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Differential Toxicity of Atrazine to Selected Freshwater Algae

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The documented presence of atrazine in surface waters has prompted a large number of studies on its potential adverse effects on nontarget organisms such as freshwater algae, which are the most important primary producers in aquatic habitats and are potential indicators of water quality (Blaise 1993). Recently, a comprehensive database of the ecological effects of atrazine, including 85 freshwater organisms, was compiled (Solomon et al. 1996). Based on this compilation of acute (≤ 4 day) or chronic (> 9 day) toxicity values, algae are the most susceptible aquatic organisms to atrazine, although it is apparent that different species and divisions of freshwater algae exhibit varying levels of response to atrazine exposure.

Numerous studies have indicated that atrazine inhibits growth and photosynthesis of freshwater algae and algal responses to atrazine vary widely depending upon concentrations used, duration of exposure, and algal species tested. However, there are few studies that directly compare the effects of atrazine between different divisions of freshwater algae, and most studies have focused on short-term (up to 96 hrs and 7 days) growth inhibition with a limited number algal species (Larsen et al. 1986, Walsh et al. 1987, Hersh and Crumpton 1989, Abou-Waly et al. 1991, Kasai et al. 1993, Kirby and Sheahan 1994). Very few studies have focused on long-term (over 14 days) toxicity tests (Kirkwood and Fletcher 1970, Johnson 1986, Megharaj et al. 1987, Okay and Morkoc 1994). Atrazine is relatively persistent in water, and its concentrations would not be expected to vary greatly over time, especially in short-term bioassays (Solomon et al. 1996). More recent testing procedures have recognized that, because of the wide range of sensitivity observed, a battery of species is recommended to improve algal toxicity detection and predictability in chemical evaluation (Boutin et al. 1993). Therefore, in the present study eight freshwater algal species from two divisions were chosen and the effects of atrazine on these algae were assessed over 28 days. The objectives of this study were to examine the effects of atrazine on growth of four green algae and four diatoms and to quantify the differences to atrazine exposure between the tolerance levels of these two algal divisions.

MATERIALS AND METHODS

Four species of green algae and four species of diatoms were selected based on their availability in culture collections and their ability to grow under similar conditions (temperature, lighting, and nutrition). The test species represented a wide range in taxonomy, morphology and physiology. Green algae

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(Chlamydomonas sp., *Chlorella* sp., *Pediastrum* sp., and *Scenedesmus quadricauda)* were obtained from Carolina Biological Supply Company (Burlington, NC). Diatoms *(Cyclotella gamma, C. meneghiniana, Synedra acus,* and *S. radians*) were obtained from the Loras College culture collection (Dubuque, IA). All algae were grown under axenic conditions. Green algae were grown in WC freshwater medium (Guillard 1975), and diatoms were grown in Chu #10 medium (Nichols 1973). Cultures were incubated at 20°C with an alternate 12/12 light:dark cycle using cool-white fluorescent lamps at ca. 100 $\mu M\ m^{-2} s^{-1}$. Cultures were shaken at 150 rpm for 7- 10 days to obtain algal cultures in exponential growth phase before initiating bioassays.

Technical atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) (99.8% pure) was obtained from Supelco, Inc. (Belletonete, PA) and used in all bioassays. Screw-capped glass tubes (150 mm x 22 mm diameter), each containing 14.5 mL autoclaved medium, were inoculated with 0.5 mL aliquots of fresh cell suspension (7-10 days old). Fifteen µL of atrazine solution (dissolved in acetone) was added to each tube providing nominal concentrations of 1, 10, 100, 250, 500, and 1000 µg atrazine/L medium. The final concentration of acetone was 0.1%. Preliminary analysis indicated that this concentration had no effect on algal growth. Three replicate tubes were used at each atrazine concentration. Untreated controls received only 0.1% acetone. The final volume in each experimental system was 15 mL (Kent and Currie 1995). All tested species exhibited sustained growth based on both cell density and chlorophyll *a* for up to 28 days (data not shown). Samples were withdrawn after 7, 14, 21 and 28 days. For each sample, growth was determined using two methods: (1) spectrophotometric determination of optical density, and (2) fluorometric measurement of chlorophyll *a* content.

