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THE RELATIVE SUSCEPTIBILITIES OF
BULINUS TRUNCATUS (AUDOUIN) AND
SAROTHERDON KOSSAMBIGUS (PETERS)
TO CERTAIN MOLLUSCICIDES

A Thesis Submitted for the Degree of
Doctor of Philosophy in the University of London

by

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ABSTRACT.

In spite of varying opinions as to the effectiveness of molluscicides in the control of schistosomiasis, the search for more effective and highly selective molluscicides should continue, so that adverse effects on non-target organisms caused by commercially available molluscicides may be reduced and the possibility of resistance guarded against.

To this end, the differences in susceptibility to Frescon and 4'-chloronicotinanilide between the schistosome-bearing snail, Bulinus truncatus and a representative tropical food fish, Sarotherodon mossambicus, were examined and discussed in relation to the rate of uptake of these compounds and their distribution among different organs.

The rate at which water is taken up by B. truncatus and S. mossambicus has also been calculated and related to the rate of uptake of molluscicides. This part of the work was an attempt to elucidate the mechanism of carriage of the chemicals into the animals being studied.

It has been demonstrated that B. truncatus and S. mossambicus concentrate Frescon and 4'-chloronicotinanilide to high levels. For B. truncatus, both Frescon and 4'-chloronicotinanilide were concentrated in the pseudobranch, while in S. mossambicus the highest concentration of Frescon was found in the liver and that
of 4'-chloronicotianilide in the bile.

The high tolerance of _B. mossambicus_ to 4'-chloronicotianilide was found to be due to the rapid metabolism of the compound in fish to more polar forms which are more easily disposed of via the bile.

Comparison of the relative susceptibilities of two field collections of _B. truncatus_ showed that snails collected from the Frescon-treated area of the Gezira display a higher tolerance to Frescon than do snails collected from the untreated area. Frescon uptake rate was found to be lower in the less susceptible snails, and this is tentatively suggested as the basis of the observed difference in tolerance. It is additionally shown that _B. truncatus_ infected with _Schistosoma haematobium_ is more susceptible to Frescon than uninfected snails.
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CHAPTER 1

GENERAL INTRODUCTION
1. Schistosomiasis.

Schistosomiasis is a chronic disease caused by digenetic trematodes of the genus, *Schistosoma* and transmitted by fresh water pulmonate snails belonging to the family, Planorbidae and by amphibious operculates belonging to the sub-family, Hydrobiinae. There are two clinical forms of the disease, urinary schistosomiasis caused by *Schistosoma haematobium* (Weinland) and intestinal schistosomiasis caused by *S. mansoni* (Sambon) and *S. japonicum* (Katsurada).

The adults of *S. haematobium* are usually found in the veins of the pelvic plexuses. On the other hand, the adults of *S. mansoni* and *S. japonicum* commonly inhabit the mesenteric veins. The female has the capacity to lay large numbers of eggs. For example, a single *S. haematobium* female is able to lay up to 250 eggs per day and its life span is thought to be less than two years, but a proportion of the parasites may live for longer periods (Nelson, 1975). An infected person may harbour a large number of worms (up to 250 pairs) which, with the great mass of eggs they lay and the host reaction to such bodies, can cause considerable perturbation in associated host tissue (Cheever, 1968).

Conservative estimates place the number of individuals infected with schistosomiasis at 150 million (World Health Organisation [WHO], 1965). The true figure may eventually be shown to be considerably larger with the
more widespread use of the improved diagnostic and epidemiological techniques which have become available in recent years. The increase in the prevalence of the disease in relation to the development of water resources (McNullen et al., 1952; Sturrock, 1965; Berrie, 1970), has caused concern among health authorities and has stimulated much needed investigations into the prevalence and transmission of the disease, the development and evaluation of different control measures, and improvements in the planning of new irrigation schemes (Webbe, 1959). The public health significance of the disease may be evidenced by the recent impetus by WHO to fund research into selected priorities, in the fields of epidemiology and control, chemotherapy and immunology (WHO Scientific Working Group, 1977).

2. Control of schistosomiasis.

Webbe, 1969, stated that the objective of control of schistosomiasis is to limit the spread of infection, to reduce morbidity or to control transmission and thereby reduce the intensity and prevalence of infection. Schistosomiasis can be controlled by employing one or more of four general methods: first, mass treatment of human cases to reduce or abolish release of eggs; second, sanitation measures; third prevention of contact with water; and fourth reduction or elimination of the snails which are essential to transmission of schistosomiasis to man.
2.1. Mass chemotherapy.

The mass treatment of human cases sounds as promising a measure for control as any, but alone would not be effective under the conditions presently existing in most of the endemic areas reviewed by WHO in 1961. The lack of a suitable drug has made mass chemotherapy in most endemic areas impracticable (Jordan and Webbe, 1969).

Lucanthone hydrochloride was first used about a quarter of a century ago both in experimental animals and in humans (Archer and Dennis, 1969). It was the first effective drug that could be given orally and it required a shorter period for treatment than the classical antimonial drugs (Rosi et al., 1967). Hycanthone, the hydroxy-methyl derivative of lucanthone (Rosi et al., 1965), shows considerable promise for the treatment of \textit{S. mansoni} infections, producing a considerable reduction in egg output and a high cure rate. Many physicians regard niridazole (Ambilhar) as the drug of choice for \textit{S. haematobium} and providing that \textit{S. mansoni} and \textit{S. japonicum} infections are early and confined to the intestinal phase, moderately good results can be expected in treatment of these infections (Davis, 1966; Wolf, 1967; Webbe, 1969; Farid et al., 1970). Metrifonate (Bilarcil) is the most interesting addition to the range of schistosomicides seen in recent years. It is of monospecific activity in being effective only against infections with \textit{S. haematobium}. There is either minimal or absent activity against \textit{S. mansoni} in experimental models and in infected patients (Davis and Bailey, 1969; Pleština et al., 1972). On the basis of therapeutic
efficacy, estimated population coverage, tolerance, case of administration and cost, metrifonate was considered to be marginally superior to niridazole and more so to hycanthone in the treatment of *S. haematobium* (WHO, 1973). Jowbury and Cooke (1977) reported field trial results which showed that metrifonate has a prophylactic effect, is highly effective against *S. haematobium*, is easy to administer and safe.

Webbe and James (1977) demonstrated that a new compound Praziquantel (Embay 8440) exhibits a high degree of activity against the 3 major schistosome species in the hamster with no apparent significant differences in efficacy against the different geographical strains of the parasite. They noted that the efficacy of the compound against *S. haematobium* is significantly greater than that of metrifonate. It is clear that, although many compounds are known to possess schistosomicidal activity, the approach of mass treatment has the following limitations; first, multiple examinations are necessary if all infected persons are to be found; but since this is often impracticable, a large number of cases are generally missed; second, inadequate treatment due either to failure of the patient to complete the course of treatment or the failure of the drug to cure the patient; third, reinfection is common.

2.2. Sanitation measures.

To prevent contamination of snail-infested water by schistosome eggs, it is important to provide the community with a good system of sewage disposal. But,
this is expensive to install and often requires considerable modification of human habits. Again, *S. japonicum* can be maintained in non-human hosts (Pesigan *et al.*, 1958 and WHO, 1961) and *S. mansoni* is also zoophilic (Riggan and Berrios Duran, 1956; Vianna Martins, 1958; Nelson, 1960; Fenwick, 1969) so that provision of a sewage disposal system probably would not entirely prevent the contamination of snail-infested water by schistosome eggs.

2.3. Prevention of contact with water.

It is true that schistosomiasis could be prevented if man did not come into contact with infected water. Unfortunately, most native populations, especially children, come into contact with infected water casually or through agricultural practice, bathing and other household functions. Provision of safe water for drinking, bathing and domestic use goes a long way towards reducing human infections, but this does not help the farmers who face occupational hazards in that they are regularly exposed to schistosome cercariae. However, evidence from Brazil (Barbosa *et al.*, 1977) and South Africa (Pitchford, 1970) suggests that provision of safe water will reduce the prevalence of schistosomiasis. Jordan *et al.* (1976) and Jordan (1977) confirm this suggestion from longitudinal studies in St. Lucia.

2.4. Snail control.

Various methods for snail control have been tried with varying degrees of success. These include the extermination of snails through biological, physical or
2.4.1. Biological control.

Although biological control has been suggested from time to time during the past 50 years (Bequaret, 1928; Mozley, 1939), the subject has not attracted serious consideration despite the extensive literature on the predators, parasites and diseases of molluscs which might furnish leads for experimental investigations. Various workers have discussed controlling schistosome-bearing molluscs through the use of leeches and rotifers (Michelson, 1957) dipteran sciomyzid larvae (Berg, 1953, 1964; Echblad, 1973; Beaver, 1974) turtles (Michelson, 1957 and Hopkins, 1973) ducks (Cawston, 1921; Humphreys, 1932; Malone, 1965; Shiff and Mowbray, 1970) and various fish (Mozely, 1951, 1953; Bowmaker, 1968; Andrade and Antunes, 1969; Barnish, 1971; Blackburn and Sutton, 1971).

One of the few cases in which biological control seems to have been effective is the successful introduction of the ampullariid snail, *Marisa cornuarietis* (L.) for the control of *Biomphalaria glabrata* (Say). The history of the utilisation of *Marisa* for the control of Puerto Rican *B. glabrata* has been amply presented and its biology has become relatively well known (Chernin *et al.*, 1956; Oliver-Gonzalez *et al.*, 1956; Radke *et al.*, 1961; Bartelt, 1970; Blackburn and Sutton, 1971; Ferguson, 1972).

2.4.2. Physical control.

Physical control is a specific form of sanitation of the water channels so as to make them unsuitable for snail
life. This can be achieved by the removal of snail habitat by destroying water plants at lake and stream edges either manually or with herbicides and by modification of agricultural practice and water management techniques such as increasing velocity of water, stream straightening, canal lining and channel design (Jobin and Ippen, 1964 and McJunkin, 1970). But this is found to be expensive and becomes increasingly difficult as new irrigation schemes are set up (McMullen, 1961).

Some notable successes have been recorded, however, in the Philippines and in Japan, where control of irrigation water, proper drainage and better agricultural practices have been instituted (Okabe, 1957; Pesigan et al., 1958; Hairston and Santos, 1961; Yokogawa, 1972). The Chinese under the guidance of the Thoughts of Chairman Mao Tse-tung have scored great successes in the prevention of schistosomiasis in different counties through environmental alterations. The methods used include soil burial, snail drowning, 'anti-snail belts' and burning of waterside vegetation (Anon, 1968a, b; 1977).

2.4.3. Chemical control.

Chemical control is probably the most rapid and feasible procedure for the elimination of snails (Webbe and Jordan, 1966) and has the advantage that it controls intermediate hosts of other diseases (e.g. fascioliasis). Japanese workers (Miyagawa, 1913, 1916) were the first to use chemical compounds such as calcium cyanamide for snail control. Chandler (1920), in the United States of
America, found that certain aquatic snails were killed in 48 hours by a solution of copper sulphate as low as 1-2 ppm and suggested that the chemical might be effective against schistosome-bearing snails. Since then many countries have adopted copper sulphate as a molluscicide and it is still in use in some countries (Venezuela, Egypt and Sudan). As a result of screening tests made on a series of compounds, McMullen et al. (1948) reported that sodium pentachlorophenate (NaPCP) was molluscicidal. McMullen et al. (1951) and McMullen (1952) demonstrated that dinitro-o-cyclohexylphenol (DN-1) was highly molluscicidal.

New and more effective molluscicides have been introduced into the field during the last two decades. According to WHO (1973), two outstanding compounds, niclosamide ethanolamine salt (Bayluscide) and N-tritylmorpholine (Frescon) are currently the molluscicides of choice against aquatic snails.

Niclosamide has the advantage that, although it kills snails, snail eggs and schistosome cercariae, it is not toxic to man and has limited biocidal effect (Gönnert and Schraufstätter, 1959). It is non-corrosive, reasonably persistent but not residual, and is degraded by sunlight into harmless organic chemicals. Its disadvantages are its high cost and its lethal effect on fish and some other aquatic animals. N-tritylmorpholine has the advantages of being effective at an extremely low concentration and having limited biocidal action (Boyce et al., 1968).
The ideal molluscicide.

Colwill (1957) defined the ideal molluscicide as one that remains effective in water at low concentrations for a prolonged period of time; it must be non-toxic to man and warm-blooded animals and innocuous to fish and wildlife; it must not repel snails and it must be cheap, readily available, easily transported, chemically stable and preferably agreeable to handle. He goes on to point out that in practice all prospective molluscicides fall short of this ideal. Furthermore, Duncan (1969) stated that if the molluscicide is insoluble in water, it should, if possible, be formulated as an emulsion concentrate or wettable powder and that it should be non-phytotoxic as far as crops are concerned, though herbicidal action in irrigation canals could be additionally useful.

When a compound is a new biocide it is essential to investigate the possible hazards to man, domestic animals and wildlife. This is usually done by exposing laboratory animals in standardised acute and chronic tests and examining them for toxicological effects. Biochemical studies are also usually conducted with the most active compounds to determine the metabolic fate of the compound.

