INTRODUCTION

The role that molluscicides can play in the effective control of schistosomiasis has been well established. Partly as a consequence of the rising costs of synthetic molluscicides, there has been an increasing interest in plants showing molluscidal properties. An appealing aspect of natural plant products is that, while they may be highly toxic, they degrade very rapidly when released into the environment.
This report is a review of both the present and potential roles of plant molluscicides; screening methods; problems of evaluation; needs in the field of toxicology; and methods of breeding, selection and cultivation of suitable plants. Finally, it provides a set of guidelines for research on plant molluscicides.

1. MOLLUSCICIDES IN THE CONTROL OF SCHISTOSOMIASIS

Substantial and scientifically acceptable data are available showing that, during the past 20 years, successful control of schistosomiasis has been achieved in many projects, using single and combined methods of intervention and underlining the valuable role of synthetic molluscicides in the strategies applied. During the 1960s the use of molluscicides provided the only reliable approach to control of schistosomiasis, but with the advent of new and more efficient antischistosomal drugs during the last decade, integrated methods have been increasingly used.

Snail control by periodic area-wide applications of synthetic molluscicides is now being carried out successfully in Egypt in large irrigation schemes with controlled water management. "Transmission control", based upon the focality and seasonality of transmission in most situations, is also being successfully effected by mollusciciding and surveillance. This may be a very cost-effective approach, if it can be related to accurate knowledge of human water-contact patterns, and if it can result in an important reduction of the need for expensive chemicals. Thus a reduction in transmission may be achieved by lowering snail population density and altering its age structure, even though complete eradication cannot be attained. Available data indicate that mollusciciding may be cost-effective where the volume of water to be treated per person at risk is small.

A significant reduction of the disease may be possible using the current technology and employing the new efficient drugs. Chemotherapy will undoubtedly play a vital role in reducing the severity of the disease and contribute significantly to the control of transmission by lowering egg output. However, the effect on transmission of population-based chemotherapy as a single control measure is not known. Nevertheless in many endemic areas, integrated measures involving snail control and chemotherapy will reduce the prevalence and intensity of infection and control transmission, cost-effectively, within a short period of time. Where schistosomiasis is not given high priority, however, the cost of drugs, molluscicides and other methods may deter the implementation of any control operations.

It is doubtful that the present generation of synthetic molluscicides, with the exception of niclosamide, will continue to be available. This poses a serious problem for future snail control.

Thus, economic and ecological considerations increasingly favour the use of molluscicides with selective activity, which are biodegradable, inexpensive and readily available in endemic areas. The impetus to study possible plant molluscicides is due largely to the high cost of imported synthetic compounds and the desirability of introducing appropriate technology into endemic areas.

It is considered that in order to utilize molluscicides effectively, schistosomiasis control activities must be efficiently organized at a national level. Furthermore, plant molluscicides will probably be most useful in areas where transmission is predominantly focal, in contrast to situations where transmission is widespread and where synthetic molluscicides may continue to be used. It is believed that plant molluscicides can play an important role in integrated programmes and self-help schemes at the village level.
2. PLANTS WITH RECOGNIZED MOLLUSCICIDAL ACTIVITY

Since the 1930s, more than 1,000 plant species including nearly 600 in China, have been tested for molluscidal activity. A relatively high proportion of the active species investigated were selected on the basis of medicinal properties or general folklore knowledge of toxicity. The highest proportion of species showing molluscidal activity is in the family Loguminosae, followed by the Euphorbiaceae, Rubiaceae, Polygonaceae and Compositae. A large proportion of the Phytolaccaceae is also highly active.

The most thoroughly studied of the plant molluscicides is one derived from the berries of Phytolacca dodecandra (endod), an aqueous extract of which has been used for snail control in Ethiopia. In the Sudan, a water extract of the seeds of Croton macrostachys is found to have high molluscidal activity. In the Philippines, Jatropha curcas showed activity against Oncomelania snails. In Egypt, trials are in progress using Ambrosia maritima. The crushed shells of the cashew nut (Anacardium occidentalis) are currently being used for snail control in Mozambique.

3. GEOGRAPHICAL DISTRIBUTION AND ECOSYSTEM DATA BASE ON MOLLUSCICIDAL PLANTS

Many plants with molluscidal activity are now being documented. Information on their geographical distribution, ecological requirements and availability must be coordinated and systematized. Specimens should be submitted to a national, as well as an international, herbarium. Many international herbaria have computerized data bases, which can indicate, among other information, whether a plant is present in a given area. A system of documenting pertinent field information and the necessary plant parts to be submitted for identification are listed in the annexed GUIDELINES.