For optical density determination, three test tubes were randomly withdrawn and thoroughly vortexed for each concentration and each time point. Optical density was measured at 680 nm (OD_{680}) in a Beckman DU-65 Spectrophotometer (Beckman Instruments, Palo Alto, CA). Kasai et al. (1993) reported that cell numbers and OD_{680} are highly correlated. A strong correlation between optical densities and cell densities of the algae tested was confirmed in this experiment, with r^2 values > 0.97 for all algal species tested. Optical density was then used as a surrogate for growth (cell density) for each freshwater alga. The method of Welschmeyer (1994) without acidification was used for fluorometric measurement of chlorophyll *a* content. After optical density measurement, a known volume of suspended algal sample was passed through a Whatman GF/A filter. The filter was then placed in a sealed screw-capped glass tube containing 10 mL 90% ethanol to prevent evaporation. The test tubes were placed in a 78°C water bath for 5 min (without light) and then cooled in the dark at room temperature. The extract was decanted into a clean cuvette, and fluorescence was read with a Model 10-AU Fluorometer (Turner Designs, Sunnyvale, CA) calibrated with standard solutions of chlorophyll *a* obtained from Sigma Chemical Company (St. Louis, MO). The chlorophyll *a* content in each sample was expressed as µg/L.

The percent inhibition (%I) of algal growth for each atrazine concentration was calculated by comparing mean OD_{680} values and chlorophyll *a* content to control samples (U.S. EPA 1989). Atrazine concentrations that inhibit algal growth (cell density and chlorophyll *a* content) by 50% relative to controls (EC_{50}s) were calculated using the Probit Procedure (SAS Institute 1994) with log transformed values of test atrazine concentrations. The statistically significant effects of atrazine on growth of different algae species was determined using analysis of variance (ANOVA), in conjunction with Duncan's multiple range test (SAS Institute 1994) with 95% confidence intervals ($p < 0.05$).

RESULTS AND DISCUSSION

The inhibitory effects of atrazine on algal growth as measured by optical density and chlorophyll *a* fluorescence of green algae and diatoms are presented in Figures 1 and 2, respectively. Based on comparisons with controls, various growth-response patterns were observed following atrazine treatment. At 10 µg/L, atrazine was slightly inhibitory based on both cell density and chlorophyll *a* in a number of species. However, this same concentration stimulated cell density and chlorophyll *a* for others. For example, both cell density and chlorophyll *a* content were consistently higher for *Chlamydomonas* sp. and *Synedra acus* at 1 µg/L (data not shown) and 10 µg/L at all time points. The growth of all species was inhibited almost completely by atrazine at 1,000 µg/L after 14 days, but did not exceed 80% for the 7-day readings. Growth inhibition became most evident at 100 µg/L for most of the green algae, although a sharp increase in growth inhibition did not occur until 250 µg/L for most diatoms. Optical density and chlorophyll *a* measurements produced similar inhibition curves indicating that cell density and chlorphyll *a* content responded similarly to atrazine exposure.

EC_{50} values (effective concentrations that caused 50% inhibition compared with the controls) were calculated for each species at 7, 14, 21 and 28 days after treatment for both opitical density (Table 1) and chlorophyll *a* (Table 2) measurements. The EC_{50} values varied significantly among the different species and divisions of the algae tested, and the EC_{50} values for diatoms were generally higher than those of green algae. At 7- to 14-day exposures, the green alga *Chlamydomonas* sp. was the most susceptible species among all the species tested, and the diatom *C. meneghiniana* was the most tolerant. The EC_{50} values for all species except *Chlamydomonas* sp. were generally higher for the 7-day exposure than for other time points, and differences in EC_{50} values among species were more apparent at 7 days than at the other time points.

In the evaluation of ecotoxicological risk of herbicides, values of sensitive species have been an important consideration (Huber 1993). EC_{50} values are useful to more exactly determine the range of pesticide concentrations that cause growth inhibition in an algal population (Anton et al. 1993). EC_{50} values from this study are similar to those reported for other algal species (Solomon et al. 1996, Huber 1993). Atrazine was highly toxic to all the freshwater algal species tested. Growth inhibition was detected in some species at concentrations as low as 10 µg/L and complete inhibition was observed at 1,000 µg/L. Although atrazine was clearly toxic to all species tested, there was a broad range of sensitivity among species, and in general, green algae were more susceptible to atrazine than diatoms. Previous studies at the community level have suggested similar differences in atrazine toxicity to freshwater algae. Herman et al. (1986) observed a shift from a chlorophyte- to a diatom-dominated community after atrazine treatment to an in situ, enclosed periphyton community. Shehata et. al. (1993) also reported that green algae and blue-green algae declined while diatoms increased in the presence of 0.01 mg/L of the triazine herbicide, gardoprim. This differential sensitivity to toxins among freshwater algae could have serious impacts on community structure and seasonal successional patterns (Hersh and Crumpton 1987), and any inhibitory effects of atrazine to freshwater algae should be considered environmentally significant.