Wright (1959) and Muller (1965) concluded that a better understanding of the mode of action of molluscicides gained through the study of hormone and enzyme systems of snails, may uncover some peculiarity in snail metabolism or function which would lend itself to specific chemical attack. In this way it should be possible to expand our
knowledge of biochemical mechanisms and to design new compounds.

The WHO Scientific Working Group on Schistosomiasis (1977) recommended that the search for new and more effective compounds should be continued and listed a number of selected research subjects for early attention. Amongst these was included studies of mode of action, molluscan physiology and biochemistry and molecular modification and structure–activity relationships, all of which presumably being aimed at improving molluscicidal specificity if not achieving the ideal molluscicide.

4. Detoxication mechanisms.

The majority of foreign organic compounds undergo definite chemical changes in the animal resulting in the excretion of specific metabolites usually via the kidneys. There are however, some compounds which are excreted unchanged, i.e. biochemically inert compounds, although they may be pharmacologically active (Brodie and Maickel, 1962). Excretion by other channels such as the expired air, the bile and the faeces, the saliva and the skin may also occur. The type of change which occurs depends primarily upon the structure of the compound but other factors such as the species, route of administration and diet may also be involved (Williams, 1959). These changes or biotransformations are usually divided into 4 main types: oxidation, reduction, hydrolysis and synthesis. The first three are many and varied, but in general compounds of similar structure are oxidised, reduced and hydrolysed in a qualitatively similar manner. The
synthesis reactions, or conjugation processes appear to be relatively few in number and are mainly reactions involving carbohydrates and amino acids. Whether or not a given compound will undergo any of the above syntheses will depend upon its possessing a particular chemical group or "centre for conjugation". If the compound does not carry such a group it may acquire one, for example by oxidation or reduction. It has been found that some synthetic processes are confined to particular classes of animals or even species.

To maintain a state of homeostasis the body must be capable of excreting unwanted material. This may be achieved through the kidney in the case of unchanged materials which are water soluble. Lipophilic compounds do not leave the body by this route, either because they are bound to proteins or lipid in the blood and therefore cannot enter the glomerular filtrate, or having entered the kidney tubules, they are reabsorbed by the lipid membranes of the cells lining the ducts. In order to be excreted, they must therefore be altered in the direction of increased water solubility i.e. the lipophilic compounds must be modified into derivatives of greater polarity. It has been usual in the past to refer to such mechanisms as detoxication mechanisms. This is not a complete statement of the case however, as the metabolites can be more toxic than the original compound. For example, Davson (1955) found that in the body the insecticide, parathion produces the more toxic compound, paraoxan.
The conventional view holds that drugs are detoxified by enzymes of intermediary metabolism which cannot distinguish foreign compounds from their normal substrates. The non-specificity of a series of enzymes for the oxidation of foreign compounds would raise an ancillary question of how normally-occurring substrates would be protected from attack by these non-selective enzymatic scavengers. This problem appears to have been met by the microsomal enzymes (Brodie and Maickel, 1962). It is said that the oxidative enzymes, despite their low specificity have a predilection for foreign compounds and do not molest normal substrates even when these have similar chemical structure. A possible explanation is that the microsomal enzyme might be segregated by the lipid membrane of the microsome which can be penetrated only by fat-soluble substances.

Again Brodie and Maickel (1962) found that a number of varieties of fish, both from fresh and salt water, cannot oxidise drugs in vivo nor can their liver microsomes carry out in vitro the oxidative mechanisms of N- or O-dealkylation, hydration, deamination or sulphotoxidation. After a series of experiments, they found that the fish do not need the help of oxidative enzymes for disposal of lipid soluble compounds for these compounds are rapidly disposed of by passive diffusion through the lipoidal gill membrane. They stated that generally, aquatic animals do not possess the same oxidative enzymes as do terrestrial animals (reptiles, birds and mammals) which have developed such mechanisms in the course of evolution to dispose of lipid-soluble, unwanted
substance ingested in food. For terrestrial animals, without these enzymes, the foreign substance would gradually accumulate to toxic levels and life would cease.

5. Toxicity of molluscicides.
A much fuller understanding of the toxicity of molluscicides might be achieved if relevant information on molluscan physiology and biochemistry were available. An understanding of several basic phenomena associated with the uptake of compounds would be helpful, for example. This information might include, first, the possible modes of entry of molluscicides from the aqueous environment into snails and secondly the relationship between chemical structure and biological activity. Thirdly, the identification of the target cells and (or) tissues which are most vulnerable to the toxic action of the molluscicides or their metabolites might be established and whether the death of the mollusc results from the effect of the chemical on such cells or tissue. Fourthly, the mode of action of the molluscicide which causes lethality could be determined. These are some of the basic data that must be looked into before a more rational approach can be taken in developing new molluscicides which possess the desirable properties of the ideal molluscicide discussed above.

5. The possible modes of entry of molluscicides into snails.
One of the first clues to the character of living membranes was supplied by Overton (1902). Since then considerable evidence has accumulated in support of
Overton's thesis that the cell membrane is mainly lipoid in character (Gorter and Grendel, 1925; Parpart and Dziemian, 1940; Davson and Danielli, 1952; Mayer and Maickel, 1960). Recent evidence from electron microscope pictures and X-ray diffraction patterns (Singer, 1975; Singer and Nicolson 1972; Capaldi, 1974) has revealed that all cell membranes (i.e. outer and inner) are essentially alike, being composed of protein and fatty acid substances and that lipid accounts for about half of the mass of the membranes. In inner membranes, the lipid is entirely phospholipid. Substances which are lipid-soluble might therefore be expected to pass into membranes by dissolving in them. However, much remains to be learned before there can be a good understanding of the relation between membrane structure and membrane permeability. For example, there is the question of the nature of membrane pores; their presence in the lipoid layer seems necessary to explain the passage of water and the ready diffusion of small, lipid-insoluble molecules and ions across cell membranes (Andersen and Ussing, 1957; Berliner, 1959; Paganelli and Solomon, 1957; Solomon, 1958).

There is also the question of how certain inorganic ions and lipid-insoluble molecules, such as glucose and amino acids, rapidly cross the cell boundary. Giese (1959) has discussed the factors which may influence movements of solutes through cell membranes. Amongst these he includes the effect of molecular size and ionisation. He pointed out that charged molecules enter much more readily than ions. The cell membrane is accordingly envisaged as a
mosaic of negative and positive charges with a net positive charge. An anion might, therefore, be expected to enter more rapidly than a cation. The valency of an ion will also play a part in its entry. The differences which are recordable between entry rates for various ions of the same valency could be explained by hydration of the ion which will alter the size of the various particles. The entry of such molluscidal ions as copper may be catalysed in this way, though as will be seen later, Azevedo et al. (1958) provide an alternative explanation based on their finding that radioactive copper appears to be concentrated and absorbed through the intestinal tract.

Schanker (1962) concluded that the ways by which solutes move across membranes may be grouped into two general categories, passive transfer processes, and specialised transport processes. In the passive transfer processes, the membrane behaves as an inert solvent layer or system of aqueous channels through which the solute passes and thus many substances can move across the membrane by either simple diffusion or filtration. In specialised transport, the membranes display an active character, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane; the substances passing through the membrane by either active transport, facilitated diffusion, exchange diffusion or pinocytosis.

Duncan (1969) reviewed the methods or routes by which the molecules might enter the body of the snail as follows:
5.1.1. Ingestion with food and water followed by transport across the gut wall. This may be the case with molluscicides formulated as very fine particles as was indicated by Hopf et al. (1963) for copper compounds of low solubility.

5.1.2. The ingestion of particulate matter by the outer surface cells of mollusc mantle is reported by Nakahara and Bevelander (1967). The mantle epithelium of the calico clam, Macroscollista maculata, was seen to ingest colloidal gold and carmine particles. Using colloidal thorium dioxide (thorotrast) and electron microscopy, these authors have also observed a similar phenomenon in two related species of bivalves, Pinctada radiata and Isognomon alatus.

5.1.3. Alternatively, chemicals could pass through the external membrane of the snail as molecules rather than particles. Harris (1960) has outlined the ways in which this might occur. He states that the passage of molecules or ions from a solution into a membrane when no chemical forces operate, is a consequence of the collision between the particles and the membrane which gives rise to a certain surface concentration followed by diffusion within the membrane material. Somers (1963) working on uptake of copper fungicides into fungal spores reported that the uptake of $^{64}$Cu by the cells is probably an ion-exchange reaction which is followed by permeation throughout the cell. There was no evidence of an adsorption process and copper is therefore considered to be accumulated passively by the spores by unspecific reactions with cell
constituents. Cheng and Sullivan (1974) using \(^{67}\text{Cu}\) revealed that the accumulation of copper intermingled with mucus on the surface of the foot region of B. glabrata is probably not the primary site of entry of the metal ion. On the other hand, the accumulation of copper on the surface of the rectal ridge appears to be of significance since copper in the aqueous environment is brought into contact with tissue by the water current entering the dorsal mantle chamber, and it is postulated that this area may be the site of entry.

5.2. Structure-activity relationships.

Drug action had previously been correlated with partition coefficient (Overton, 1901) but it is only in the last twelve years that serious attempts have been made to quantify relationships between physicochemical properties of compounds and the biological responses they elicit. In this way, not only the mechanisms of action might be explained but the activity of unprepared congeners may also be predicted (Dunlop, 1976). Free and Wilson (1964) suggested that biological activity is an additive property of the substituents on a molecule. Everytime a substituent appears in a molecule it is assumed that it will play a constant role either contributing to or detracting from the overall biological activity.

Dixion (1948) found that the greater the electron withdrawl from the halogen atom, X, in compounds of type \(\text{R-C-CH}_2\text{X} \), the more active the system will be as a lachrymator and thus the more reactive it will be towards
sulphydryl groupings. De Villiers and Mackenzie (1963), applying the same argument to the molluscicidal activity of the phenacylhalides, proved that phenacylbromide is more active than phenacylchloride. This is also the case with two p-iodo-phenacyl compounds and two m-nitrophenacyl derivatives. They conclude that increasing the positive nature of the halogen atom, either by going from chlorine to bromine or by the correct substitution on the phenyl ring, increases the molluscicidal activity. Similarly, Wain (1963) made a systematic investigation, of the effects on herbicidal action, of replacing the -NO₂ groups in DNC(2:4-dinitro-6-methylphenol) by -CN. A number of 2- and 4-hydroxybenzonitriles were subsequently synthesised and their activity as weed killers was assessed by standard methods. In general, only poor performance was shown by derivatives of salicylnitrile but a number of compounds derived from 4-hydroxybenzonitrile were shown to possess outstanding herbicidal activity and moderately good molluscicidal activity; e.g. 3;5-diiodo-4-hydroxybenzonitrile (ioxynil) and the 3;5-dibromo analogue (broxynil). Dunlop (1976), examining quantitative structure-activity relationships between molluscicidal activity and the physical and chemical properties of nicotinanilides, revealed a positive correlation between molluscicidal activity and partitioning.

5.3. Distribution of molluscicides among organs.
Little work has been done on this line of investigation, which could be an important one in providing clues as to the mode of action of molluscicides. Application of
radiotracer methods can tell whether compounds accumulate in any particular tissue or cells and the rate of accumulation in the various organs might also be related to molluscidial effect.

Azevedo et al. (1958), using radioactive sodium orthophosphate, showed that the radioactivity of the soft parts of the snail was x4 that of the shell. Using solutions containing $^{32}$P, $^{131}$I and $^{64}$Cu they succeeded in showing by means of autoradiographs of Helisoma duryi tissue, that the radioactive compounds were absorbed by the intestinal tract and became diffused through the various organs, with a tendency to accumulate in the hepatopancreas. Duhm et al. (1963), using $^{14}$C-niclosamide, found that storage of activity could not be established in any organ of the rat, not even following repeated application. Less than 0.5% of the applied activity could be traced in the animal 48 hours after the final application. Of this 0.5%, the greater part was found in the gastrointestinal tract. Somers (1963) and Martin (1969) used radioactive copper to show that fungal conidia may concentrate copper by a factor of up to 100-fold over that in the immediate environment.

Beynon (1971), using Rasbora spp., related the build up of $^{14}$C-Frescon (up to x50 the actual concentration in the water in 4 hours) to the filtration of the solid particles of the chemical from the water by the gills of the fish. Matthiessen (1977), using Sarotherodon mossambicus, found that after 55 hours exposure to a sub-lethal solution of Frescon, approximately 40% of the Frescon was found in
the bone, 16% in the muscles, 16% in the gut and 10% in the gill. He suggested that the relatively high concentration found in the bile, liver and gut indicates that liver is involved in Frescon excretion.

