THE EFFECT OF SIX HERBICIDES ON THE LONGEVITY OF *SCHISTOSOMA MANSONI* MIRACIDIA

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ABSTRACT

The effects of various concentrations of the herbicides Sutan, Lasso, Vernam, Eptam, Ro-Neet, and 2,4,5,-T on longevity of miracidia of *Schistosoma mansoni* were determined in depression slides. Observations were made at hourly intervals on pond water controls and lower concentrations of herbicides, while in higher concentrations the exact time of miracidial death was recorded. During the bioassays, no major changes were observed at the beginning and end of each trial in temperature, dissolved oxygen, and pH. Toxicity was measured by determining half-life values from mortality curves. The most toxic herbicides at low concentrations (1 ppm) were Sutan, Lasso, and Vernam which reduced half-life values over 50% when compared to pond water controls. At concentrations between 20 and 30 ppm, Sutan and Ro-Neet were the most effective miracidicides. Although Eptam and 2,4,5-T were the most soluble herbicides, they were the least effective at killing miracidia.

The blood fluke, *Schistosoma mansoni*, is one of the causative agents of schistosomiasis. This disease affects millions of people in the world and control efforts to stop its transmission have been of major interest to scientists and government officials in many countries. At present most of these efforts have been directed at ridding humans of the adult worm by chemotherapeutic drugs and eliminating the snail intermediate host with molluscicidal agents. In recent years some attention has been paid to elimination of the aquatic stages of the life cycle, the cercariae and miracidia, as a possible means of control. Shortly after DDT became available as an insecticide it was evaluated as a possible control agent for cercariae of *Schistosoma mansoni* (Kuntz and Stirewalt, 1946). Later a study carried out by the U.S. Army (1959) in Japan showed that miracidia of *Schistosoma japonicum* were sensitive to low concentrations of two molluscicides, dinitro-o-cyclohexylphenol (DN-1) and sodium pentachlorophenate (Na-PCP).

In this study attention was focused on the effect of several different herbicides on the miracidial stage of *S. mansoni*. The compounds used were Sutan, Lasso, Ro-Neet, Vernam, Eptam, and 2,4,5,-T. Since these herbicides are present in small concentrations as pollutants in agricultural runoff water, the effect of different concentrations on miracidial longevity were assessed.

MATERIALS AND METHODS

The life cycle of *S. mansoni* was maintained in mice and the snail host, *Biomphalaria glabrata*. Miracidia were obtained by diluting homogenized liver tissue containing eggs with pond water in a hatching flask (an Erlenmeyer flask covered with black tape and topped by a one-holed rubber stopper fitted with a vertical glass tube). Miracidia that hatched in the flask concentrated in the tube and were removed from the open end with a micropipette.

The herbicides Sutan (Butylate), Ro-Neet (Cycloate), Vernam (Vernolate), and Eptam (EPTC) were obtained from the Stauffer Chemical Company while Lasso (Alachlor) and 2,4,5-T were supplied by Monsanto, Inc. Herbicides were diluted with pond water to give solutions ranging from 1 ppm to maximum solubility of active ingredient. Miracidia were tested at room temperature in 1 x 3" elliptical depression slides with a well volume of 1 ml. For each concentration of herbicide two slides, each containing 15 miracidia, were used. A slide containing miracidia in pond water was observed concurrently with each experimental trial as a control. Counts of free-swimming miracidia were made at hourly intervals. At higher concentrations of herbicides, where mortality of miracidia was higher, the slides were monitored constantly and deaths recorded in minutes and hours.

For each bioassay the pH of the chemical solution and the pond water used were determined with a Corning pH meter. These readings were found to vary from pH 7.2 to 7.9 for the various herbicides, well within the range for normal activities of *S. mansoni* miracidia (Maldonado, Acosta-Matienzo, and Herrera, 1950). Dissolved oxygen levels of the pond water and herbicide solutions were determined before and after each trial with a galvanic cell oxygen analyzer (Yellow Springs) and found to range from 7.2 to 6.6 ppm. Bruce et al. (1971) reported the oxygen requirements for *S. mansoni* miracidia to be 6 l/hr/1,000 miracidia. Thus there was more than adequate oxygen for the metabolic requirements of the miracidia in the test vessels.

