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Fate and Effects of Atrazine in Small Aquatic Microcosms

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Heavy use of the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) (Eichers et al. 1978) has resulted in concentrations of up to 42 µg/L in natural waters (Richard et al. 1974). This widespread usage combined with reports of toxicity to algae at concentrations as low as 1 µg/L (O'Kelly & Deason 1976) prompted this investigation of the ecological hazard of atrazine as evaluated in small aquatic microcosms.

The objectives of this study were to determine the fate and effects of atrazine on small laboratory microcosms, to evaluate the resilience of the systems after removal of the atrazine, and to compare the responses of different types of test systems to the herbicide. This study was carried out with mixed biota communities in either continuous-flow aquaria or static beakers.

MATERIALS AND METHODS

Continuous-flow experiments were carried out in 12 seamless glass aquaria (20 cm x 30 cm x 18 cm) placed in a temperature-controlled room at 22°C. Light was supplied by daylight-balanced, high intensity fluorescent lamps at an intensity of 6500±500 lux. Water temperature varied from 19°C during the dark period (12 hr) to 24.5°C during the light period (12 hr).

Initially each microcosm received a 0.5 cm layer of pond sediments, 7L of pond water and an inoculum of net plankton plus aufwuchs from a mesotrophic, soft-water, 2-ha pond. Subsequently all microcosms received a weekly inoculum of net plankton from this pond for the duration of the 49-wk experiment.

The nutrient medium was modified Freeman's standard reference water (Brockway et al. 1979). This medium was added through a glass and stainless steel system at a rate of 0.6 mL/min giving an 8.1 day retention time. All microcosms were mixed continuously by chain-driven, 50-rpm stainless steel stirrers.

The 12 microcosms were organized into 4 sets of 3 containers, with each set on a separate nutrient feed system. After a 39-week development period, atrazine concentrations of 0, 0.5, 5, and 50

µg/L were introduced through the nutrient supply system into the respective microcosm sets. The microcosms themselves were not spiked with atrazine so the concentrations increased gradually. When atrazine concentrations in the microcosms were to be increased abruptly (test for acclimation, described in Results), a concentrated spike was added to each microcosm. To lower the atrazine concentration abruptly (test for resilience), 50% of the medium in a microcosm (3.5L) was removed and the tank refilled with nutrient medium. This was done on two successive days and normal medium input diluted the atrazine thereafter.

Six parameters were monitored throughout the experiment. Dissolved oxygen (DO) and pH were measured before and after the 12-hr light period using an Orbisphere[1] DO meter and an Extech Model 609 digital pH meter. Total dissolved phosphorus, nitrate, nitrite, and ammonia concentrations were determined weekly, on the same day and at the same time using a Technicon Auto Analyzer II (USEPA 1979). Samples for N and P were taken 1 hr after lights-on.

Atrazine of 98.2% purity was obtained from the Reference Standards Repository, USEPA, Research Triangle Park, NC. Atrazine analyses were done by high performance liquid chromatography using a 2% water-methanol mobile phase, a Whatman Partisil ODS-2 column, and a variable wavelength UV-VIS detector at 222 nm.

Two static tests were carried out using 1-L glass beakers containing 600 ml of the previously described medium. The beakers were inoculated with 2-g aliquots (wet weight) of mixed biota from 1-yr old laboratory flow systems established for stock purposes. The inoculum was predominantly Spirogyra sp., but, also included Oedogonium, Microcystis, Apthanothece, Scenedesmus, many other algal species, flagellate protozoans, and nematodes.

Temperature and lighting were the same as for the flow studies. Dissolved oxygen and pH measurements were made at lights-on and lights-off daily as previously described and atrazine was from the same source and concentrations were measured as previously described.

The first experiment tested atrazine concentrations of 0, 50, 500 and 5000 µg/L (4 replicates). The assay lasted 12 days with a set of replicates sacrificed on days 1 and 7. An abiotic control series (1 replicate) was included to test for non-biological losses (e.g. hydrolysis, photolysis, sorption). The second experiment lasted 7 days and tested atrazine concentrations of 0, 0.5, 5 and 100 µg/L (3 replicates).