5.4. Action of molluscicides.

The process leading to the production of energy i.e. to the synthesis of adenosine-5'-triphosphate (ATP), starts in the cytoplasm with the biotransformation of glucose to pyruvate. Energy production and biosynthesis are also required for growth. Lower molecular weight compounds such as amino acids and purines are produced in the cytoplasm; protein synthesis takes place in the ribosomes; DNA in the nucleus and RNA partly in the nucleus and the remainder in the cytoplasm. Kaars Sijpesteijn (1970) summarised the process which leads to the production of ATP into four main stages; first, alteration of glucose to pyruvate, second, oxidation of pyruvate to carbon dioxide via the tricarboxylic acid cycle (TCA cycle), third, hydrogen or electron transfer via flavin enzymes, cytochromes and cytochrome oxidase to form water using up oxygen and fourth, oxidative phosphorylation to form the energy rich compound, ATP from adenosine-5'-diphosphate (ADP). These four processes are coupled in such a way that inhibition of any of them leads to inhibition of respiration as well as inhibition of ATP production.

Brand et al. (1949) surveyed 72 compounds for their inhibitory effect on the oxygen consumption of
Australorbis glabratus ( = B. glabrata ). They could distinguish 4 types of reaction: no effect; transitory inhibition; slow inhibition, rapid and lasting inhibition. But, due to the sharp initial retraction of snails into their shells and the implications of this retraction on oxygen uptake, the mode of action of the compounds could not be ascertained with certainty. There were some indications however, that enzyme inhibition is involved in the latter two reaction types listed above. The most effective inhibitor reported by them was α-nitrostilbene which, at a concentration of 10 ppm, reduced oxygen consumption by more than 99%. Wain (1963), investigating the mode of action of the 3,5-dihalogeno-4-hydroxybenzonitriles as herbicides found that these compounds were strongly active in uncoupling oxidative phosphorylation. Since this property is shown also by 2,4-dinitro-6-methyphenol and pentachlorophenol, both of which are well known molluscicides, he examined them for molluscicidal activity and found that ioxynil had a LC₅₀ of about 0.5 ppm and broxynil about 2.0 ppm when tested, as their sodium salts, against B. glabrata.

5.5. Mode of action of some molluscicides in current use.

5.5.1. Sodium pentachlorophenate (NaPCP).

Weinbach (1954) suggested that the molluscicidal activity of pentachlorophenol (PCP) is due to the interference with the oxidative phosphorylation process. He indicated that it is a powerful uncoupler of oxidative phosphorylation in both snail and rat tissues. Weinbach and Nolan (1956) found that PCP, unlike 2,4-dinitrophenol (DNP), inhibits
the ATP-ase activity in a variety of mitochondrial preparations including 'soluble systems'. Ishak et al. (1970) revealed that the activity of NaPCP is due to its uncoupling action when used in low concentrations (3 ppm) but at higher concentrations (above 3 ppm) this could be attributed to the inhibition of the glycolytic pathway.

5.5.2. Copper compounds.

Hopf et al. (1963) tested various copper compounds of low solubility and found the activity increased with decreasing pH. It was concluded that toxic action was due to cupric ion (Cu^{2+}). Martin (1969) also suggested that some copper compounds probably act via the cupric ion which means that cuprous oxide must be oxidised before becoming effective; the mechanism, enzymatic or otherwise, by which this is done is unknown however.

Ishak et al. (1970) found that the oxidation of various substrates by homogenates of B. glabrata was inhibited by concentrations of copper sulphate at micromolar levels but that neither pyruvate nor α-ketoglutarate were involved. Kaars Sijpesteijn (1970), in a review of the mode of action of agricultural fungicides, suggests that copper ions might be fungitoxic through interference with pyruvate dehydrogenase. Although copper sulphate has been shown to inhibit oxygen uptake by some fungal spores at concentrations that just prevent germination (McClen et al., 1954) there do not appear to be any studies on whether pyruvate or α-ketoglutarate accumulates in fungi treated with inorganic copper. Corbett (1974) concluded
that the inhibition of dihydrolipoyl dehydrogenase, and hence the pyruvate (and possibly α-ketoglutarate) dehydrogenase system, provides a possible explanation of the effect of cupric ions on fungi, but that other mechanisms may operate as well.

If copper does work by inhibiting keto-acid oxidation, one would expect copper salts to be generally biocidal. However, possibly due to lack of uptake by higher plants and insects, inorganic copper salts are not widely used to control pests other than fungi, though copper sulphate has been used as a selective herbicide and algicide (Martin and Worthing, 1972) besides its use as a molluscicide.

5.5.3. Organotin compounds.

Trisubstituted-tin compounds, whose activity and mode of action has been reviewed by Kaars Sijpsteijn et al. (1969) are thought to work by inhibiting oxidative phosphorylation. This is mainly based on the evidence by Aldridge and Street (1971) that these or related compounds are active inhibitors of oxidative phosphorylation in isolated mammalian mitochondria. There is evidence that triethyltin inhibits ATP synthesis by binding to a component of the energy conservation mechanism, and by interfering with the exchange of hydroxide ions and anions across the mitochondrial membrane (Rose and Aldridge, 1972).

5.5.4. Niclosamide.

Günnett and Schraufstätter (1959) showed that the concentrations of niclosamide which are lethal to snails
strongly inhibit oxygen uptake by the whole snail, while lower concentrations were found to stimulate respiration by up to 40%. Ishak et al. (1970) explained the molluscicidal activity of the molluscicide on the basis of its powerful inhibitory action on the oxidative processes of the snail. They showed that it stimulates respiration at very low concentrations (0.000033 ppm) and that at a concentration of 0.3 ppm it inhibits succinate oxidation by 45%, glutamate oxidation by 70% and reduced tetramethylparaphenylene diamine (TMPD) oxidation by 15%. Later, Ishak and Mohamed (1975) confirmed the inhibition of the respiratory rate by niclosamide and they were able to demonstrate that sub-lethal concentrations markedly reduced the respiration rate and that the rate of oxygen consumption decreased at increasing concentrations of the chemical.

5.5.5. Frescon.

Beynon (1971) stated that Frescon breaks down readily to form triphenylcarbinol (TPC) and morpholine in water, soil and plants and that these compounds are known to be of a low order acute and sub-acute toxicity to mammals. Both Frescon and TPC are lipophilic but they will not be stored in animal fat since mammals convert them to hydroxylated derivatives which are readily excreted.

Brown and Hubble (1969) associated the toxicity of Frescon with the N-tritylmorpholine molecule rather than with the hydrolysis products, TPC and morpholine. Beynon (1971) found that the metabolism of Frescon itself in rats and dogs was similar to that of a mixture of TPC and
morpholine and he suggested that it is probable that Frescon is hydrolysed in the gut prior to adsorption. The morpholine is excreted largely unchanged in urine but TPC is metabolised mainly by hydroxylation in the para-position followed by conjugation with glucuronic acid. These conjugates are hydrophilic compounds unlike Frescon which is lipophilic. Moreover, Griffiths (1968) found that Frescon is more toxic to rats when administered intravenously than orally and this may be due to gastric acids speeding up the hydrolysis rate. He also showed that the mammalian liver can hydrolyse Frescon and that traces of hydroxycarbinols could be detected as metabolites both free and conjugated.

Using $^{14}$C-Frescon, Beynon (1971) suggested that its metabolism in fish would probably occur with longer exposure times, as he was able to identify only unchanged Frescon in fish exposed to treated water for 30 minutes. Similarly, using thin layer chromatography (TLC) on ether/ethanol extracts of fish exposed to $^{14}$C-Frescon for 30 minutes, Griffiths (1968) showed that the material in the fish was unchanged, since the radioactive peak co-chromatographed with unlabelled Frescon. He also reported that the rate of removal of Frescon or its metabolites from the fish body appears to be slower than in mammals, possibly due to the inability of the fish to convert Frescon and TPC to more hydrophilic compounds and this may be due to the absence of drug-metabolising enzymes in fish as has been reported by Maickel (1960) and Brodie and Maickel (1962).
It seems that Frescon does not directly affect respiration like many other molluscicides e.g. niclosamide and pentachlorophenol by interfering with oxidative phosphorylation and it appears more likely that it acts on the nervous system of the snail (Shell Chemicals, 1974). It was added that Frescon appears to act on the synapses, either by increasing the amount of transmitter-substance released from the presynaptic terminals, or by potentiating the action of the transmitter at the post-synaptic cell membrane. It is not yet known whether the actual concentrations of Frescon required to produce these effects on the isolated nervous system can account for the molluscicidal activity in Frescon. Moreton and Gardner (1976) have suggested the nervous system as a possible site for Frescon action in freshwater snails. They showed that Frescon modifies the action of synaptic networks so as to cause intermittent massive discharges affecting the entire nervous system. These "Frescon bursts" were shown to occur after a minimum of 10-20 minutes in nerve cells from the visceral or right parietal ganglion of the isolated central nervous system of Lymnaea stagnalis exposed to 10 ppm of molluscicide. Banna (1977) working on both Archachatina and Bulinus was able to demonstrate these "Frescon bursts" in some neurons together with the inhibition of normal activity in others. Accordingly he suggested that the chemical may also be affecting the interneurone pools.

6. Present work.

It is evident from the above survey that a large amount
of information exists on the different aspects of the mechanisms of action of molluscicides. However this information is diffuse, lacking the coordination in direction and continuity which would help in achieving a better understanding of the susceptibility of molluscs, aquatic biota or domestic animals to molluscicides.

With the above points in mind, the present study was made in order to examine the difference in susceptibility between the schistosome-bearing snail, Bulinus truncatus and a representative tropical food fish, Sarotherodon mossambicus to two molluscicides; Frescon, which is known to be toxic to both snails and fish at more or less the same concentrations and 4'-chloronicotinanilide, a candidate molluscicide which is relatively non-toxic to fish (Matthiessen, unpublished).

The ratio of uptake and loss of the two compounds were studied in order to find out whether these rates influence molluscicidal activity and also whether they are the cause of the difference in susceptibility between the two species. It is also essential to study their metabolism in order to determine the nature of their metabolites and whether they are excreted or retained in the body.

The relative susceptibilities to Frescon of various groups of B. truncatus collected from the Gezira were also examined and the implications of these results on future application of Frescon in the Gezira are discussed.
CHAPTER 2

MATERIALS AND METHODS
MATERIALS AND METHODS

1. Molluscicides.
Strictly speaking N-tritylmorpholine or N-triphenyl-
methylmorpholine is the active ingredient of the
commercial emulsifiable concentrate formulation, Frescon.
The names are often used interchangeably, however, as
they are in this text.

1.1. N-Tritylmorpholine (Frescon).
N-Tritylmorpholine was kindly supplied as a 16.5% w/v
emulsifiable concentrate (Frescon FX 28) by Shell
Research Ltd., Sittingbourne, Kent; sample FC 5730.

1.2. Radioactively-labelled N-tritylmorpholine (3H-Frescon).
Radioactively-labelled N-tritylmorpholine, (N-triphenyl-
methyl[^3]H morpholine), with universal tritium label in
the morpholino ring was also supplied by Shell Research
Ltd. (Specific Activity = 21.85 µCi/mg on 1st June, 1974).

1.3. 4'-Chloronicotinanilide.
This compound was prepared by slowly adding nicotinyl
chloride-hydrochloride to 4-nitroaniline in a minimum of
dry pyridine and refluxing for 90 minutes. The precipitate
gave a satisfactory elemental analysis (C,H,N ± 0.3%)  
(Dunlop, 1976).

This was synthesised by an acid catalysed exchange reaction
between tritiated water and the substituted aniline,
followed by acylation with nicotinylchloride. The chemical
had chemical and radiochemical purities > 99% (Specific Activity = 47.0 µCi/mg on 21st August, 1974) (Dunlop, 1976).

2. Other radioactive compounds.

2.1. n-[1,2(n)-\textsuperscript{3}H]hexadecane.

- Tritium labelled n-hexadecane (Specific Activity = 2.27 µCi/gm on 1st December, 1973) was obtained from The Radiochemical Centre, Amersham, Buckinghamshire.

2.2. Tritiated water (\textsuperscript{3}H\textsubscript{2}O).

This was from stock from The Radiochemical Centre, (Specific Activity = 11.2 mCi/ml on 15th March, 1977).


Insta-gel and Dirilume-30 (universal liquid scintillation scintillants) and Solven-350 (tissue solubiliser) were obtained from Packard Instrument Ltd., Caversham, Berks.


Silica gel GF(250 microns) precoated analytical thin layer chromatography plates were obtained from Anachem, Luton, Bedfordshire.

5. Reagents and solvents.

All reagents and solvents were of analytical grade and were obtained from British Drug Houses Ltd., Poole, Dorset.

6. Animals.


These were collected from two different locations; from Hebeika canal in the Frescon-treated area of the Gezira
Schemo, Sudan and from Abu Cucli canal in the non-treated area of the same scheme.

6.2. *Biomphalaria glabrata* (Jay).
This is a Brazilian strain reared under standard conditions (Duncan and Brown, unpublished). This snail replaced *Bulinus truncatus* in one experiment on the distribution of radioactive material among organs. This was considered acceptable since they are of the same family though it is also recognised that species differences may occur.

The strain originated from the Wonji-Shoa Sugar Plantation, Ethiopia and has been bred in London at the C.O.P.R. for 2 years.

This is a laboratory bred strain (= *Tilapia mossambica* (Peters)) originally obtained from the Malaysian Agricultural Research and Development Institute, Malacca.

