THE FATE OF AQUAZINE IN A SMALL POND

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Gas chromatographic analysis of samples from a simulated small pond treated with Aquazine showed rapid loss of the herbicide from the solution, but herbicide concentration gradually increased with time in soil and pellet samples. Microorganism composition changed rapidly after treatment with the herbicide.

INTRODUCTION

The wide use of pesticides in recent years has caused concern about the fate of the chemicals in the environment. There is little evidence that herbicide residues are accumulating in aquatic environments, but the amount of herbicides used yearly has been increasing faster than any other group of pesticides. Many herbicides are applied to the soil and thus may eventually enter aquatic systems. This may occur in run-off water by movement in solution or as a soil-herbicide complex. During a period immediately after application, sediment-free water was found to contain dieldrin in concentration 5 times that occurring in later runoff events (1). For some pesticides, the loss from the land surface and their ultimate entry into an aquatic system is associated with the sediment phase. The adsorption of many of these chemicals has been demonstrated (2). Once a pesticide enters a large aquatic system, it is difficult to determine its effect on all organisms in that system.

Aquazine, a new formulation of simazine (2-chloro-4,6-bis(ethylamino)-s-triazine), has been used to control aquatic weeds in streams and lakes (3). It is expensive and difficult to treat and sample large bodies of water because of changing macroorganisms, water volume, aeration, nutrients, and light. For these reasons a plastic tank was used to simulate a small pond. To determine the fate of this chemical, samples of water, algae, and soil in the tank were obtained at intervals and analyzed for the presence of Aquazine.

MATERIALS AND METHODS

A large (90 × 50 × 60 cm) fiber glass tank in a greenhouse was used to simulate a small pond environment. A 5-cm layer of Zaneis loam, a reddish brown silty loam with pH of 6.1, was placed in the tank as an algae inoculum. To enhance algal growth, 94.6 1 of Bristol's (4) solution were added. An aerator was placed in the tank and a 30 W cool white Westinghouse fluorescent bulb provided constant illumination. After 10 days, flagellates, bacteria, and algae from the soil were observed in the solution. No attached algae were observed in the system. Temperature, pH, and optical density (O.D.) of the pond solution were recorded daily. When the O.D. had reached about 0.5 at 678 nm, the tank was treated with $10^{-5}$ M Aquazine. Daily samples of water and soil were taken for the next two weeks.

Water samples were centrifuged and the supernatant and pellet containing flagellates, bacteria, and algae were extracted twice with chloroform. The extracts were evaporated to dryness in a vacuum evaporator and then 1 ml of methanol was added. Soil samples were taken with a hollow, 12-mm (i.d.) tube and placed in a plastic container. Excess water was decanted and the soil pellets were extracted twice with chloroform by shaking on a Vortex mixer. The combined extracts were evaporated to dryness and 1 ml methanol was added.

A Varian 2740 gas chromatograph with an electron capture detector was used. One µl of each methanol solution was injected into a 1.5 m × 3 mm (i.d.) stainless steel column packed with 1.5% OV-17 on 100/ 200 mesh Gas Chrom G. Injection, column, and detector temperatures were 232, 200, and 257 C respectively. Carrier gas, filtered nitrogen, was used at a rate of 30 ml/min. Standard solutions of Aquazine were prepared and aliquots were injected into the gas chromatograph. A standard calibration curve for Aquazine was thus obtained by plotting peak area against concentration (ng/µl). This curve was used to determine the concentration of sample peaks.
One experiment was begun in January and concluded in February. A second trial was begun in March and concluded in April.

RESULTS AND DISCUSSION

Even though the tank was kept in a greenhouse, there was a wide variation in water temperature. The average temperature for trial 1 was less than that for trial 2 (Figures 1 and 2). However, the growth rate of the algae, as measured by O.D., was not directly associated with the temperature. There was a general increase in O.D. until it reached about 0.5. After treatment with $10^{-5}$ M (2 mg/l) Aquazine, there was a sharp, continuous decline in O.D. Similar results were obtained by Hawxby et al. (5) when uni-algal cultures were treated with s-triazine herbicides in various concentrations. Wells and Chappel (6) noted that 1 ppm of simazine completely inhibited the growth of *Chlorella pyrenoidosa*. Ashton, et al. (7) reduced the growth of *Chlorella vulgaris* 85% by treating with 70 mg/l atrazine for 72 hr. Kruglov (8) found that less than 2.5 mg/l atrazine reduced growth of *Chlorella*-like algae by 95%.