[1]Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

RESULTS AND DISCUSSION

The fate of atrazine in the flow systems was determined by closely monitoring the increase in concentration of atrazine in the aqueous phase. There was very little variability between replicates and the measured concentrations of atrazine were less than 5% below the projected concentration during the 50 days of constant input (Fig.1). The close agreement between the projected and actual concentrations indicated there was no significant sorption or degradation of the compound. There was more variability in the data from the static tests but the results still were similar to those from the flow-microcosms and showed that little sorption or degradation of atrazine occurred during the study.

The results of both studies are in general agreement with field data, which indicate slow degradation and low bioconcentration of atrazine (Klassen & Kadoum 1979.)

Net DO production during the day was the main parameter used to evaluate ecosystem effects. The results of the two static tests run 2 months apart fit together very well (Fig. 2). When compared to the controls, there was no difference in oxygen production in the 0.5 µg/L and 5.0 µg/L treaments. Oxygen production was decreased 25-30% in the 50 µg/L treatment, 40-50% at 100 µg/L, 90% at 500µg/L and showed zero to negative net production at 5000 µg/L. There was excellent replicability throughout the two static tests with coefficients of variation (CV) typically below 7%.

Biotic community composition was similar in all treatments. Spirogyra sp. was the dominant producer and dominant organism, and rotifers and nematodes were the dominant consumers. All communities at 500 µg/L and below appeared healthy, though there was little net production at the 500 µg/L level as indicated by DO measurements. After 12 days at 5000 µg/L the algae were totally bleached, but viable rotifers and nematodes were still present.

The development, functioning, and replicability of the flow-microcosms were similar to systems we have studied in the past (Brockway et al. 1979). The net 12-hr oxygen production increased from 4 mg/L for each of the 12 microcosms during week 1 to about 11 mg/L during week 14, at which point it leveled off until about week 24 when a slow decrease started that lasted until the end of the pretreatment phase in week 39.

The data on oxygen production in all the atrazine perturbed systems are shown in Fig. 3 as a percentage of the production in the control tanks. The mean of the controls fluctuated between 8 and 12 mg/L/12 hr for the 72 days of treatment and the relationships between the treated and control systems are obvious when the data are normalized to the control tanks. The treatment regimes were: Control (0 µg/L); Treatment 1 (5 µg/L); Treatment 2 (50 then 0 then 50 µg/L); and Treatment 3 (0.5 then 50 then 100

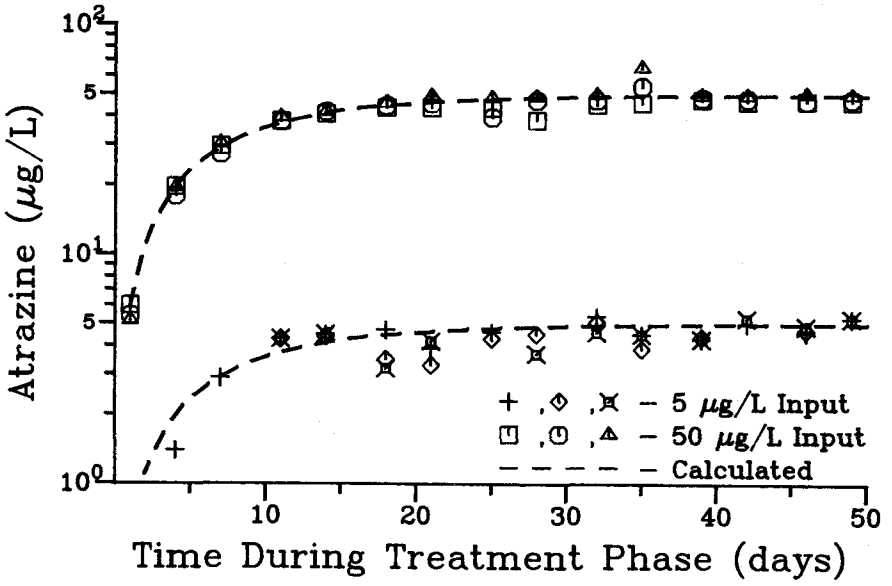

Figure 1. Actual and calculated concentrations of atrazine in flow microcosms.