6.5. *Schistosoma haematobium* (Weinland) miracidia.
These were hatched from eggs collected from the urine of infected schoolboys in Kereiba Village, Gezira, Sudan.

7. Rearing methods.

7.1. *Bulinus truncatus*.
The two collections were bred separately in identical perspex aquaria containing 9 litres of an aerated, artificial hard water (AHW: 0.104 gm CaCl and 0.26 gm MgSO₄·7H₂O per litre of distilled water (Hopf and Muller, 1962)) at 25°C and under fluorescent light on a
12 hr/12 hr diurnal cycle. To rear the snails for experimental use, about 200 adults were transferred to a new tank for about 24 hours and then removed again, leaving behind egg masses whose age was therefore known within 1 day. The eggs began to hatch after 5 days and the hatchlings were fed on small additions of ground TetraMin fish food (Tetra Werke, West Germany). When the snails were 10 days old, 100 snails were moved to a new tank and from then on were fed on dried lettuce twice a week with frequent additions of TetraMin fish food. This method arranged that all snails in any one aquarium were of the same age, presumably in the same physiological condition and more or less of the same size and weight. The relationship between the shell height and body weight was examined. The shell height was measured at the greatest distance between the basal margin and the uppermost point of the shell, the apex, taken parallel to the axis (Mandahl-Barth, 1962). It is evident from Fig. 1 and Appendix I that a curvilinear relationship represented by the biparametric equation $y = ax^b$ exists (where $a = 0.16$ and $b = 3.03$) and that a straight line relationship seems best satisfied by plotting logarithms of the two parameters (Fig. 1a).

7.2. *Biomphalaria glabrata.*

These were cultured at 26°C and under fluorescent light on a 12 hr/12 hr diurnal cycle. About 100 snails were put into 8 litres of AMW when they were 3-4 days old, fed on boiled lettuce and provided with under-gravel and airlift filtration through charcoal (Duncan and Brown, unpublished).
Figure 1. RELATIONSHIP BETWEEN SHELL SIZE AND SNAIL TOTAL WEIGHT OF BULINUS TRUNCATUS.
Figure 1a. RELATIONSHIP BETWEEN SHELL SIZE AND SNAIL TOTAL WEIGHT OF BULINUS TRUNCATUS.
7.3. *Sarotherodon mossambicus.*

These were reared and bred under standardised laboratory conditions (Matthiessen, unpublished). A maximum of 10 adults was kept in a 30 litre glass aquarium through which pre-heated (27-28°C), dechlorinated and air saturated London tapwater (total hardness = 291 mg/l as CaCO₃, pH = 7.9-8.0) was pumped at a rate of 6 litres per hour. The tanks were illuminated by fluorescent light on a 12 hr/12 hr diurnal cycle. The fish were fed on Main Stream Trout Diet, Standard 2 expanded, pellets (BP Nutrition Ltd., Witham, Essex). For the first four weeks, after the fry have been removed from the female, they are kept in 20 litre perspex tanks at a density of about 5 fish per litre. The fry were fed on ground Trout Diet twice a day. All the fish used were the first generation descendants of the original stock.


In order to expose snails to radioactive molluscicides under constant conditions of molluscicide concentration, oxygen tension and temperature, the flow-cell apparatus described by Duncan et al. (1977) was used.

The apparatus (Fig. 2) consisted of a cylindrical glass cell 5 cm high x 2.5 cm diameter. A glass tube with a bell-shaped end was clamped so as to stand 1 mm away from the bottom and sides of the cell. Another glass tube whose end had been turned through 90°, was held within the first tube by a polythene collar. The exposure cell was held level with the tops of the two brown-glass, Winchester bottles. A peristaltic pump was used to drive air in to
Figure 2. Single Cell Unit With Inflow And Outflow Connections.
displace water from one of the bottles and so into the cell. The air line passed almost to the bottom of the Winchester thus helping to keep the water aerated. After passing downwards under the bell, the water was evacuated through the stainless steel tubing by means of a second peristaltic pump set to work slightly faster than the first. A three-way tap was employed to redirect the air flow to a second Winchester bottle which contained the molluscicide solution so that the molluscicide was passed into the cell. The exposure cell was replicated 10 times and the entire apparatus (Fig. 2a) was contained in a 135 litre water bath which was maintained at constant temperature by means of 6 thermostatically controlled heaters distributed evenly on the bottom of the bath, and 2 electric mixers, one on each side of the bath, to distribute the heat evenly.


9.1. Introduction.

Liquid scintillation counting is a method of assessing the radioactivity of beta-emitting isotopes (e.g. $^3$H, $^{14}$C, $^{32}$P). The samples are dissolved in a solvent containing a solute that fluoresces or scintillates when excited by beta-particle energy. Solubiliser may be necessary in order to dissolve some samples and the whole mixture is termed a 'cocktail'. Since the radioactive sample is in intimate contact with the cocktail constituents, the efficiency of the energy transfer is maximised. The beta particles excite the solvent molecules which in turn transfer the energy to the scintillator resulting in the
Figure 2a. Replicated Cell Arrangement Housed In Water Bath.
production of photons of light which pass through the wall of the vial containing the sample to be detected and converted to electrical pulses by photomultipliers. The pulses produced are proportional to particle energy and are sorted into channels by analyser units incorporating pulse height discriminator circuits.

With experimental samples, a number of undesirable effects occur. These include interference with the sample-solvent-scintillator energy transfer mechanism by chemical substances (e.g. dissolved oxygen and solubilisation chemicals) or the colour of the solution absorbing emitted light. The result of these effects is to cause a reduction in the detector photomultiplier pulse height voltage, resulting in a shift of the pulse height spectrum as observed by the system analyser circuits. These effects are termed chemical and colour quenching respectively.


After exposure to labelled compounds, whole adult *B. truncatus* and fry *S. mossambicus* were removed from the solution, blotted dry, weighed and measured, then wrapped in a small piece of tissue and folded into a Packard 'Combustocone' and immediately burnt in a Packard model 305 Oxidiser set to wash the condenser with distilled water (1 ml) and add Instagel scintillator (14 ml) to a scintillation vial.
9.2.2. Solubilisation.
Weighed portions of various tissues of *S. mossambicus* and *B. glabrata* were placed in counting vials and Soluene-350 (1 ml) was introduced into each vial which was kept at 28°C for 24 hours. To ease the solubilisation the vials were frequently agitated. Dimilume (14 ml) was then placed in each vial and the samples were left to stand over night. Dimilume is a commercial scintillant which also suppresses chemiluminescence.

9.3. Liquid scintillation spectrometer.
The samples were counted at 13.5 ± 1°C in a Packard model 2425, liquid scintillation spectrometer using the automatic external standard ratio method to correct for quenching.

9.4. Quench calibration.
The model 2425, liquid scintillation spectrometer incorporates an external gamma-ray emitting source (266 Radium) in a shielded compartment which is automatically positioned close to the vial for quench calibration. The gamma source interacts with the solvent and scintillator and gives rise to a spectrum of photon energies very similar to the ³H beta spectrum. The emission spectrum due to the external source is affected by quenching agents in a similar way to that of the sample beta source and this fact is employed in developing an automatic quench correction for experimental samples. Calibration curves of counting efficiency versus external standard ratio (ESR) are generated as described below.
9.4.1. Combusted sample preparations.

Different volumes of distilled water (0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 ml) were pipetted into separate vials and Insta-Gel (14 ml) was added to each vial. An internal standard or 'spike' of 50 µl of 3-n-hexadecane (100% efficiency = 174010 disintegrations per minute (dpm)) was then introduced using a microsyringe. The samples were then counted in the spectrometer. From the counts per minute (cpm) obtained for each vial the % efficiencies were calculated

\[
\% \text{ Efficiency} = \frac{\text{cpm}}{174010} \times 100
\]

The calculated % efficiencies were then plotted against the external standard ratio obtained for each vial (Appendix 2). The result as shown in Fig. 3 was a linear-relationship represented by the equation

\[ y = 0.0644 + 0.0150x \]

9.4.2. Solubilised B. glabrata.

Eight snails were crushed in 8 ml of Soluene-350 and kept at 28°C for 24 hours. Different volumes (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml) of the solubilised sample were pipetted into separate vials and 14 ml of Dimilume were added. A spike of 50 µl 3H-n-hexadecane (100% efficiency = 179445 dpm) was introduced using a microsyringe. Single snails were also crushed and solubilised in 1 ml of Soluene-350 and treated in the same manner. The samples were then counted in the spectrometer. From the cpm obtained for each vial the % efficiencies were calculated and these were plotted against the E3R obtained for each vial (Appendix 2a). The result as shown in Fig. 3a is a
Figure 3. TRITIUM QUENCH CALIBRATION CURVE FOR COMBUSTED SAMPLE PREPARATIONS.
Figure 3a. TRITIUM QUICK CALIBRATION CURVE FOR SOLUBILISED B. GLABRATA.
linear relationship represented by the equation

\[ y = 2.57 + 59.21x \]

9.4.3. Solubilised *S. mossambicus*.

*S. mossambicus* was dissected and between 0.1 and 0.6 cm of various tissues were placed in counting vials. Six ml of Soluene-350 were introduced into each vial and the tissues were left to solubilise at 28°C for 24 hours. Varying amounts (0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 ml) of solubilised tissue were pipetted into separate vials. Dimilume (14 ml) was placed in each vial followed by 50 μl of \(^{3}\)H-n-hexadecane (% efficiency = 182004 dpm) using a microsyringe. The samples were left overnight to reduce chemiluminescence resulting from the interaction between the solubilised tissue and the scintillant (Peter Matthiessen, personal communication) and were then counted in the spectrometer. From the cpm obtained for each vial the % efficiencies were calculated.

It is evident from Fig. 3b that a linear correlation is best obtained when the logarithm of the calculated % efficiencies were plotted against ESR obtained for each vial (Appendix 2b). From the relationship the % efficiency can be calculated by the equation

\[ \text{% efficiency} = \text{antilog} (0.734 \times \text{ESR}) + 1.072 \]

10. Recording of snail activity.

10.1. Time-lapse cinematography is one of a number of techniques which can be used to record animal activity. Single snails were held in 4.5 mm petri dishes partially submerged in a water bath at 27°C, while groups of 6 snails were held
Figure 3b. TRITIUM QUENCH CALIBRATION CURVE FOR SOLUBILISED TISSUE OF S. MOSSAMBICUS.
in glass vials (similar to those used in the flow-cell apparatus) through which water was made to flow by means of a peristaltic pump (Watson-Marlow Ltd.). In order to differentiate individual snails in each cell, their shells were painted different colours with a non-poisonous enamel (Humbrol, Hull) (Fig. 4).

Direct illumination was provided by a 100w bulb placed 1 metre from the water bath when a monochrome (Ilford Mark V) 16 mm. motion picture safety negative was used and by 2 photoflood lamps (Philips, Photolita No. 1) placed 2 metres from the cell when Ektachrome (Kodak, commercial film 7252) film was used. The heat generated by the lamps was absorbed in a 3 litre round-bottom flask filled with tap water and placed midway between the lamps and the cell.

Photographs were taken using a M16 Reflex Bolex camera fitted with an electric motor drive (Paillard-Wild motor MBF-A). The camera was triggered at regular intervals of 30 seconds by an electronic timing device (Paillard-Wild variometre control unit MBF-B).

The films were developed by Filmatic Laboratories Ltd., London. A remote controlled motion analysis projector was used for the analysis of the snail's activity. Each frame was projected onto a piece of paper, at right angles to the projected image, and the position of each snail was recorded. From the position of each snail on consecutive frames the distance moved by each snail in 30 seconds could be measured.
Figure 4. Colour-coded B. truncatus In The Flow Cell.
10.2. Recording snail trails.

The method of Ullyot (1935), for recording the direction of the movement of the flatworm, *Dendrococlum lacteum*, was used with slight modifications. The bottom of the aquarium was covered by a sheet of clean glass on which the snail crawled, leaving a mucus trail. This mucus track was normally quite invisible, but if the glass plate was put into a suspension of fine mud, the fine particles became entangled in the mucus, so that after gentle agitation of the plate in the clean water the path traced by the snail stood out as a brown line. The plate was then dried and photoprints were made from it to give a permanent record (Fig. 5).

11. Thin layer chromatography.

The plates were activated before use by heating to 150°C for 4 hours. Using a spotting jig, test materials and markers were spotted onto the plates 1.5 cm from the bottom by means of a 10 µl micropipette. The plates were run, in ascending mode, in closed tanks. The atmosphere was equilibrated with the solvent by using a large area of filter paper round the inside of the tank and dipping into the solvent. The solvent mixtures used routinely for N-tritylmorpholine and its hydrolysis products were 10% v/v diethylether in hexane or ethylacetate/diisopropyl ether (1:1, v/v) and for 4'-chloronicotianilide and its hydrolysis products, 20% v/v ethanol in benzene or dioxan/acetone (1:1, v/v). Ultra-violet light was used to locate individual spots after the plates had been run.
Figure 5. Trails Of B. truncatus.
12. Infection of snails with *S. haematobium*.