4. PLANTS WITH POTENTIAL MOLLUSCICIDAL ACTIVITY

Some 42 molluscidal compounds have been isolated from plants. In addition to saponins, thought until recently to be the major active molluscidual constituents, flavanoids, alkaloids and terpenoids have shown encouraging results. The recent findings that bidesmotic saponins and some dammarane glycosides and alkaloid saponins are inactive may help to explain variations in the results obtained by different investigators.

Further studies of other structure/activity relationships of compounds may result in the identification of plants with more selective effects. Molluscidal compounds, like other phytotoxins, may be present and synthesized more frequently in some plant groups than in others, although many compounds do occur independently. Thus, sesquiterpene lactones are most common in the Compositae, but triterpenoid saponins are widespread in nature.

There is a lack of information on the distribution of the molluscidal activity in different plant parts. Such information might have predictive value, but is not available due to the failure of most investigators to systematically study all parts of plants. Among the Phytolaccaceae, the berries are most active, the leaves less so and the roots not at all; this correlates with the distribution of saponins and inactive alkaloids. In most species in the family Euphorbiaceae, the highest activity levels are in the seeds, while in the Solanaceae the leaves and fruits contain the highest molluscidal activity. Efforts should be made to test all parts of plants for molluscidal activity. However, for sustained use in snail control programmes, emphasis in the case of perennial plants should be placed on fruits, seeds, flowers and regenerating leaves, rather than on roots, stems and non-regenerating bark.
5. BIOCHEMISTRY AND MODE OF ACTION OF MOLLUSCICIDES

Little is known about snail metabolism or physiology which can be used to predict the structure of potential synthetic molluscicides, the less for substances of plant origin. The known active water soluble principles of plant molluscicides act on the snail surface membrane, the dose/response being an "all or none" phenomenon over a very narrow range. Regarding the biochemistry of recognized molluscidal compounds of plant origin, saponins have been emphasized, but others including tannins, flavonoids, alkaloids and terpenoids should be thoroughly investigated.

As is true for the mode of action of most insecticides, the action of a molluscicide may conceivably be a multi-component process, affecting more than one system. Several such responses indicative of this have been reported in the literature, as for example: reduction in heart rate, swelling of tissues and alteration of water balance. At present there is no reason to believe that these responses are due to a common mode of action, suggesting that there may be a range of actions and that the mode of action of molluscicides may be the last aspect to be elucidated.

6. TOXICOLOGY

Safety testing should parallel developmental stages of a plant molluscicide. Only plant strains with consistent molluscicidal activity under known cultivation conditions deserve to be tested. Plants having any toxic effects resulting from direct human contact during handling and processing do not merit further development. The toxic effects on non-target organisms, particularly fish, must be at acceptable levels. There should be no evidence of toxicity in short-term tests for mutagenicity carried out simultaneously with tests for molluscicidal activity or following demonstration of such activity. In the case of water extracts, the persistence of toxicity in the aquatic environment and the toxic properties of any hydrolysates of a lipophilic nature which could accumulate in food products should be determined. If contamination of food products is likely, the chemical nature of the toxic compound(s) must be known before any meaningful toxicity testing can be undertaken.

7. LABORATORY AND FIELD EVALUATION

Plants which fulfill certain practical criteria (see annexed GUIDELINES) may be considered for field testing if the molluscicidal activity has been evaluated with the following minimal results from laboratory screening tests:

- the crude preparation of the plant material should be active at 100 mg/litre* or less and kill 90% of snails exposed for 24 hours at a defined water temperature;
- a cold and/or hot water extract of the plant material should be active at 20 mg/litre or less, to kill 90% of snails exposed for 24 hours at a defined water temperature;
- optionally alcoholic (methanol) or lipophilic solvent extracts of the plant material should be active at 20 mg/litre or less, to kill 90% of snails exposed for 24 hours at a defined water temperature; and
- the molluscicidal activity against amphibious snails should be at 100–200 mg/litre and kill 90% or more snails exposed within three days in the preliminary test.

* mg/litre = ppm
If the molluscicidal activity is found only in the alcoholic and/or lipophilic solvent fractions, the potential for further development may be limited by the processing required for use in endemic areas. *Biomphalaria glabrata*, or any other exotic snail which is an intermediate host of *Schistosoma* species, should not be introduced into a laboratory in an endemic or potentially endemic area for testing purposes.

When research on a plant product has established defined molluscicidal activity with acceptable toxicity levels and feasible cultivation potential, the responsible research group should be encouraged to collaborate with laboratories having the expertise and facilities capable of determining chemical structures and their function. A full description of the chemical characteristics of the plant product is desirable, but this is not a prerequisite for field evaluation and development of the plant molluscicide.