At low concentrations, atrazine stimulated both growth and chlorophyll *a* content of the green alga *Chlamydomonas* sp. and diatom *Synedra acus*. These results are consistent with other reports indicating stimulatory effects of atrazine at low concentrations. El-Dib et al. (1989) reported that gardoprim, which is structurally very similar to atrazine, showed stimulatory effects on chlorophyll *a* content of a

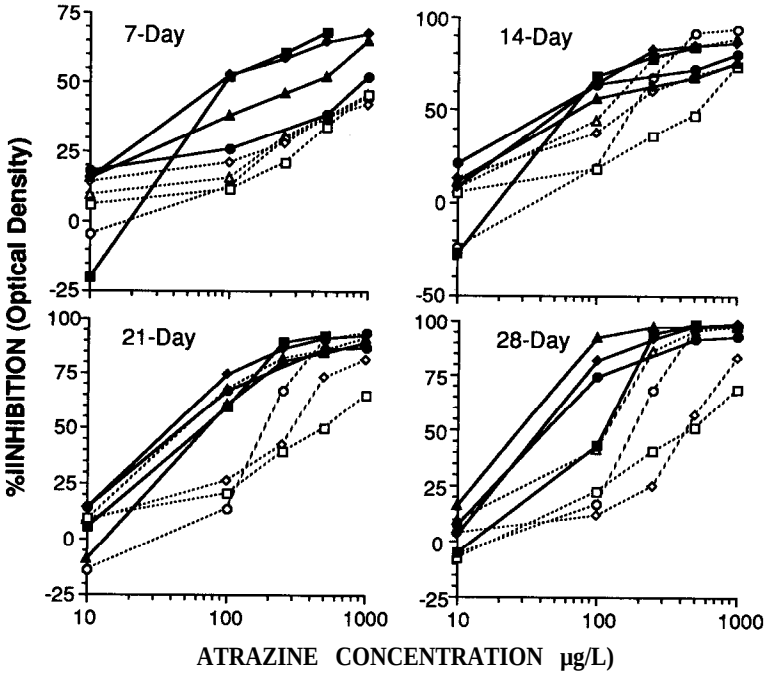

Figure 1. Atrazine inhibition of optical density in green algae (—■— *Chlamydomonas* sp., —◆— *Chlorella* sp.,—●— *Pediastrum* sp.,—▲— *Scenedesmus quadricauda*) and diatoms (- - □ - - *Cyclotella gamma*, - - ◇ - - *Cyclotella meneghiniana*, - - △ - - *Synedra acus*, - - ○ - - *Synedra radians*). Each point represents the mean of three determinations.

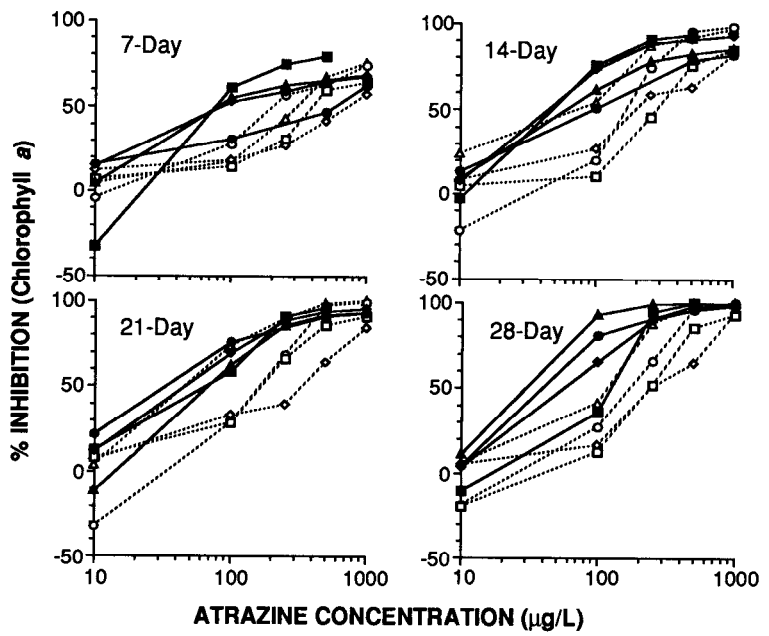

Figure 2. Atrazine inhibition of chlorophyll *a* in green algae (—■— *Chlamydomonas* sp., —◆— *Chlorella* sp., —●— *Pediastrum* sp., —▲— *Scenedesmus quadricauda*) and diatoms (- - □ - - *Cyclotella gamma*, - - ◇ - - *Cyclotella meneghiniana*, - - △ - - *Synedra acus*, - - ○ - - *Synedra radians*). Each point represents the mean of three determinations.

Table 1. EC_{50} values for atrazine toxicity to selected freshwater algae based on optical density.