*B. truncatus* was exposed to miracidia hatched from *S. haematobium* eggs. The sample was rapidly washed, in urine flasks, several times with tap water until it was a clear suspension. The suspension was transferred from the urine flasks into several petri dishes. The dishes were left under artificial light for 1 hour at room temperature (20-25°C) to stimulate hatching. The hatched miracidia were picked up with the aid of a Pasteur pipette under a binocular microscope.

Snails (4.0-5.0 mm shell height) were exposed separately to 5-8 miracidia in a 2 cm depth of water for 4 hours at room temperature. The snails were returned to the rearing tanks and 4 weeks later, they were examined for infection. They were placed separately in glass tubes (5 cm high x 2.5 cm diameter) containing 2 cm depth of water (AHW) and exposed to artificial light for 6 hours at room temperature (McClelland, 1967) to stimulate shedding of cercariae. The positive snails were grouped together and transferred to new tanks. The rest were tested every other day for about a month to see if they were infected.
CHAPTER 3

PRESENT WORK (PART I)

Comparative Toxicity and Uptake of N-tritylmorpholine (Frescon) and 4'-chloronicotinanilide by Bulinus truncatus and S. rotheroidon mossambicus.
Comparative Toxicity and Uptake of N-Tritylmorpholine (Frescon) and 4'-Chloronicotinanilide by B. truncatus and S. mossambicus.

1. Introduction.

Susceptibility to molluscicides is known to vary from species to species of snail (Shiff and Ward, 1966). It is of interest therefore, to know whether this is due to different rates of uptake of molluscicides, their distribution into various tissues, detoxication mechanisms or simply a function of size or body weight. N-Tritylmorpholine is a molluscicide which is used to control schistosome-bearing snails (Boyce et al., 1966). It is frequently applied to snail-infested water bodies as a 16.5% w/v emulsifiable concentrate (Frescon, FX 23). It has the advantage of being extremely effective at low concentrations. The applied dosage however, depends on the pH of the water since it is highly susceptible to hydrolysis, to triphenylcarbinol and morpholine at acidic pH (Boynon et al., 1967). The acute toxicity of Frescon to snails and fish (Tables 1 and 2) is variable and depends on the species as well as physical factors such as temperature and pH of the treated water.

4'-Chloronicotinanilide is one of a number of chemicals developed by Dow Chemicals, U.S.A., as candidate molluscicides (Ehrenford, 1969). It has been shown that these compounds have low water solubility and low oral toxicity to mice; and that they are non-toxic to fish (de Souza and Paulini, 1969). The LC$_{50}$ of 4'-chloronicotinanilide against B. glabrata after exposure for
Table 1. Toxicity of Frescon to snails on 24 h exposure.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>Water pH and temp.</th>
<th>Toxicity (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomphalaria glabrata</td>
<td>Boyce et al. (1966)</td>
<td>pH 7.8, 21°C</td>
<td>LC₉₀=0.042</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC₅₀=0.025</td>
</tr>
<tr>
<td>Biomphalaria pfeifferi</td>
<td>Boyce et al. (1966)</td>
<td>pH 8.0, 22°C</td>
<td>LC₉₀=0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC₅₀=0.014</td>
</tr>
<tr>
<td>Riom. sudanica tanganicensis</td>
<td>Webbe and Sturrock (1964)</td>
<td>pH 7.5-7.8, 22°C-24°C</td>
<td>LC₉₀=0.078</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC₅₀=0.044</td>
</tr>
<tr>
<td>Bulinus (Physopsis) nasutus productus</td>
<td>Sturrock (1964)</td>
<td>pH 7.5-7.8, 22°C-24°C</td>
<td>LC₅₀=0.090</td>
</tr>
<tr>
<td>Bulinus (Physopsis) globosus</td>
<td>Boyce et al. (1966)</td>
<td>pH 8.0, 22°C</td>
<td>LC₉₀=0.085</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC₅₀=0.050</td>
</tr>
<tr>
<td>Bulinus truncatus</td>
<td>Boyce et al. (1966)</td>
<td>pH 7.8, 21°C</td>
<td>LC₉₀=0.100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC₅₀=0.053</td>
</tr>
</tbody>
</table>

24 hours, was found by de Souza and Paulini (1969) to be 0.14 ppm and by Dunlop (1976) to be 0.37 ppm. Matthiessen (unpublished) found that x200 the dosage required to give an LC₅₀ for B. glabrata is necessary to obtain a similar LC₅₀ for S. mossambicus.

The display of apparent selectivity is of great importance because no other molluscicide shows such differential toxicity between snails and fish, and it was therefore chosen for study in view of this unique property.

2. Results.

2.1. The susceptibility of B. truncatus and S. mossambicus to Frescon and 4'-chloronicotinanilide.

Adult B. truncatus and fry of S. mossambicus were tested
Table 2. Toxicity of Froccon to fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>Water pH &amp; temp</th>
<th>Toxicity (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carassius auratus</td>
<td>Deschions &amp; Floch (1966)</td>
<td>pH 7.0</td>
<td>LC&lt;sub&gt;700&lt;/sub&gt; = 0.50 (24 hrs)</td>
</tr>
<tr>
<td>Carassius auratus</td>
<td>Shiff et al. (1967)</td>
<td>pH 7.8, 23°C</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; = 0.07 (24 hrs)</td>
</tr>
<tr>
<td>Lebistes reticulatus</td>
<td>Shiff et al. (1967)</td>
<td>pH 7.8, 23°C</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; = 0.25 (24 hrs)</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>Willomitzer &amp; Lucky (1970)</td>
<td>pH 6.6, 21°C</td>
<td>LC&lt;sub&gt;100&lt;/sub&gt; = 1.00 (10 hrs)</td>
</tr>
<tr>
<td>Rasbora heteromorpha</td>
<td>Shiff et al. (1967)</td>
<td>pH 7.8, 23°C</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; = 0.03 (24 hrs)</td>
</tr>
<tr>
<td>Sarotherodon mossambicus</td>
<td>Shiff et al. (1967)</td>
<td>pH 8.1, 20°C</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; = 0.11 (10 day)</td>
</tr>
</tbody>
</table>

under identical conditions against Froccon and 4'-chloronicotinanilide. In all instances, dosage was calculated as active ingredient.

The snails (6.5 mm shell height) and fry (17 days old) were of the same body weight (40 ± 1 mg). They were exposed in 200 ml of molluscicide solution prepared in aerated, dechlorinated tap water, pH 8.1, at 25°C. Ten animals were exposed singly at each concentration so that dead or dying animals could not affect the response of others. After an exposure period of 24 hours, the animals were left to recover for 48 hours in fresh tap water.

Death was presumed to have occurred in snails when
prodding with a seeker elicited no response, while in fish it was assessed either by their floating on the surface or sinking to the bottom with complete inactivity. Controls were established for each test although it is noteworthy that in fact no deaths ever occurred in controls for fish or snails.

The dose-mortality data from each test (Appendix 3) were analysed using log-probit plot computer program (Scoppa, 1972). The results are shown in Figure 6; the lethal concentration values and data describing the line fit are given in Table 3.

Table 3. A comparison of the dose-mortality data for Frescon and 4'-chloronicotinanilide to *Bulinus truncatus* and *Sarotherodon mossambicus* after 24 hour exposure and 48 hour recovery period.

<table>
<thead>
<tr>
<th></th>
<th>Frescon</th>
<th>4'-Cl-nicotinanilide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Bulinus truncatus</em></td>
<td><em>Sarotherodon mossambicus</em></td>
</tr>
<tr>
<td>LC50 (ppm)</td>
<td>0.034</td>
<td>0.019</td>
</tr>
<tr>
<td>LC90 (ppm)</td>
<td>0.060</td>
<td>0.021</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>F test</td>
<td>88.00</td>
<td>11.88</td>
</tr>
</tbody>
</table>

It is found that the susceptibility of the two species to Frescon and 4'-chloronicotinanilide differs significantly (*p* = 0.05), the susceptibility of the two species to Frescon being of the same order while that of *S. mossambicus* to 4'-chloronicotinanilide is x100 < that of *B. truncatus*. 
Figure 6. SUSCEPTIBILITY OF *B. TRUNCATUS* AND *S. MOSSAMBIcus*
TO FRESCON AND 4'-CHLORNICOTINANILIDE.
2.2. The acclimatisation period in relation to the activity of snails.

Yager and Harry (1964) reported that the uptake of metals by *B. glabrata* may be in part a function of the activity of the snails. Duncan et al. (1977) showed that the uptake of 4'-chloronicotianilide by *B. glabrata* is influenced by the activity of the snails and that activity decreased with time after a snail was placed in a new environment. Thus, they allowed for an acclimatisation period of 2 hours prior to measuring the rate of uptake. It was considered desirable to investigate this in more depth before proceeding to the present studies on rates of uptake. To establish the length of the acclimatisation period required for snails, before exposure to molluscicides, the activity of *B. glabrata* and *B. truncatus* was studied under different conditions by recording the snail's mucus trails and by time-lapse cinematography.

2.2.1. Snail trails.

It is apparent from the recording of snail's trails (Fig. 5) using a modification of the method due to Ullyot (1936) that the snails do not follow trails laid by other individuals of the same species. This may be seen by the trails crossing one another instead of the original trail being followed.

2.2.2. Time-lapse cinematography.

The movement of single, and groups of, *B. truncatus* and *B. glabrata* was recorded in 4.5 mm diameter petri-dishes containing static water and in cylindrical, glass tubes
arranged with water inflow and outflow as in the flow cell apparatus, in which the water was kept flowing at the rate of 1 ml per minute by means of a peristaltic pump. In subsequent experiments, the water flow was increased to 10 ml per minute for 10 minute intervals after 2 and 3 hours from the start of the transfer of the snails to the cylindrical tubes. This replicates the change-over to molluscicide exposure as used in the flow cell apparatus. The distance moved by each snail every 20 minutes was summed (Appendix 4a and b) and the results are illustrated graphically in Fig. 7a and b.

It is apparent that increased activity is demonstrable at the start of the transfer to the new environment in the two species, B. glabrata being more active than B. truncatus. This activity decreases to an almost constant level after approximately 2 hours in the two species. This was found to be independant of the number of snails in the container and the shape of the container. The snails on reaching the constant activity level were found to be undisturbed by changing the speed of pumping water over them.

It is concluded therefore, that a 2 hour acclimatisation period is necessary in rate of uptake experiments and that the system for quick change-over to molluscicide exposure media in the flow cell apparatus will not influence rate of uptake measurements.

2.3. Uptake and loss of Frescon and 4'-chloronicotinamide. The rates of uptake and loss of Frescon and
Figure 7a. **ACTIVITY OF B. GLABRETA.**
Figure 7b. ACTIVITY OF BULinus TANgATUS.
4'-chloronicotinanilide by *R. truncatus* and *S. moseambicus* were studied in order to find out whether these rates influence molluscicidal activity and also whether they are the cause of the difference in susceptibility between the species.

3H-Frescon and 3H-4'-chloronicotinanilide of the same Specific Activity (18.7 µCi/mg) were formulated in dimethylsulphoxide (DMSO) and dispersed in aerated, dechlorinated tap water, pH 8.1, to give 0.0152 µM solutions (0.005 ppm Frescon and 0.0037 ppm 4'-chloronicotinanilide). This concentration was judged from the bioassay results to be sub-lethal to snails and fry at least for the exposure periods which were to be used.

Groups of 7 snails of shell height 2-9 mm were exposed to the labelled molluscicide solution in the flow cell apparatus described in the previous chapter. Fry weighing 40 ± 1 mg were exposed in 100 ml beakers through which the solutions were kept flowing by means of a peristaltic pump. A steady loss of 21% of Frescon after 3 hours was found to be due to adsorption onto the polythene tubing of the pump (Appendix 5). The amount adsorbed has been calculated and allowed for. All the exposures were made at 25.5°C and an acclimatisation period of 2 hours was allowed before exposure to molluscicidal solutions. In loss experiments, the initial exposure to molluscicide solutions was 4 hours.

The rates at which the molluscicides are taken up and lost has been measured over different periods of time.
The results are given in Appendices G–I and are shown graphically in Figs. 8 and 9. In the case of snails, the amount of chemical per mg of body weight was calculated from the amount of chemical present in each group of snails in a cell divided by the total weight of the snails in that cell. For fish, the mean of the chemical present in approximately 6 fry was divided by the mean weight of fry i.e. by 40 mg. It can be seen from Fig. 8 that the uptake rates of Frescon and 4'–chloronicotinanilide by B. truncatus and S. mossambicus are all non-linear relationships which can be represented by the general biparametric equation \( y = a + bx \). At the same time, it is evident that the rates of loss are also of a non-linear relationship of the form \( y = \frac{1}{(a + bx)} \). The coefficients describing each curve are given in Fig. 9. These curves were chosen by computer-program (Vostry, 1972) to give the closest fit from a range of 15 possible equations; they are not necessarily the best fit. The uptake rate of 4'–chloronicotinanilide is lower than that of Frescon for both species, the uptake by S. mossambicus being the lowest of all. The rates of loss of the chemical are of the same order. Fifty per cent of the Frescon absorbed by B. truncatus and S. mossambicus was eliminated by 32 and 23 hours respectively. Similarly, 50% of the 4'–chloronicotinanilide absorbed by B. truncatus and S. mossambicus was eliminated by 23 and 30 hours respectively.