When the criteria already discussed have been fulfilled, field evaluation should be carried out in the endemic area, with the approval of national authorities, to demonstrate the efficacy of the plant. The steps of the evaluation should be carried out in the following sequence:

- the molluscide should be applied by one or several different techniques in defined snail habitats and according to a standard protocol, with quantitative evaluation of the effects upon snails;
- following a successful first evaluation, the plant molluscide should then be tested in an epidemiologically-defined endemic area in association with chemotherapy, in comparison with a similar area where an acceptable reference molluscide is used in combination with chemotherapy, and/or with an area where chemotherapy is used alone; and
- the second evaluation should then be based upon the comparative longitudinal measurement of human epidemiological parameters to determine changes in prevalence, intensity of infection and, if feasible, incidence.

The reference molluscicide in all testing procedures should be niclosamide.

8. CULTIVATION

Successful and cost-effective cultivation of plants on a large scale, exclusively for snail control, remains to be demonstrated. The agronomic success achieved in the 1970s with *Phytolacca dodecandra* suggests that, at least in Ethiopia, such cultivation at the village level is feasible.

Successful cultivation of a "wild" plant requires careful breeding and selection for a high-yielding and high-potency type (strain) adapted to a wide variety of environmental conditions – soil, temperature, moisture, shade, etc. Differences in molluscicidal activity has been observed in the same plants grown in diverse environments. This evaluation can be accomplished only under strict supervision, preferably at agriculture research stations or in experimental plots in different geographical areas.

Plant propagation techniques will vary according to species and their ecological requirements. Methods of breeding and cultivation should be well documented so that the information gained can be readily transferred to other countries. After initial breeding and selection, cell culture techniques may play an important role in the future.

Ideally the plant would be intercropped with local cultivars, thereby promoting greater farmer supervision in regard to weed, pest and disease
control. A self-help programme should, if possible, be integrated into the
daily activities of local farmers.

Plant materials in their natural state are not generally known to be
molluscicidal. For the most effective use, minimal processing, such as
grinding and crushing, is normally required.

Large-scale cultivation may not be feasible in some endemic countries,
where the need for the recurrent use of machinery, fertilizers and possibly
pesticides requires scarce hard currency which may not be available.

9. CONCLUSIONS

The availability of safe, effective oral antischistosomal drugs and the
high cost of imported synthetic molluscicides used in the control of
schistosomiasis provide the incentives for examining the potential role of
plant molluscicides. It is clear that an inexpensive and effective method of
snail control in combination with chemotherapy is desirable. Although natural
products have been used for millennia, their toxic effect on non-target
organisms has rarely been determined and evaluated. This is true also for
plant molluscicides.

Phytolacca dodecandra, Ambrosia maritima and Anacardium occidentalis are
several of the plants which have shown promising molluscicidal activity. Field
trials of these species are currently being carried out in Ethiopia, Egypt and
Mozambique respectively.

Based on present knowledge of the distribution of recognized plant
molluscicides, there is no justification for future "blind screening" of
plants in order merely to identify new taxa. At present, certain saponins,
specifically those with an oleic acid derivative, seem to offer the greatest
potential as molluscicides.

There has been great progress in the development of extraction procedures
for plant products. However, there is a need to standardize methods for
phytochemical screening and bioassay to permit more comprehensive evaluation
of plants and greater comparability of the results obtained by different
investigators.

Phytolacca dodecandra provides a good example of the possible problems
associated with different extraction methods and their relation to toxic
effects. This plant has been reported not to be mutagenic when extracted in
water, but the alcohol extract of the plant is mutagenic.

The success of the Phytolacca dodecandra breeding and selection programme
in Ethiopia clearly demonstrated that saponin quantity and potency, as well as
its resistance to insects can be increased over several years. The occurrence
of varietal differences among plant species with promising molluscicidal
activity should be investigated - possibly using cell culture techniques.

A long harvest period is preferred as this promotes more efficient
processing, a ready supply of molluscicide, and reduced storage costs.

Probably the first criterion in assessment of any potential plant
molluscicide is that it be water soluble. Such a property enhances bio-
degradability and reduces possible build-up of toxic residues in the
environment.

Production of plant molluscicides by an inexpensive and efficient method
will be necessary at the village level. A self-help programme, using a
locally produced molluscicide and coupled with chemotherapy, could
significantly reduce prevalence and intensity of infection to acceptable levels and do so at a reasonable cost.

The papers reviewed at the workshop provide a basis from which a set of guidelines has been prepared. These guidelines are intended to indicate the information required in research proposals to be submitted for consideration of the Steering Committee of the SWG on Schistosomiasis.

10. LIST OF WORKING PAPERS*

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* The working papers are not available individually, but will be published in a single volume by the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases.
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ANNEX

GUIDELINES FOR EVALUATION OF PLANT MOLLUSCICIDES

These guidelines are intended to provide a general background to scientists involved in research on molluscicides of plant origin. The interpretation of them is meant to be flexible to accommodate local needs.