Algae	Atrazine EC_{50} (µg/L)[1] 7-Day	14-Day	21-Day	28-Day
DIATOMS				
Cyclotella gamma	1552.4 ± 26.2 a	447.6 ± 16.7 a	493.3 ± 24.6 a	429.7 ± 21.7 a
Cyclotella meneghiniana	3213.7 ± 95.6 b	180.4 ± 3.3 b	244.9 ± 16.7 b	417.3 ± 11.3 a
Synedra acus	1173.2 ± 23.9 c	180.1 ± 6.7 b	203.4 ± 5.8 c	204.3 ± 5.6 b
Synedra radians	1488.6 ± 88.6 c	113.8 ± 4.4 c	68.7 ± 2.2 d	88.9 ± 4.7 d
GREEN ALGAE				
Chlamydomonas sp.	80.6 ± 3.6 f	26.2 ± 2.6 e	73.4 ± 3.8 d	110.6 ± 8.5 c
Chlorella sp.	155.2 ± 4.5 f	56.2 ± 2.8 e	46.8 ± 2.6 e	53.1 ± 2.3 e
Pediastrum sp.	1282.2 ± 38.9 c	66.6 ± 4.6 b	51.7 ± 2.2 de	51.9 ± 2.3 e
Scenedesmus quadricauda	333.8 ± 18.4 e	124.2 ± 7.5 b	50.7 ± 5.3 de	27.6 ± 2.1 f

[1] Mean ± SEM of three separate determinations. Differences among treatment means determined by analysis of variance ($P<0.05$). Means followed by the same letter within columns are not significantly different ($P>0.05$; Duncan's multiple range test).

Table 2. EC_{50} values for atrazine toxicity to selected freshwater algae based on Chlorphyll *a* content.

Algae	Atrazine EC_{50} (µg/L)[1] 7-Day	14-Day	21-Day	28-Day
DIATOMS				
Cyclotella gamma	494.4 ± 21.6 b	282.9 ± 9.7 a	149.0 ± 7.5 b	241.8 ± 21.7 a
Cyclotella meneghiniana	959.4 ± 56.8 a	224.6 ± 7.6 b	265.6 ± 9.7 a	255.4 ± 11.3 a
Synedra acus	259.3 ± 11.5 d	167.3 ± 4.6 b	159.4 ± 8.1 b	168.7 ± 5.6 b
Synedra radians	336.9 ± 7.8 c	49.4 ± 2.5 c	60.5 ± 6.7 c	92.0 ± 4.7 d
GREEN ALGAE				
Chlamydomonas sp.	46.5 ± 3.0 f	33.1 ± 1.6 f	58.1 ± 1.8 c	116.5 ± 9.8 c
Chlorella sp.	72.9 ± 4.6 f	52.6 ± 2.4 e	52.2 ± 1.4 c	67.8 ± 1.8 e
Pediastrum sp.	536.6 ± 24.4 b	88.8 ± 6.3 d	28.0 ± 3.0 d	42.4 ± 2.2 f
Scenedesmus quadricauda	171.0 ± 8.9 e	80.7 ± 2.9 d	54.9 ± 1.7 c	28.8 ± 1.2 f

[1] Mean ± SEM of three separate determinations. Differences among treatment means determined by analysis of variance ($P<0.05$). Means followed by the same letter within columns are not significantly different ($P>0.05$; Duncan's multiple range test).

green alga *Scenedesmus* sp. at low concentrations (10 and 20 µg/L) after a 7-day exposure. Stimulatory effects were also detected when a culture of *Scenedesmus* sp. was exposed to the phenylurea herbicide, patoran (El-Dib et al. 1991). The stimulation of growth observed at low concentrations of toxicants may be the result of adaptation of the photosynthetic pigments (Hatfield et al. 1989, Gustavson and Wangberg 1995, Koenig 1990) that allows for increased growth at lower concentrations and longer exposure periods.

Some plants, such as maize or millet, possess efficient detoxification mechanisms to detoxify atrazine (Ebert and Dumford 1976). As a consequence, atrazine can be used as a selective herbicide in production agriculture. It is apparent from this study that different species and perhaps divisions of freshwater algae exhibit different responses to atrazine exposure. Therefore, selective toxicity of pesticides or other organic contaminants of aquatic systems could alter species composition, decrease diversity, interfere with normal successional patterns, and alter food webs as a whole. The phenomenon of differential algal sensitivity to contaminant stress described by Wurster (1968) asserts that certain species or groups within phytoplankton communities possess the inherent ability to resist pollutant stress far better than do others. The results of the present experiment suggest the need for further research into the interaction of atrazine and freshwater algae to better understand the ecotoxicity of atrazine to a broader range of taxa. An understanding of the factors responsible for differential toxicity in algae will improve our ability to predict the impact of aquatic contaminants on freshwater ecosystems.

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