2.4. The rate of water uptake by B. truncatus and S. mossambicus

The rate of water uptake by B. truncatus and S. mossambicus was studied in order to find out whether
Figure 8. UPTAKE OF N-TRITYLMORPHOLINE AND 4-CHLORONICOTINILIDE BY B. TRUNCATUS AND S. MOSSAMICUS.
Figure 9. LOSS OF N-TRITYLMORPHOLINE AND 4-CHLORONICOTIANILIDE BY B. TRUNCATUS AND S. MOSSAMBICUS.
water influx is responsible for the carriage of chemicals into the animals and whether this might be a basis for selective action between compounds.

Weighed, adult P. truncatus and fry S. mossambicus were held singly in 50 ml beakers containing 25 ml of aerated, dechlorinated tap water. The beakers were themselves held in the surface of a water bath running at 25.5°C. After an acclimatisation period of 2 hours the water in the beakers was removed by suction by means of a peristaltic pump and immediately replaced with tritiated water (Specific Activity = 57600 cpm/ml). Temperature changes were avoided by keeping the tritiated water in the same bath. The snails and fry were allowed to remain in the radioactive solution for a known time before being removed and oxidised as described previously. The results are shown in Appendices 14 and 15 and in Fig. 10. Each point on the graph was determined from at least 3 replicates. The results indicate that both snails and fry take up water very rapidly and that equilibrium is established in both animals after approximately 6 minutes. The graph continues to rise slightly after this which is probably due to the final establishment of equilibrium between influx and efflux. The amount of water taken up per unit time is measured either by calculating the amount of water taken up in the first second using the equation describing the curve or by drawing a tangent to the curve at zero time i.e. before equilibrium begins to develop. Using the first method, it is found that 1.0 mg of snail tissue takes up 225 μl of water in 1 minute

\[ = 0.225 \text{ μl min}^{-1} \]
Figure 10. RATE OF WATER UPTAKE BY B. TRUNCATUS AND S. MOSSA-MICUS.
and 1.0 mg of fry tissue takes up 74 µg of water in 1 minute

\[ = 0.074 \mu l \text{ min}^{-1} \]

using the tangent method, it was found that 1.0 mg of snail tissue takes up 210 µg of water in 1 minute

\[ = 0.210 \mu l \text{ min}^{-1} \]

while 1.0 mg of fry tissue takes up 70 µg of water in 1 minute

\[ = 0.070 \mu l \text{ min}^{-1} \]

2.5. Distribution of Frescon and 4'-chloronicotinilide within the tissues and organs of \textit{B. glabrata} and \textit{S. mossambicus}.

Other work presented in this study has shown that snails and fish absorb Frescon and 4'-chloronicotinilide from water and concentrate it to relatively high levels but it is not known how these concentrations are internally distributed and whether their distribution bears any relation to the susceptibility of the species. It would also be of interest to relate the distribution of these molluscicides to possible modes of action and routes of excretion.

In this experiment, \textit{B. glabrata} was used instead of \textit{B. truncatus}. The former species being greater in size would obviously be expected to produce larger amounts of tissue and would therefore be easier to manipulate in this type of experiment. Adult \textit{B. glabrata} (shell diameter 25 mm) and juvenile \textit{S. mossambicus} (mean weight = 1.30 gm) were exposed for 4 hours to 0.02 ppm solutions of \textsuperscript{3}H-Frescon (Specific Activity = 20.9 µCi/mg) and
$^3\text{H}-4'$-chloronicotinanilide (Specific Activity = 52.8 µCi/mg). At the end of the exposure, the snails were killed by immersion in hot water ($75^\circ C$) for 20 seconds and the snail body was then removed from the shell using forceps; the fish were killed by a blow on the head. Both animals were dissected immediately. Most tissues and organs were used completely, while some were merely sampled. Due to limitations, such as the amount of tissue which can be dissolved by tissue solubilisers and the volumes which can be incorporated into the scintillation cocktail, it would be impracticable to solubilise whole fish tissues for example, skin, muscle and bone. Each sample was immediately weighed and placed in a counting vial for solubilisation and counting as described in the previous chapter.

The amount of chemical per mg of tissue was calculated from the mean of three samples and from this, the concentration factor was calculated as:

$$\frac{\text{amount of chemical per mg wet weight of tissue}}{\text{amount of chemical per mg of solution}}$$

The results are summarised in Table 4 and show that the highest concentration of Frescon in $B. \text{labrata}$ was found in the pseudobranch with intermediate levels present in mantle collar, head and foot regions. In $S. \text{mossambicus}$, the highest concentration was recorded in the liver with moderate concentrations in the gut, spleen, heart, kidney and brain. For $4'$-chloronicotinanilide, again the highest concentration was found in the pseudobranch of $B. \text{labrata}$ with intermediate concentrations in kidney, mantle and mantle collar. In $S. \text{mossambicus}$, $4'$-chloronicotinanilide
was concentrated in the gall bladder with moderate concentrations in the kidney, liver and spleen.

Table 4. Distribution of Frescon and 4'-chloronicotin-anilide in *B. glabrata* and *S. mossambicus* after exposure for 4 hours to 0.02 ppm solutions. The results are shown as concentration factors: μg/mg wet weight.

<table>
<thead>
<tr>
<th>Tissues</th>
<th><em>B. glabrata</em></th>
<th><em>S. mossambicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frescon (μg)</td>
<td>4'-chloronicotin-anilide (μg)</td>
</tr>
<tr>
<td>shell</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>foot</td>
<td>170</td>
<td>118</td>
</tr>
<tr>
<td>head</td>
<td>147</td>
<td>128</td>
</tr>
<tr>
<td>mantle</td>
<td>41</td>
<td>150</td>
</tr>
<tr>
<td>mantle collar</td>
<td>122</td>
<td>158</td>
</tr>
<tr>
<td>pseudo-branch</td>
<td>433</td>
<td>515</td>
</tr>
<tr>
<td>kidney</td>
<td>44</td>
<td>153</td>
</tr>
<tr>
<td>heart</td>
<td>39</td>
<td>74</td>
</tr>
<tr>
<td>liver</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>gut &amp; content</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>albumen gland</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>ovotestis</td>
<td>44</td>
<td>22</td>
</tr>
<tr>
<td>genitalia</td>
<td>35</td>
<td>95</td>
</tr>
</tbody>
</table>
2.6. Metabolism of Frescon and 4'-chloronicotinanilide by

*B. glabrata* and *S. mossambicus*.

*B. glabrata* (shell diameter > 25 mm) and *S. mossambicus*
(mean wt = 11.0 gm) were exposed for 4 hours to 0.02 ppm
solutions of Frescon and 4'-chloronicotinanilide. At the
end of the exposure, the animals were killed and
dissected immediately. The gall bladder and the liver from
each fish were taken separately, while the pseudobranchs
from 10 snails were collected together. The tissues were
then weighed, homogenised in chloroform (1mg/20ml) and
allowed to extract for 24 hours at room temperature. The
supernatant was then reduced to approximately 1 ml in a
rotary evaporator at 30°C. The extract, standard
solutions of N-tritylmorpholine and its hydrolysis
products (triphenylcarbinol and morpholine) and 4'-chloro-
icotinanilide and its hydrolysis products (4-chloro-
aniline and nicotinic acid) were chromatographed using
the solvent systems described in the previous chapter.

Qualitative analysis of the chromatograms of all the
chloroform extracts of both the gall bladder and liver of
*S. mossambicus* and the pseudobranch of *B. glabrata*
indicated the presence of unchanged parent compound,
except in the extract of the gall bladder of fish exposed
to 4'-chloronicotinanilide where spots similar to those
recorded for 4-chloroaniline and nicotinic acid were
produced.
3. Discussion.

Many countries where schistosomiasis is endemic cannot afford to meet the financial requirements for the control of the disease due to their limited health budgets and, in some cases, due to the allocation of resources to other more pressing disease problems. It seems that because of the lack of an assured market for molluscicides, the necessary research by industry on either improving the available molluscicide formulations or looking for new chemicals has been neglected. However, in the last 20 years, considerable effort has been made in screening different compounds for molluscicidal activity. Any candidate molluscicide must at least equal the standard set by those commercially available.

In the laboratory, 4'-chloronicotinanilide was found to be less toxic than Frescon. Its 24 hour LC$_{50}$ of 0.30 ppm against _B. truncatus_ compares unfavourably with an LC$_{50}$ of 0.034 ppm for Frescon. Against _B. flabrata_, the LC$_{50}$ was found by Dunlop _et al._ (in press) to be 0.37 ppm compared to 0.044 ppm for Frescon (Webbe and Sturrock, 1964). However, the compounds must not be compared on this factor alone. The standard requirements for an ideal molluscicide described by a number of authors (Colwill, 1957; Wright, 1959; Ritchie, 1973; Duncan, 1974) include many other criteria which are of relevance. Specificity of the compound is a high ranking criterion among these requirements. Since fish and snails have, to some extent, the same habitat, it is important from both the economic and public health point of view that fish should not be affected by molluscicidal applications. But, the
currently used molluscicides are known to be toxic to fish to a greater or lesser degree.

The present study has shown that Frescon is more toxic to *S. mossambicus* than *B. truncatus* while 4'-chloronicotin-anilide was shown to be non-toxic to *S. mossambicus* at concentrations about x100 those required to kill *B. truncatus*. The differences in susceptibility between snails and fish to both Frescon and 4'-chloronicotin-anilide were thought to be influenced by the amount of chemical penetrating each species.

Since it has been suggested that uptake of chemicals by snails is in part a function of the snail activity (Yager and Harry, 1964; Duncan et al., 1977), the understanding of the behaviour of schistosomae-bearing snails on exposure to molluscicides is of importance in studies of rates of chemical uptake.

Wells and Buckley (1972) have reported that the pulmonate, *Physa acuta* and the prosobranchs, *Monodonta lineata* and *Littorina saxitalis* follow trails laid by the individual and also respond to the trails left by others of the same species. The trails apparently fade in 30 minutes. The limited experiments undertaken here, using Ulyott's modified technique for recording the movement of snails, show that both *B. truncatus* and *B. glabrata* do not follow trails laid by other snails, even though these were fresh trails less than 15 minutes old.

The finding that a significantly higher activity is
demonstrable during the first 2 hours following the transfer of the snails to a new environment indicates that the snails are probably adapting after transfer to the new environment. Further work needs to be done in order to ascertain whether this adaptation is associated with differences between, for example, the quality or temperature of the water in which the snails were reared and that of the cell.

The studies described here with Frescon and 4'-chloronicotianilide indicate that these compounds are rapidly taken up from water by B. truncatus and S. mossambicus and assimilated into various tissues. It is obvious from the longer-term exposure that the amount of Frescon taken up was higher than that achieved with 4'-chloronicotianilide; the amounts taken up being greater in B. truncatus than in S. mossambicus. Comparison between these rates of uptake and the relative susceptibilities of B. truncatus and S. mossambicus to Frescon and 4'-chloronicotianilide indicate that some connection between rate of uptake and potency of molluscicides might be possible. It is apparent that S. mossambicus which is highly tolerant to 4'-chloronicotianilide takes up this compound less rapidly than Frescon. On the other hand, both B. truncatus and S. mossambicus, which are highly susceptible to Frescon, take this compound up more rapidly than 4'-chloronicotianilide. The plateau in the amount of chemical taken up, which developed after approximately 24 hours (Fig. 8), should not be taken as a steady level of chemical, it is more likely to be due to simultaneous removal of chemical from the tissue rather than a true
steady state due to saturation of tissue. Fifty per cent of the chemicals taken up were found to be eliminated after 23-32 hours of recovery.

The rapid uptake of molluscicides by both snails and fish has been mentioned by other authors. Azvedo et al. (1957) and Cheng and Sullivan (1974), using radioactive copper sulphate, demonstrated the rapid uptake of this compound from the environment by planorbid snails and verified its diffusion into different tissues and organs. Duhm et al. (1963), studying the accumulation of 14C-niclosamide by *B. glabrata* found that within 24 hours up to 0.2% active ingredient was absorbed from a 1 ppm solution. Boynon (1971), using *Rasbora* spp., found that after 4 hours, the fish concentrate Frescon up to x50 the concentration in water. Similarly, Matthiessen (1977) reported that *S. mossambicus* concentrates Frescon up to x1300 from a 0.005 ppm solution. Glickman et al. (1977) also reported the rapid uptake of pentachlorophenol by the rainbow trout (*Salmo gairdnerii*). Dunlop (1976), Duncan et al., (1977) and Duncan and Brown (unpublished) were able to measure rates of uptake of Frescon and nicotinanilide and some substituted nicotinanilides by *B. glabrata*. They hinted that the rate of uptake of molluscicides might be directly dependant on the rate of water influx.