1. The identification of the plant must be confirmed by a botanist and specimens submitted to a national herbarium and at least one international herbarium. The voucher specimen should include the following information:

a) name of collector, collector number, herbarium number;
b) date and season of collection;
c) geographical location of collection (longitude and latitude);
d) altitude of collection and habitat;
e) local name of the plant;
f) the specimen submitted should be fully flowered and/or with ripe fruit as appropriate;
g) uses and reason for collection; and
h) type of plant (tree, shrub or herb).

2. All subsequent communications, particularly scientific publications, concerning the plant should include:

a) the names of the herbaria where voucher specimens are deposited;
b) genus, species and authority;
c) season in which the plant was collected;
d) whether wild or cultivated tree, shrub or herb, and
e) part of the plant tested.

3. The molluscicidal activity must be evaluated and include the following minimal results from laboratory screening tests, if a candidate plant is to be considered for field trials. The species and origin of the laboratory snail should be fully described. The exact methods and conditions of experimentation should be carefully documented.

3.1 Aquatic Snails

a) The crude plant material \* should be active at equal to or less than 100 mg/litre to kill 90% of snails exposed for 24 hours at constant water temperature.

b) A cold and/or hot water extract of the plant material should be active at equal to or less than 20 mg/litre \*\* to kill 90% of snails exposed for 24 hours at defined water temperature.

c) Optionally, an alcoholic solvent (methanol) extract of the plant material should be active at equal to or less than 20 mg/litre \*\* to kill 90% of snails exposed for 24 hours at defined water temperature.

d) Optionally, a lipophilic solvent extract of the plant material should be active at equal to or less than 20 mg/litre \*\* or less to kill 90% of snails exposed for 24 hours at defined water temperature.

\* Plant material used should be recorded as dry weight.
\*\* 1 ppm = 1 mg/litre
3.2 Amphibious Snails

The molluscicidal activity on amphibious snails may be further evaluated when a concentration at 100-200 mg/litre or less kills 90% or more snails exposed within three days in a preliminary screening test.

3.3 If the molluscicidal activity is found only in the alcoholic and/or lipophilic solvent fractions, the potential for further industrial development may be limited by the processing required for use in endemic areas.

4. The level of safety testing should parallel developmental stages of a plant molluscicide. Before it is used operationally, all safety testing requirements should be fulfilled which take into account the degree of exposure of operational personnel, general population and the environment.

   a) The plant should not cause dermal toxic effects upon exposure to those involved in handling and processing it.

   b) Toxicity to non-target organisms must be at acceptable levels.

5. When research on a plant product has:

   a) defined molluscicidal activity;

   b) shown it to be acceptably safe; and

   c) reached the point of showing feasibility of cultivation,

the research group is encouraged to collaborate with laboratories having competence for determination of chemical structures. It is concluded that a full description of the chemical characteristics of active molluscicide constituents is desirable, but not a prerequisite, for field evaluation and development of the plant molluscicide.

6. The agriculture aspects to be considered include:

   (a) cultivation of the plant under a wide range of local environmental conditions while maintaining high, consistent molluscicidal activity;

   (b) possibility of propagation from seeds or cuttings;

   (c) a high yield of molluscicidal activity per plant or unit area cultivated;

   (d) resistance to diseases and pests; and

   (e) maintenance - in storage conditions for at least up to one year - of molluscicidal activity of the plant material, which is produced seasonally.

7. Other General Considerations:

   a) If preparation of the plant material is necessary, ideally this should require only crushing or grinding by appropriate local technology.

   b) If extraction is necessary, the active chemical principle should be extractable by a simple apparatus and commonly available solvents, preferably water.

   c) Knowledge on the part of the local population of growing habits and requirements, toxicity and any medicinal properties of plants is an asset.

   d) Absence of cultural uses of candidate plants and aversions based on
folklore and magic, which might interfere with their use for snail control, is desirable.

e) Suitability of the plants for other uses, such as medicines, pesticides and other manufactured goods, food and other domestic uses, erosion control and reforestation, is highly desirable.

f) Appropriate environmental and plant conservation should be observed in collection of the candidate plant so as to maintain satisfactory ecological balance.

8. Field Evaluation

When the above criteria have been met and approval has been obtained from national authorities, the following sequence of field evaluations is suggested:

a) Application of plant molluscicide by one or several different techniques in defined natural snail habitats according to a protocol similar to that described in this document.

After successful completion of this first evaluation, operational evaluation becomes appropriate as follows:

b) Use of the plant molluscicide in a defined endemic area in combination with chemotherapy and in comparison with a similar area where:

- chemotherapy alone is used; and/or

- an acceptable synthetic molluscicide (niclosamide) is used in combination with chemotherapy (this trial would be optional, according to requirements of the specific endemic countries).