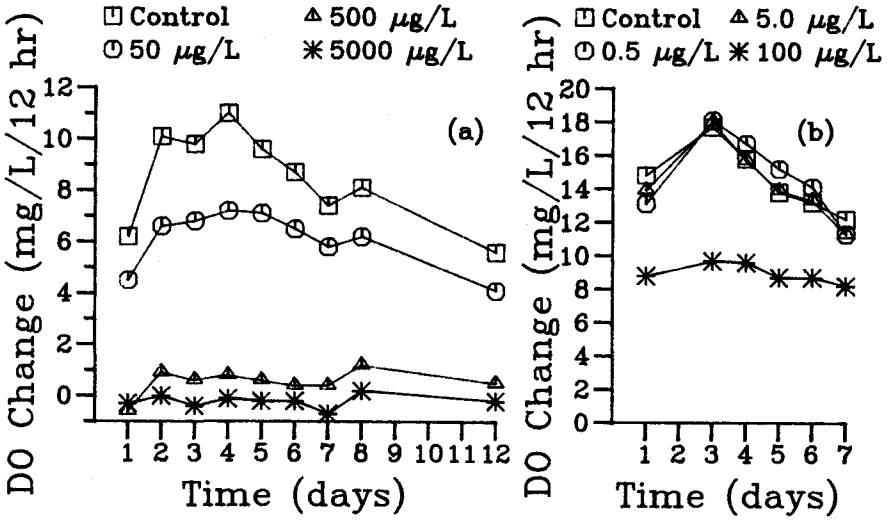

Figure 2. Net diurnal oxygen production in two static tests with the listed concentrations of atrazine.