The rate of water uptake by *B. truncatus* and *S. mossambicus* was, therefore, studied in an attempt to relate water and molluscicide uptake. The experiments show that both *B. truncatus* and *S. mossambicus* are freely permeable to water and that the flow of water through the animals when
they are in their normal environment is very fast. Van Aardt (1968), using _Lymnaea stagnalis_, found that tritiated water equilibrates in the snail within 30 minutes after exposure while Potts _et al._ (1967) estimated the biological half-life for the uptake of tritiated water by _S. mossambicus_ (given as _Tilapia mossambica_) to be well below 1 hour. The high influx rates of water observed for _B. truncatus_ and _S. mossambicus_ undoubtedly are related to their existence in water and the relative unprotectedness of the body surface for water permeation. In contrast, the biological half-life for tritiated water for the rat under normal feeding and housing conditions is 3-4 days (Horzer and Maborich, 1966). The flow of water into _B. truncatus_ is greater than that into _S. mossambicus_. However, the equilibrium is established in both at the same time. Values of 0.224 µl min⁻¹ and 0.074 µl min⁻¹ per mg of tissue were calculated for _B. truncatus_ and _S. mossambicus_ respectively. Using weight as a basis for comparison, the value calculated above for _B. truncatus_ is remarkably close to the one derived from the value of 17.74 µl min⁻¹ calculated by Duncan (1969) for an 8.5 mm _B. glabrata_. An 8.5 mm _B. glabrata_ weighs 84.0 mg which therefore gives an uptake rate of 0.210 µl min⁻¹ mg⁻¹. The calculated rates of influx of water have been used to find a value for the amount of molluscicide expected to enter after a certain time. Such a calculation rests on the assumption that the molluscicide enters with the water in the same concentration as it exists in the medium. It was hoped that such calculations would give values similar to those obtained from the uptake of Fresscon and 4'-chloronicotin-
anilide by *B. truncatus* and *J. mcosaebius*. However, the values calculated, i.e. the amount of chemical expected to enter the animals, were found to be less than the values obtained for uptake (Appendix 16). This indicates that the rate of uptake of the compounds was faster than the rate of water influx.

The mechanism involved in the diffusion of the compounds through the animal membranes might be a specialised transport process in which the membrane plays an active part, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane (Schanker, 1962). Specialised transport processes, for example, pinocytosis, are known to occur in snails. Nakahara and Bevelander (1967) reported the ingestion of particulate matter by the outer surface cells of mollusc mantle. The mantle epithelium of the calico clam, *Macrocallista maculata*, was seen to ingest colloidal gold and carmine particles. These authors have also observed a similar phenomenon in 2 related species of bivalves, *Pinctada radiata* and *Isognomon alatus*. But, pinocytosis appears to operate too slowly to account for the rapid uptake of these compounds. Harris (1960) stated that the passage of molecules or ions from a solution into a membrane, when no chemical forces operate, is a consequence of collision between the particle and the membrane which gives rise to certain concentrations followed by diffusion within the membrane and across it to redissolve in the aqueous phase on the other side. Lieb and Stein (1959) explained the diffusion of non-
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Poor text in the original thesis.
electrolyte molecules through membranes by the concept of porous behaviour i.e. the membrane behaving as homogenous polymer networks.

The present experiments have demonstrated high levels of Frescon and 4'-chloronicotinanilide in the pseudobranch of B. glabrata, the accumulation in other organs being relatively slight by comparison. The presence of large concentrations of molluscicide in the pseudobranch may be indicative of uptake and/or elimination. However, the TLC analysis of a chloroform extract of snails exposed to Frescon and 4'-chloronicotinanilide shows that the pseudobranch contained unchanged N-tritylmorpholine and 4'-chloronicotinanilide respectively. This would imply that this organ is an uptake rather than an elimination one since metabolism to form more hydrophilic compounds might be expected in a tissue responsible for excretion.

The gastropod epithelium functions not only for protection but also in respiration (Moss, 1954). The uptake of compounds through the epithelium has been reported on many occasions. Zylstra (1972) demonstrated the uptake of colloidal ferritin by the dorsal head epidermis and inner mantle epithelium of Lymnaea stagnalis. Furthermore, Sullivan and Cheng (1974), believe that the rectal ridge greatly increases the area of the mantle epithelium-water interface and postulated that the cilia-mediated water flow pattern, made possible by its contiguity with the dorsal ridge, strongly suggests that the rectal ridge is involved in the uptake and elimination
of substances by the snail.

The distribution of Frescon and 4'-chloronicotinamidocarbonilide within the body of snails and fish does not show any relation with the fat content despite the fact that both molluscicides are lipophilic in character. This seems to be in agreement with the findings of Matthieussen (1977) who exposed *S. mossambicus* to Frescon. The general dependance of distribution of pesticides on fat content was however confirmed by Matthieussen *et al.* (1976) when *S. mossambicus* was exposed to the organophosphate insecticide, Abate. Anderson and Fenderson (1970) and Ernst *et al.* (1976) have shown that DDT residues in fish are broadly dependant on fat content, with high concentrations in adipose tissue and brain.

The high concentration of Frescon found in the fish liver indicates that the compound is possibly metabolised and excreted via this organ. Statham and Lech (1975) reported that Bayluscide is metabolised by fish liver, and Griffiths (1958) has shown that mammals also metabolise Frescon in the liver and excrete its metabolites via the bile and urine. However, the presence of a low concentration of Frescon in the bile and the fact that most of the Frescon found in the liver after 24 hours exposure consisted of unchanged N-tritylmorpholine does not support the hypothesis of metabolism and excretion of this compound via this route. Brodie and Maickel, (1962) suggested that aquatic animals do not have the capability of detoxifying drugs and that
lipid-soluble compounds are rapidly disposed of by passive diffusion through membranes into the water. Consequently, it is suggested that the liver may be the site of action of the molluscicide which causes fish mortality by interfering with the normal function of the organ. That is to say, Frescon accumulates in the liver until a threshold of toxicity level is attained. This 'target organ' hypothesis has been suggested by Sullivan and Cheng (1974, 1975). In an autoradiographic study of the uptake of $^{67}$Cu in _B. glabrata_ they concluded that the principal site of copper action is located in the rectal ridge. Moreton and Gardner (1976), experimenting with _Lymnaea stagnalis_, reported the nervous system as a possible site for Frescon action in fresh water snails. However, the validity of such suggestions remains to be seen; the studies needed to further investigate these points were beyond the scope of the present study.

The high concentration of 4'-chloronicotinamidide reported in the fish gall bladder suggests that this compound is most probably excreted via the bile after being hydrolysed into 4'-chloronilene and nicotinic acid as shown by the TLC analysis of chloroform extracts of the bile of _S. rosaceus_ exposed to 4'-chloronicotinamidide. The metabolism and excretion of molluscicides in the bile has been previously reported in mammals (Griffiths, 1968) and in fish (Statham and Lech, 1975). A number of insect pests such as the tobacco wireworm (_Conoderus xanthicus_), differential
Grasshopper (Eulamprus differentalis) and the cigarette beetle (Lasioderma serricorne), have a high capacity to degrade pesticides e.g. nicotine to non-toxic metabolites such as continine (Eke et al., 1964). Rollinworth (1976) states that it is quite rare for any foreign compound (xenobiotic) to enter the body and not be converted, at least in part, to metabolites that are usually more polar than the parent compound which can then be rapidly excreted from the body. He adds that differences in metabolic rate between organisms represents probably the most important force behind selective toxicity. Therefore, the capability of fish to withstand high concentrations of 4'-chloronicotinanilide and its analogues may be related to the rapid breakdown of the compound in fish to more polar forms which are easily disposed of via the bile.
CHAPTER 4

PRESENT WORK (PART II)

Comparison of the Relative Susceptibilities of Various Groups of Bulinus truncatus to Prosocon.
1. Introduction.

The Gezira lies between the Blue and White Niles to the South of Khartoum. Its consists of an area of 1 million hectares of irrigated land with a further million hectares potentially irrigable. It is supplied from the Blue Nile via three main types of distribution canals, main, major and minor canals. The function of both the main and major canals is to transport the water rather than to irrigate the land. The minor canals, positioned at right angles to the major canals, provide a reservoir for irrigation of the field via field channels known as Abu eshreens and Abu sittas. It was realised from the outset of the scheme in 1925, that schistosomiasis could become a major public health hazard and copper sulphate was therefore introduced as a molluscicide in 1956 (El-Fager, 1958). The disease has spread nevertheless and large numbers of snails can be found in the minor canals (Varley, 1976). In 1969, a comparison between copper sulphate and the then new molluscicide, N-tritylmorpholino or Frescon showed that the latter was more effective and easier to apply (Amin, 1972). As a result of this study, copper sulphate was gradually replaced by Frescon. The regimen of Frescon application has since evolved through the use of drip-feed dispensers backed up by knapsack spraying of the tail ends of minor canals to the use of aerial spraying in which applications are made 5 times a year in early September, mid-November, late January, mid-March and early June. However, the 'blanket' applications
of Frescon have had limited success in the Gezira and have proved to be uneconomic (Penwick, personal communication). During 1977, a pilot study indicated a strong correlation between human habitation, well defined water contact sites and the presence of infected snails. Infected snails were often found in the Abu eshreens near villages and dwelling sites. Following this study, 'focal' control was thought to be an alternative strategy which will be economically viable and feasible.

The complete elimination of snails by focal mollusciciding is unlikely to be achieved in many of the defined transmission habitats and a proportion of the snail population may therefore receive only a reduced concentration of the molluscicide. A study of the susceptibility of infected snails to the molluscicide was therefore thought to be essential for the evaluation of a focal control strategy.

As the phenomenon of pesticide resistance has become more prevalent with the exposure of pest populations to widespread chemical treatments, the possibility of development of resistance in snail hosts of schistosomiasis has become an intriguing question for those concerned with the application of mollusccides (WHO, 1975). A pest population is described as resistant when it has lost its original susceptibility to a toxic chemical to the point that it can no longer be controlled with that chemical (Brown, 1969). With these points in mind, it was decided to investigate whether any level of Frescon resistance has developed in the snail populations of the treated area of
the Gezira and to provide base-line information with which to examine the situation at any given time in the future.

2. Results.

2.1. The susceptibility of *B. truncatus* to Frescon; data for snails either infected or uninfected with *S. haematobium*.

Two groups of *B. truncatus*, either infected or uninfected with *S. haematobium*, were bioassayed at the same time and under identical conditions in order to determine their relative susceptibilities to Frescon e.c. The snails used were of the same size (shell height = 6.5 ± 0.05 mm). They were exposed singly at 25°C in molluscicide solution (200 ml) prepared in aerated, dechlorinated tap water (pH 8.10), 10 snails at each concentration. After an exposure period of 24 hours, the snails were left to recover for 48 hours in fresh, aerated tap water.

The dose-mortality data from each test (Appendix 17) were analysed using a log-probit computer program. The results are shown in Fig. 11; lethal concentration values and data describing the line fit are given in Table 6. It is apparent from the results that infected snails are more susceptible to Frescon than uninfected ones. Comparison of the dose-mortality data for slope and position showed a significant difference in position at the 5% level (*F* = 32.81).
Figure 11. SUSCEPTIBILITY OF B. TRICULARIS TO MASCON.
Table 5. A comparison of the dose-mortality data of Froscon against infected and uninfected B. truncatus after 24 hour exposure and 48 hour recovery period.

<table>
<thead>
<tr>
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<th>Infected B. truncatus</th>
<th>Uninfected B. truncatus</th>
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<tbody>
<tr>
<td>LC50</td>
<td>0.025</td>
<td>0.032</td>
</tr>
<tr>
<td>LC90</td>
<td>0.044</td>
<td>0.055</td>
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<td>Degrees of freedom</td>
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<td>F test</td>
<td>77.35</td>
<td>256.19</td>
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</table>

2.2. The rate of uptake of Froscon by B. truncatus either infected or uninfected with S. haematobium.

The rate of uptake of Froscon by two groups of B. truncatus either infected or uninfected with S. haematobium was studied using $^3$H-Frescon (Specific Activity = 20.36 µCi/mg). Batches of 6 snails (shell height 2-9 mm) were exposed to labelled molluscicidal solution (0.005 ppm) in the flow cell apparatus described previously, 5 cells for each of the two groups. An acclimatisation period (2 hours) was allowed prior to exposure. Two batches at a time, one from each group, were removed from the flow cell apparatus after known exposure times.

The results are given in Appendix 18 and are shown graphically in Fig. 12. Each point on the graph represents the total amount of chemical (µCi) taken up by a batch of 6 snails in a cell divided by the total wet
Figure 12. UPTAKE OF N-TERTIARYMORPHOLINE BY B. TRIGATUS.

1 S. haematobium infected.
2 uninfected.
weight (mg) of the snails in the cell. It is apparent from the results that uninfected snails take up Frescon more rapidly than infected ones. Comparison of the rates of uptake indicated a significant difference at the 5% level \(F = 20.77\).

2.3. Susceptibility of *B. truncatus* to Frescon; data for snails collected from two different locations of the Gezira scheme.

Two groups of *B. truncatus* were collected during January, 1976; one group from Hobeika canal in the Northern Group of the scheme and the other from the Abu Guoli canal near Hassaheisa. The two canals are 50 km apart though they both receive water from the main canal. Hobeika canal is in an area treated with Frescon for 6 consecutive years; Abu Guoli has never been treated.