Half-life values or the time required for 50% mortality of miracidia were determined graphically from longevity curves plotted for each herbicide and control trial. This parameter was used because of its frequent appearance in literature on miracidial longevity and for comparative purposes.

RESULTS

A normal longevity curve for miracidia used in all the pond water controls was constructed (Figure 1). This indicated that miracidia die at almost a constant rate rather than during a short period of time when their glycogen may become exhausted. Thus, some natural mortality can be expected in the first hours of the experimental trials. The average half-life of untreated miracidia was 5 hours as determined from Figure 1.

In the experimental trials the least toxic herbicides were Eptam and 2,4,5-T. Half-life data plotted against concentrations of these two herbicides are given in Figure 2. Because of the high maximum solubility of these herbicides, larger concentration intervals were used than for the other herbicides. For Eptam half-life figures for miracidia decline steadily with increasing concentration. At the highest concentration used (175 ppm), miracidia were killed in less than a minute. In general, 2,4,5-T was the least toxic herbicide of those tested. The half-life observed at 5 ppm was approximately 2 hours but increased concentration of herbicide did not give the dramatic reduction in half-life that was observed with the other herbicides. Even at maximum solubility (150 ppm) miracidia showed a half-life of 30 minutes.

The half-life data for the most toxic herbicides are given in Figure 3. Of these four herbicides, Sutan was the most efficient at killing miracidia.

The half-life curve descends from 2.5 hours at 1 ppm to death in minutes at 40 ppm. Lasso and Vernam were equally effective at concentrations of 1 ppm but were not quite as toxic to miracidia at higher concentrations, both requiring 60 ppm concentrations to reach the toxicity of Sutan at 40 ppm. Ro-Neet was the least effective of all the herbicides tested at 1 ppm, the miracidial half-life being the same as the pond water control. However, at concentrations of 20, 30, and 40 ppm, Ro-Neet was as toxic as Sutan and at 20 ppm killed miracidia faster than either Lasso or Vernam.

DISCUSSION

Combined data on the longevity of *S. mansoni* miracidia in pond water gave an average maximum life span and half-life of 11.9 and 5.0 hours, respectively. The maximum life span of the miracidia was 2 to 3 hours longer than previously reported by Maldonado and Acosta-Matienzo (1947). In contrast, Lampe (1927) stated that the miracidia of *S. mansoni* survive 40 hours at 33° C, while Schreiber and Schuster (1949) reported a half-life of 8 hours and a maximum life span of 22 hours. Miracidia do not feed but their life span can be increased by adding glucose to the aqueous medium (unpublished data). Natural death occurs when stored glycogen is exhausted (Maldonado et al., 1950). Differences in longevity data from various studies may be explained by the quality of water used or environmental factors including temperature, dissolved oxygen, pH, and light conditions. Another important difference may be the time the eggs have been confined within the host tissue before hatching. It is possible aging of the miracidia began while eggs were still within the host and the more extended this phase is the higher will be the death rate of miracidia soon after hatching. In support of this, Maldonado and Acosta-Matienzo (1947) found miracidia from old eggs live 1 to 2 hours, even under the most favorable conditions, while viable young eggs produced miracidia which had a longer life span.

All of the herbicides used in this study caused an increased mortality of miracidia when compared to the pond water controls. Sutan, Lasso, Ro-Neet, and Vernam were considered the most effective miracidicidal agents, while Eptam and 2,4,5-T required greater concentrations of active ingredient to reduce half-life determinations below one hour. Sutan, Lasso, and Vernam reduced by one half half-life values at concentrations of 1 ppm when compared to controls, while Sutan and Ro-Neet were the most effective miracidicides at 20 to 30 ppm.