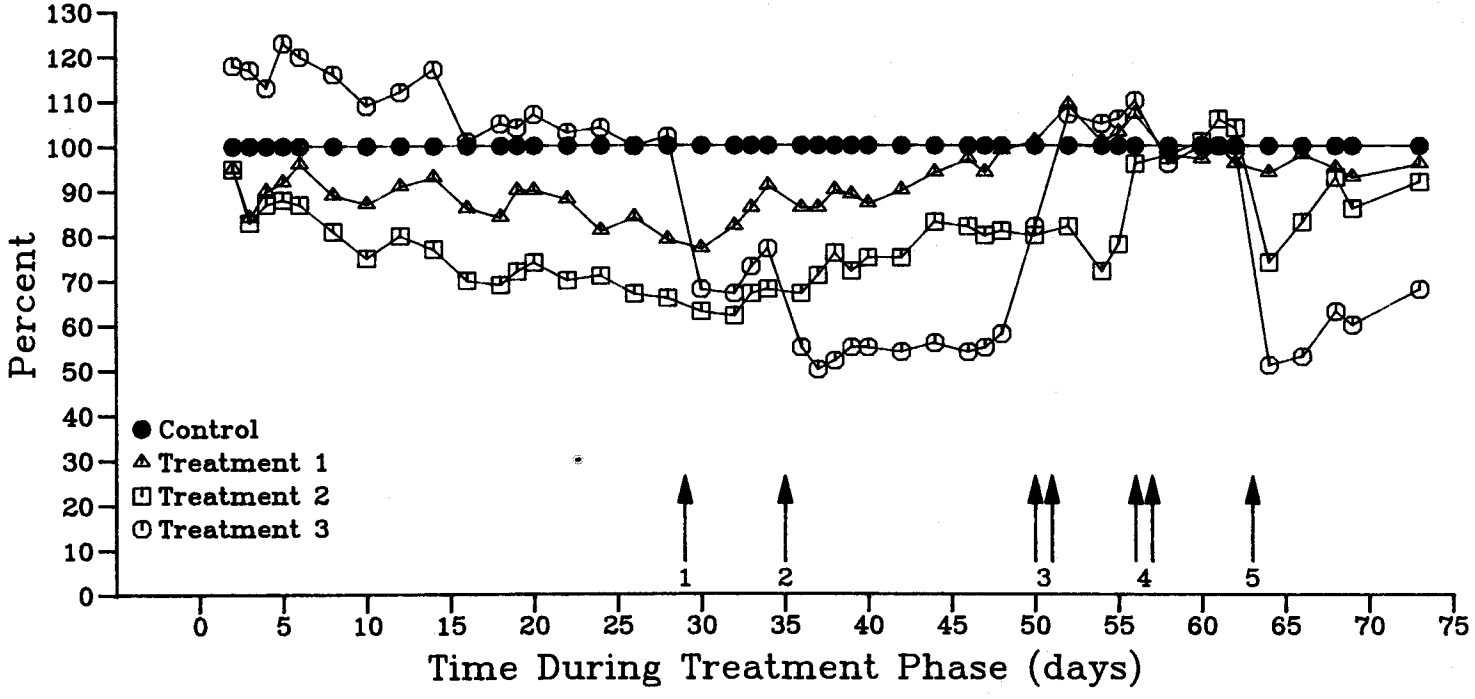

Figure 3. Net diurnal oxygen production in atrazine treated microcosms as a percentage of the controls. Treatment 1 = 5 μg/L; Treatment 2 = 50, then 0, then 50 μg/L; Treatment 3 = 0.5, then 50, then 100, then 0, then 100 μg/L. Arrow 1 = 1st spike of Treatment 3, Arrow 2 = 2nd spike of Treatment 3, Arrows 3 = dilution of Treatment 3, Arrows 4 = dilution of Treatment 2, and Arrow 5 = spikes of Treatments 2 and 3. n = 3 for each point.

then 0 then 100 µg/L).

The replicability of these systems was again very good with CV typically under 19%; however, over a period of several days oxygen production means for the controls typically varied ±10%. Therefore, we concluded that in comparing two sets of means (i.e. control versus treatment) deviations of 20% could not reasonably be judged as treatment effects.

During the first 4 weeks of the treatment phase, oxygen production in all of the treated tanks was declining as atrazine concentrations increased (Fig.3). This trend ceased when atrazine concentrations essentially stabilized. The degree to which the initial downward trends in the treated systems represented treatment effects is somewhat ambiguous due to the 20% error margin that should be considered. Both Treatment 1 (5 µg/L) and Treatment 3 (0.5 µg/L for 4 weeks) did not differ from the controls by greater than 20%. Treatment 2 (50 µg/L) production, however, was depressed about 30% agreeing with results from the static tests.

To corroborate the effect seen in Treatment 2 and to determine whether previous exposure to a low level of atrazine might influence subsequent response to higher concentrations of toxicant, the Treatment 3 tanks (0.5 µg/L) were increased to 50 µg/L by spiking the tanks and their medium reservoir. The response was an immediate (within 1 day) 30% inhibition of oxygen production below the control systems (day 30, Fig. 3). Five days later the atrazine concentration in Treatment 3 tanks was further increased to 100 µg/L and another immediate decrease in oxygen production to 45% below control values occurred. These data agreed very closely with the results of the static tests and indicated that previous exposure to atrazine had little impact on subsequent response to high concentrations of atrazine.

The next question of interest concerned the resilience of the communities. On days 50 and 51 for Treatment 3 and days 56 and 57 for Treatment 2, the atrazine in the tanks was diluted as described earlier and atrazine was removed from the input medium. The atrazine concentrations in the tanks decreased to less than 10 µg/L and the production of oxygen rose correspondingly to the level of the controls. The resilience was immediate and total. This is not surprising considering atrazine inhibits the Hill reaction of photosynthesis (Esser et al. 1975) and when the inhibitor is removed, oxygen production starts almost immediately as long as there has been no permanent damage to the organisms or decrease in the population.

Next, atrazine was added as a spike and in the media to those tanks from which it had been removed 6 or 13 days before. Again the response was rapid. Oxygen production was inhibited to the level at which it had been before the atrazine was removed. There was no immediate indication of any acclimation or change in magnitude of response following the previous exposure. The treat-

ment values were increasing toward the control values during the final week of the study, however, which could indicate some acclimation or resistance was finally occurring.

A comparison of the static tests and the microcosm study indicated the results are similar although the length of time is different. This could explain the lack of inhibition at 5 µg/L atrazine in the static test and indicate possible inhibition in the longer study. These results can also be compared to a pond study of atrazine (De Noyelles et al. 1982) that lasted about twice as long as our flow study. They also found a 90-95% inhibition of productivity at 500 µg/L atrazine and at their other treatment 20 µg/L, found inhibition less than at our 50 µg/L treatment. Both results are in good agreement with our static tests. In single-species studies at 5 µg/L some inhibition was found. In the pond, some resistance and succession were found, which were not clearly evident in our microcosm studies.

Of the other chemical parameters monitored, only dissolved nitrate changed significantly during atrazine treatment. Figure 4 shows that coincident with the spiking of Treatment 3 to 100 µg/L of atrazine (Arrows 2 & 5) nitrate concentrations were elevated significantly (Student's-t, P=0.01). Subsequently, reduction of atrazine in both Treatments 2 & 3 (Arrows 3 & 4) was followed by sharp decreases in nitrate with later respiking (Arrow 5) again driving nitrate up.