Two experiments were conducted, the first in June, 1976 with snails from the two groups and the second in September, 1976 with 2 batches of snails from the untreated area group and 1 batch from the treated area group. On each occasion, the snails from the two groups were bioassayed at the same time, under identical conditions. The snails used were of the same size (shell height 6.5 ± 0.05 mm) and of the same age. They were exposed singly at 25°C for 24 hours in molluscicide solution (200 ml) prepared in aerated, dechlorinated tap water \(pH 8.10\); 10 snails at each concentration.
After the exposure, the snails were left to recover for 48 hours in fresh tap water. The mortality data from all these tests (Appendix 19) were analysed by a log-probit plot as before. The results are illustrated in Figs. 13a and 13b from which it may be seen that the tolerance of the snails from the treated area was slightly higher than that of the snails from the untreated area on each of the two occasions. Lethal concentration values are given in Table 7.

Table 7. Lethal concentration values for Frescon against the two snail groups described in the text.

<table>
<thead>
<tr>
<th>Snail groups</th>
<th>First experiment</th>
<th>Second experiment</th>
</tr>
</thead>
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<tr>
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<td>$LC_{50}$ (ppm)</td>
<td>$LC_{95}$ (ppm)</td>
</tr>
<tr>
<td>Frescon-treated area</td>
<td>0.036</td>
<td>0.060</td>
</tr>
<tr>
<td>Untreated area</td>
<td>0.030</td>
<td>0.054</td>
</tr>
<tr>
<td>Untreated area</td>
<td>-</td>
<td>-</td>
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</table>

Comparison of the dose-mortality data for slope and position showed a significant difference in position, at the 5% level, between the treated and untreated area groups in both experiments ($F = 5.5$ and $20.1$ respectively) and that there was no significant difference between the two untreated area groups in the second experiment ($F = 0.6$).
Figure 13a. SUSCEPTIBILITY OF B. TRUNCATUS TO FRESON, First Experiment.
Figure 15b. SUSCEPTIBILITY OF B. TINCTORUS TO FRESCOH, Second Experiment.
2.4. The rate of uptake of Frescon by the two field collections of B. truncatus.

This experiment was also repeated twice in June and August, 1976 with exposure periods of 5 and 12 hours respectively. On each occasion, batches of snails from the treated and untreated areas were exposed in the flow cell apparatus, to 0.005 ppm of N-tritylmorpholine (Specific Activity = 21.16 µCi/mg) in aerated, dechlorinated tap water.

Results are given in Appendix 20 and are shown graphically in Figure 14. Each point on the graph represents the total amount of chemical (µg) taken up by each group of snails in a cell divided by the total wet weight (mg) of the snails in the group. It is clear that the rate of uptake of Frescon in the two groups is different. Comparison of the uptake rates of Frescon for the two groups indicates a significant difference in position at the 1% level (F = 189.5 in the first experiment and 65.3 for the second).

3. Discussion.

Comparison of the susceptibility to Frescon of two groups of B. truncatus, which were either infected or uninfected with S. haematobium, showed that the former are more susceptible than the latter. The difference was significant and therefore, it might be assumed that the infection is the cause of this difference in susceptibility. However, comparisons of the rates of uptake of Frescon indicate that infected snails take up the molluscicide less rapidly than uninfected ones. The slow uptake of Frescon
Figure 14. UPTAKE OF N-TRITYLMORPHOLINE BY B. TRUNCATUS
a) 5 hours exposure  b) 12 hours exposure.
by infected snails may be due to the damage caused by
the escaping cercariae in the loose, vascular connective
tissue characteristic of the molluscan pseudobranch
(Pan, 1965) which has been here identified as a possible
site of Froscon uptake.

The deleterious effect of schistosome parasites of man
on their snail hosts has been mentioned by various
workers. Brumpt (1941) observed that _B. glabrata_ infected
with _S. mansoni_ was apparently less resistant to
dessication. Paulini and Pellegrino (1956a, b) showed
that _B. glabrata_ infected with _S. mansoni_ was more
susceptible to sodium pentachlorophenate and copper
sulphate than uninfected snails. Hira and Webbe (1972)
demonstrated the combined effect of a molluscicide and
infection on the survival rate of snails.

It is apparent therefore, that the application of
molluscicides to potential transmission foci may
eliminate or greatly reduce the numbers of infected snails
in greater proportion than those uninfected snails even
when a complete kill is not achieved overall.

From the data obtained here, it is also evident that the
group of snails from the Froscon-treated area is more
tolerant to Froscon than that from the non-treated area.
However, this is hardly enough to mark the former group
resistant and the dosage applied in the Gezira would be
adequate to control both groups judging from the
presently reported dosage-mortality results. It might be
said, of course, that the difference uncovered between the two groups was always present and not due to selection pressure at all. Certainly, differences in susceptibility can occur between geographical strains (Olivier et al., 1962). The groups dealt with here however, were collected from confluent bodies of water and are therefore judged to have come from the same population.

The overlap in the bioassay results of the two experiments could possibly be due to seasonal variation. It has been pointed out by Tsuda (1953) and Yasuraoka et al. (1966) that the susceptibility of Oncomelania to molluscicides varies with the season of the year. This is not thought to be a factor operating in the present studies with laboratory bred animals, unless the seasonal rhythm is a very strong inherent one. It seems much more likely that the variance is inherent in the bioassay technique.

Two different and opposing views on development of resistance by snails to molluscicides once prevailed in the Far East. One group of workers argued against this as a possibility (Walton et al., 1958; Gancarz, 1953; Komiya et al., 1961; Yasuraoka and Hosaka, 1971) and another group claimed its existence (Okabe et al., 1956; Ota and Jato, 1956). The differences were finally thought to be due to variations in bioassay technique in the laboratories (Yasuraoka, 1972). More recently, however, some preliminary results have been reported, purporting to show Bayluscide resistance in B. truncatus from Iran (Jelnes, 1977).
The difference in the rate of uptake between the two groups in this study is, on the other hand, more marked suggesting that any difference in tolerance is due to differential penetration rates and that the snails with prior exposure to Frescon may have the capability to slow down the rate of penetration of the molluscicide and thus tolerate the chemical. The concept of decreased penetration has been suggested as one of several methods by which an insect becomes resistant to a toxicant (Lovell, 1963) and many resistant strains of insects have been shown to absorb insecticides at a slower rate than susceptible strains (Oppenoorth, 1953; Dinamarca et al., 1962; Sanchez and Sherman, 1966; Flapp and Hoyer, 1968; Szeicz et al., 1973).

Since the acquisition of resistance in an animal population is initially often an insidious process, there is a possibility that we are witnessing the early stages of such an event and that a strain of high tolerance, reaching the level of resistance, might develop with the protracted use of Frescon in the Gezira. It is suggested therefore, that this or other situations could be monitored from now on using the same methods outlined here. Two factors might be expected to mitigate against the development of resistance in irrigation schemes; the influx of untreated snails from the intake which will continually contribute to the gene pool and secondly, the use of focal chemical treatment of transmission sites, rather than blanket applications. Additionally, in the particular context of the Gezira, Bayluscide is being
actively considered as a replacement for Frescon on the grounds of efficiency if not specifically as a guard against incipient resistance (Amin, personal communication).
GENERAL CONCLUSIONS
Although a large body of information exists on the biochemistry and particularly on the physiology of molluscs, little has been achieved towards a better understanding of the modes of action of molluscicides. It is well known that most pesticides, at their lowest inhibiting concentrations, act rather specifically only on one vital function, localised at one particular site. Hence, one can speak of pesticides which are primarily inhibitors of energy production whereas others are inhibitors of synthesis and still others act on the structure of the cell. All the work that has been done in the field of molluscicides seems to indicate that many of these compounds are primarily inhibitors of energy production.

It must be realised that few, if any, of the compounds used as molluscicides act selectively on molluscs; many have broad spectrum activity including toxicity to other animals or phytotoxic properties. This is not surprising since at the biochemical level, processes are often basically very similar in different organisms. It is only possible to find a more selective molluscicide among compounds which act on processes which are more or less specific for molluscs e.g. shell formation.

From the results of the present study, it seems possible that the differences in susceptibility to molluscicides between species are, to some extent, influenced by the
amount of chemical taken up by the species. The mechanisms involved in the uptake of compounds are thought to be a specialised transport process, similar but faster than pinocytosis, in which the membrane plays a major part, transporting the solute in a manner that cannot be explained by the structure or physical properties of the membrane. However, cellular components involved in the processes need to be identified by further studies, using for example electron microscopy and histochemical techniques.

The pseudobranch of B. glabrata was recognised as the main site of entry for both Frescon and 4'-chloronicotinamide rather than a site of action. On the other hand, the liver of S. mossambicus was recognised as a possible site of action for Frescon, which causes mortality by interfering with the normal functions of this organ. Those findings were based on results obtained by liquid scintillation spectrometry, other techniques which are based on fundamentally different principles e.g. histochemistry, x-ray microanalysis and autoradiography need to be investigated to present more detailed information on the sites of accumulation and action within the organ.

Although no attempts have been made in this study to present quantitative data, TLC analysis of chloroform extracts of the bile and liver showed that N-tritylmorpholine absorbed by snails and fish and 4'-chloronicotinamide absorbed by snails remained chemically unchanged while 4'-chloronicotinamide
absorbed by fish was metabolised to more polar forms. Hence, the high tolerance of fish to 4'-chloronicotin-anilide is suggested as being due to the rapid disposal of these metabolites via the bile.

Evidence is presented to show that B. truncatus infected with S. haematobium is more susceptible to Froscon than uninfected snails, despite the fact that infected snails take up less chemical than uninfected ones. These results indicated that the application of molluscicides to defined transmission sites would eliminate or greatly reduce the number of infected snails within the transmission site even when a complete kill is not achieved.

A difference in susceptibility to Froscon, between B. truncatus collected from Froscon-treated and untreated areas of the Gezira, could be shown and this has been tentatively attributed to the differences in penetration rate of the molluscicide between the two collections of snails. It is suggested that methods described could be used subsequently to monitor for appearance of resistance in this or other situations.

The need for development of new and more effective molluscicides is justified by the fact that the use of molluscicide as a single measure or integrated with other control measures is considered to play a vital and continuing role in schistosomiasis control. 4'-chloronicotin-anilide and its analogues seem to offer the possibility for truly selective control of snails. The
future development of these compounds should, however, involve laboratory and field trials incorporating different formulations, investigation of field methods for analysis of low concentrations in water and feasible routes of commercial synthesis.
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APPENDICES
Appendix 1. Comparison of snail shell size and total snail weight of _B. truncatus_.

<table>
<thead>
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<th>shell size (mm)</th>
<th>total snail wt (mg)</th>
<th>log total snail wt.</th>
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<td>3.1</td>
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Appendix 2. Quench calibration data for combusted sample preparations.

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<th>% eff</th>
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Appendix 2a. Quench calibration data for solubilised Biomphalaria glabrata.

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<tr>
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Appendix 2b. Quench calibration data for *Sarotherodon mossambicus*.

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/over
## Appendix 2b. continued.

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Appendix 3. Susceptibility of *Bulinus truncatus* and *Sarotherodon mossambicus* to various concentrations of Frescon and 4'-chloronicotinanilide on 24 hours exposure.

cn = concentration
d = number dead
pr = probit

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<td>v. Frescon</td>
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Appendix 4a. Activity of *Biomphalaria glabrata*.

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<th>60</th>
<th>80</th>
<th>100</th>
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<tbody>
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<table>
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**Mean and Standard Deviation**

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**Notes:**

- The activity of *Biomphalaria glabrata* was studied under various conditions to understand its locomotion behavior.
- The table provides the distance moved every 20 minutes (in centimeters) for different groups of snails under different conditions.
- The data includes the mean and standard deviation for each condition.
- The conditions tested include whether the snails are kept separately or grouped in a cylindrical tube with constant flow rate.
- The flow rates are measured in milliliters per minute (ml min⁻¹).
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<th>180</th>
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**Distance moved every 20 minutes (cms)**

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**Distance moved every 20 minutes (cms)**

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Appendix 5. Adsorption of Frescon on polythene tubing.

A fresh 0.005 ppm solution of $^{3}_H$-Frescon (157.8 cpm/ml) was run through a peristaltic pump fitted with new polythene tubing. The activity of the solution (cpm/ml) on passing through the tubing, was measured at various time intervals.

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Appendix 6. Uptake of Frocon by *Bulinus truncatus*.

dpm = disintegrations per minute

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Appendix 14. Uptake of water by *Bulinus truncatus*

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Appendix 17. Susceptibility of *Bulinus truncatus* to Frescon; data for snails either infected or non-infected with *Schistosoma haematobium*.

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Appendix 18. Uptake of Frescon by Bulinus truncatus, either infected or non-infected with Schistosoma haematobium.

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Appendix 19. Susceptibility of *Bulinus truncatus*; collected from two different locations in the Gezira Scheme, to various concentrations of Frescon on 24 hours exposure.

\[ d = \text{number dead out of ten} \]
\[ pr = \text{probit} \]

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Bulinus truncatus.

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