Very few studies have been carried out on the effects of various chemical compounds on the longevity of miracidia. A laboratory study completed at the 406th Medical General Laboratory (1959) in Japan found that two commonly used molluscicides, DN-1 and Na-PCP, would kill *S. japonicum* miracidia at very low concentrations. Immobilization of miracidia was achieved within 30 minutes with 0.125 to 1.0 ppm of DN-1 and 2 ppm of Na-PCP. Thus these chemicals not only act in controlling schistosomiasis by killing snails but also by removing miracidia from the aquatic environment. However, both molluscicides are nonselective respiratory poisons and caution must be used when applying them to an ecosystem.

There is only fragmentary knowledge concerning the mode of action of the great array of commonly used herbicides (Ashton and Crafts, 1973). Generally they inhibit protein synthesis of plants and the available supply of ATP or other energy-rich compounds required for protein synthesis. This inactivation of protein synthesis may be the cause of decreased longevity of miracidia swimming in dilute solutions of herbicides.

Several studies reporting the effect of various herbicides on higher animals, such as fish, rabbits, and rats, have been carried out. However, the results of these studies are difficult to compare to the effects of herbicides on miracidia. First, data are calculated as LD_{50} or TLm (Median Tolerance Limit) and usually appear in units of 48- or 96-hour toxicities. Since miracidia would die naturally before this period, comparisons are difficult. Secondly, miracidia are nonfeeding invertebrates and have little in common with feeding vertebrates.

It is evident from this work that transmission of schistosomiasis could be hindered by applying an appropriate concentration of the most effective herbicides (Sutan, Lasso, Ro-Neet, and Vernam) to snail habitats. In tropical countries *S. mansoni* is transmitted by the snail, *B. glabrata*, which is found in a range of habitats such as reservoirs, banana drains, ponds, marshes, streams, and irrigation canals. Weeds in these waters provide a suitable cover and food source for these snails. Therefore, application of the appropriate concentrations of herbicides would control not only the vegetation but might hinder the transmission of schistosomiasis by reducing the possibility of snail-miracidium contact. The results of this study also indicate that runoff waters from agricultural areas where herbicides are being used for weed control in upland situations might be lethal to miracidia of *S. mansoni* and inadvertently help control the disease.

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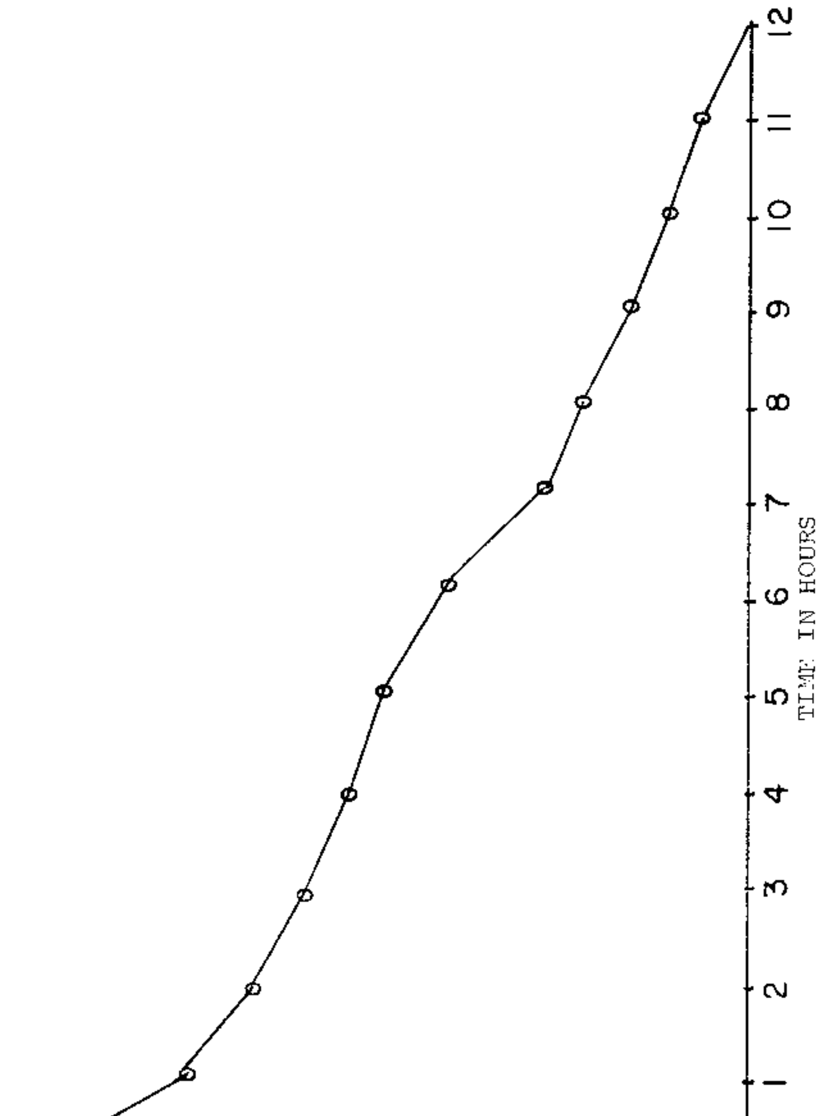

