and if any exclusion criteria has been used as may be necessitated by the study design.

7. Voluntary participation
Inform clearly that volunteers can choose to participate or not at any time of the study without any implication on or costs to the participant. State that they will still receive all the medical services they usually do whether they choose to participate or not.

8. Right to refuse or withdraw
This is a reconfirmation that participation is voluntary and includes the right to withdraw.

9. Participant protection against possible side-effects
Explain to each participant the safeguards that will be provided (e.g. personal protective equipment, training where relevant). Potential participants should be told if there are any known or anticipated side-effects caused by the molluscicide formulation. Describe the level of care that will be available in the event that harm does occur, who will provide it and who will pay for it.

10. Benefits
Mention only those activities that will be actual benefits (as an additional protection from disease) and not those to which they are entitled regardless of participation.

11. Incentives
State clearly what you will provide the participants with as a result of their participation. WHO does not encourage incentives. However, it recommends that reimbursements for expenses incurred as a result of participation in the research be provided.

12.Confidentiality
Explain how the research team will maintain the confidentiality of data, especially with respect to the information about the participant which would otherwise be known only to the physician but would now be available to the entire research team. Also explain that data will be anonymised for analysis, and how data will be stored or archived.

13. Sharing the results
Where relevant, your plan for sharing the findings with the participants should be provided.

14. Who to contact

Provide the name and contact information of someone who is involved, informed and accessible (a local person who can actually be contacted). State also that the proposal has been approved, and how. For example,

”this proposal has been reviewed and approved by [name of the local ethical committee], whose task is to make sure that research participants are protected from harm. If you wish to find about more the Local Ethical Committee, please contact [name, address, and telephone number].”

PART II: Certificate of consent

Notes:

1. In case of spray operators, if they are employed and trained by the trial institution or when molluscicide spraying is part of their job responsibility, the information sheet as suggested above should only be given and no written consent may be required.

2. This section can be written in the first person. It should include a few brief statements about the research and be followed by a statement similar to the one given in bold below. If the participant is illiterate but gives oral consent, a witness must sign. A researcher or the person reviewing the informed consent must sign each consent.

I have read the foregoing information, or it has been read to me in my chosen language. I have had the opportunity to ask questions about it, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research and understand that I have the right to withdraw from the study at any time without in any way affecting my rights. I have been given a copy of this consent form.

Print name of participant: _____

Signature of participant: _____

Date: _____ / _____ / _____

(Day / month / year)

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team).

I have witnessed the accurate reading of the consent form to the potential participant. The reading was careful and accurate, and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness: _____ AND
Thumb print of participant
Signature of witness: _____
Date: _____ / _____ / _____
(Day / month / year)

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of researcher: _____
Signature of researcher: _____
Date: _____ / _____ / _____
(Day / month / year)

A copy of this Informed Consent Form has been provided to participant _____ (initialled by the researcher/assistant).

Annex 5. Examples of large-scale field trials

Some examples of the natural habitats used in large-scale field trials of molluscicides are given below.

1. A trial in irrigation canals in the United Republic of Tanzania

A large-scale molluscicide trial was conducted on a sugar estate near Moshi in the United Republic of Tanzania for the control of aquatic snails *Biomphalaria pfeifferi* and *Bulinus* spp. (Crossland, 1963). The test site consisted of five parallel irrigation canals (*Figure A6.1*) of approximately 1 m wide and 500 m long, with facilities for controlling the flow of water (Fenwick, 1970).



Figure A6.1. A drain (top) and an irrigation canal (below) near Moshi, United Republic of Tanzania (Photo courtesy of Dr RM Oxborough)

2. Semi-field ponds, Hippo Valley, Zimbabwe

Zimbabwe has a long history of schistosomiasis control including long-term use of molluscicides in focal areas (Chimbari, 2012). Niclosamide was sprayed in the Hippo Valley between 1986 and 1998 and contributed to the reduction in the prevalence of schistosomiasis. A number of laboratory and Phase II (semi-field) trials with plant-based molluscicides have also been conducted in the area (Chimbari et al., 2007; Ndlela et al., 2007).

3. Temporary ponds, Espirito Santo, Brazil

Several small-scale studies of molluscicides have been conducted in Brazil, including the use of temporary ponds with surface area of approximately 450 m^2 each in Espirito Santo for control of Biomphalaria tenagophila, in drainage canals in a sugar estate in Rio Grande do Norte and vegetable gardens near Belo Horizonte used for tests against B. glabrata (Gilbert et al., 1973).

4. Irrigation ditches, Yamanashi Prefecture, Japan

Irrigation ditches were used for molluscicide testing against Oncomelania nosophora. The test plots consisted of part of an irrigation canal, 50–60 cm deep and 1 m wide, lined with mud and temporarily subdivided into 5 m long sections divided by mud walls (Komiya, 1961).

5. Yangtze River marshland, Jiangsu Province, China

In China, field trials of niclosamide have been conducted against the amphibious intermediate host snail, Oncomelania hupensis. A marshland area along the Yangtze river in Yangzhou, Jiangsu Province was divided into 100 m^2 plots (*Figure A6.2*). The number of snails sampled from the experimental plots was recorded before and after mist spraying with molluscicide (Yang et al., 2012).



Figure A6.2. A marshland divided into 100 m² plots for testing molluscicide against amphibious snails of *Oncomelania* spp. (Photo courtesy of Dr G-J Yang)



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&
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