After treatment, soil samples were obtained as mentioned and analyzed for Aquazine. About 5% of the total applied was present in the soil for the first few days, but herbicide concentration in the soil had increased to about 20% of the total applied when the experiment was terminated (Figure 3). Temperature fluctuation did not appear to affect the amount of herbicide left in the soil. Talbert and

![Figure 1](image1.png)  
**Figure 1.** Effects of temperature and $10^{-5}$ M Aquazine treatment (arrow) on optical density (February).

![Figure 2](image2.png)  
**Figure 2.** Effects of temperature and $10^{-5}$ M Aquazine treatment (arrow) on optical density (April).

![Figure 3](image3.png)  
**Figure 3.** Sorption of Aquazine by soil and algae. Each point is the average of two experiments.

![Figure 4](image4.png)  
**Figure 4.** Herbicide concentration in pond supernatant. Each point is the average of two experiments.
Fletchall (2) studied the adsorption of 5 s-triazines with various soils. They observed increasing adsorption with decreasing temperature. Their adsorption system reached equilibrium within one hour when the container was constantly agitated.

Pellets obtained by centrifugation were extracted and analyzed. There was no indication of herbicide for the first 8 days after treatment (Figure 3). The concentration of herbicide in the pellets then gradually increased until the experiments were terminated. At this time the pellets contained about 8% of the total concentration of herbicide applied. It is possible the procedures used were not sensitive enough to detect the herbicide during the first 8 days of treatment. King, et al (9) observed that algae and aquatic weeds sorbed significant quantities of pesticides in relation to that adsorbed by bottom sediments. Kruglov and Mikhailova (10) reported that algae can accumulate simazine in amounts exceeding 100 times its concentration in the culture medium. Others (11) have suggested that initial herbicide adsorption by vegetation followed by death and decay could account for fluctuations in herbicide concentrations.

Analysis of pond supernatant samples revealed a concentration of Aquazine the first day accounting for nearly all the herbicide applied but after 2 weeks the concentration was about 10% of the total applied (Figure 4). Aquazine is relatively soluble so a relatively high initial concentration was expected. Many herbicides are not persistent in aquatic environments. Eichelberger and Lichtenberg (12) found that most persistent substituted urea and carbamate pesticides did not remain in significant concentrations after 4 weeks. Paraquat and diquat disappear rapidly from solution with a concomitant increase in the bottom sediment (13).

pH measurements taken during the course of the treatments remained almost constant at 8.0. The pH effect on adsorption of pesticides that are neither weakly basic nor weakly acidic is usually insignificant (2). As pH was relatively constant in this experiment, it should not have been a factor in the sorption of the herbicide.

Microscopic examinations of water samples were taken during the experiment. Initial samples showed the presence of many flagellates, with a few cells of *Chlorella* sp. Just after treatment most flagellates disappeared and *Chlorella* sp. increased rapidly. Observation 10 days after treatment indicated that flagellates were increasing in number and were mostly *Paramecium* sp. and other protozoa. The algal species present were unicellular and mostly *Chlorella* sp. It appears the herbicide was more toxic to some algal species than to others, thus altering the composition of the pond as time progressed.

It is expensive and difficult to obtain uniform chemical treatment of a large pond or lake. A small pond such as the one used can solve both of these problems. Sampling can also be done much easier and faster.

Results indicated that the herbicide applied was lost rather rapidly from the solution. Some herbicide may have been volatilized or adsorbed to the walls of the tank. Algae and soil gradually sorbed the herbicide with time. The algal population gradually declined after treatment, but the concentration of herbicide in the pellets analyzed increased with time (Figure 3). This indicated a higher concentration of herbicide per algal cell as time progressed. There was a change in the pond population after treatment, indicating the herbicide may cause a change in phytoplankton composition. Further study is needed concerning this problem.

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REFERENCES