FIGURE 1. A composite longevity curve for *Schistosoma mansoni* miracida (N = 415) in pond water controls.

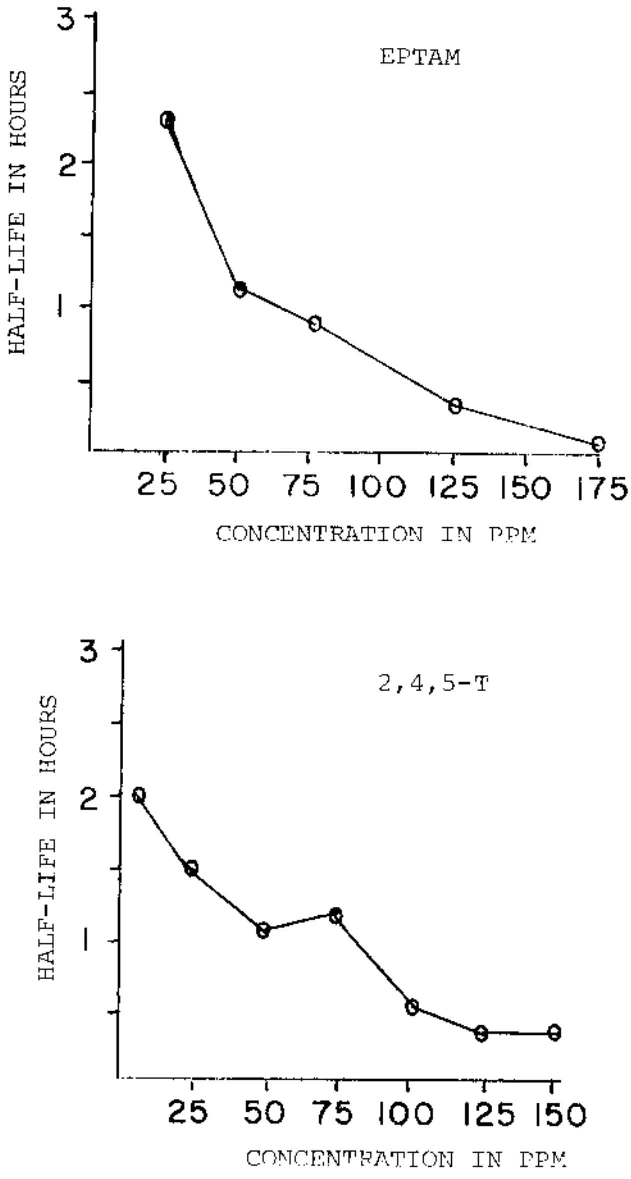

FIGURE 2. Half-life values of *Schistosoma mansoni* miracidia in various concentrations of herbicides.