These results reinforce the productivity results both by their fast response to the introduction of atrazine and by their resilience upon its removal. The concentration of nitrate reached during the 100 µg/L treatment was approximately the value we calculated based on nitrate loading from the input medium and the percent decrease in nitrate utilization if caused by inhibition of photosynthesis. The conclusion that nitrate is the most sensitive nutrient parameter in stressed systems has also been drawn for aquatic systems treated with cadmium (Hendrix et al. 1981).

The effects of atrazine on community structure were estimated by microscopic observations of biota during the study, which indicated the composition was similar throughout, relative to both time and treatment. Bluegreen algae were dominant, and greens and diatoms were numerous, with the same genera in the same relative abundance present throughout. Copepods, ostracods, and annelids were present in all systems at termination.

Our results on community structure are somewhat different from the field results of De Noyelles et al. (1982) who found definite phytoplankton population changes at 500 µg/L and slight changes at 20 µg/L atrazine. This difference from our study can probably be explained by first the high concentration, but also the longer exposure, probably more grazing pressure, and the lack of dominance in the pond by a few species. Another study (Plumley and Davis 1980) obtained very similar but not identical results between microecosystems and field systems.

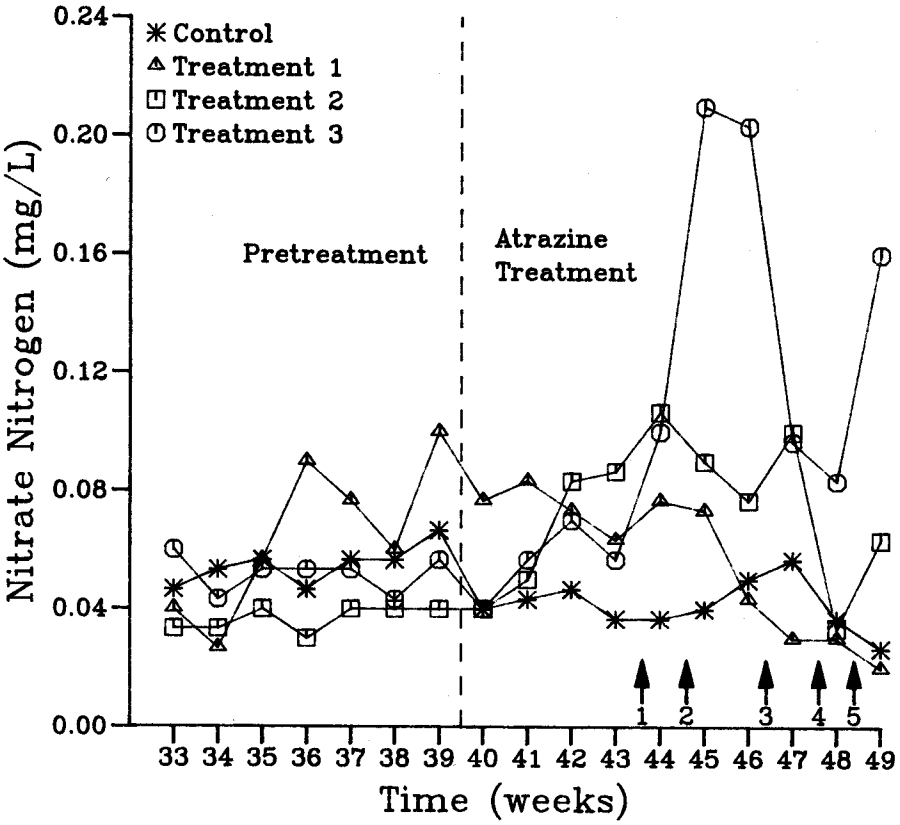

Figure 4. Dissolved NO_3–N concentrations before and during atrazine treatment. Treatments and arrows are the same as in Fig. 3. n = 3 for each point.

We can thus conclude the degradation and sorption of atrazine is very low in aquatic systems. Atrazine has a negative impact on primary producers at and above a concentration of 50 µg/L and possibly below. These effects are expressed best in oxygen production and dissolved nitrate in the systems. Our data demonstrated that small static systems can furnish results similar to those of larger more complex systems, particularly for quick response parameters.

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