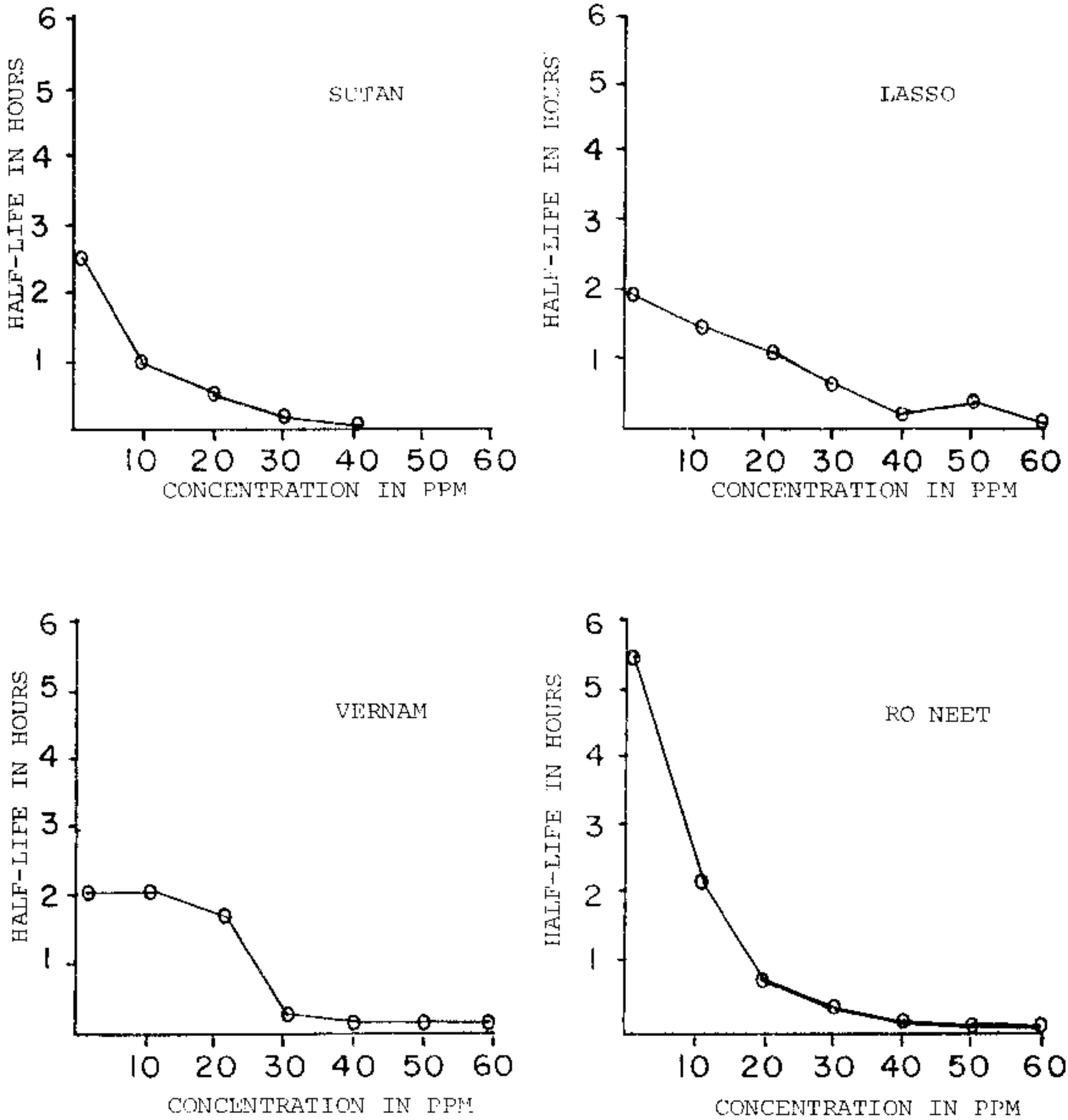

FIGURE 3. Half-life values of *Schistosoma mansoni* miracidia in various concentrations of herbicides.