Distribution and Potential Bioeffects of Atrazine in Coastal Waters

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Executive Summary

Estuaries provide critical nursery habitat for many commercially and recreationally important fish and shellfish species. These productive, diverse ecosystems are particularly vulnerable to pollution because they serve as repositories for non–point-source contaminants from upland sources, such as pesticide runoff. Atrazine, among the most widely used pesticides in the United States, has also been one of the most extensively studied. There has not, however, been a specific assessment of atrazine in marine and estuarine ecosystems. This document characterizes the presence and transformation of atrazine in coastal waters, and the effects of atrazine on marine organisms. Review of marine and estuarine monitoring data indicate that atrazine is chronically present in U.S. coastal waters at relatively low concentrations. The concentrations detected have typically been below acute biological effects levels, and below the U.S. EPA proposed water quality criteria for atrazine. While direct risk of atrazine impacts are low, uncertainty remains regarding the effects of long-term low levels of atrazine in mixture with other contaminants. It is recommended that best management practices, such as the use of vegetative buffers and public education about pesticide use, be encouraged in the coastal zone to minimize runoff of atrazine into marine and estuarine waters.

Introduction

Atrazine (2-chloro-4-(ethylamine)-6-(isopropylamino)-s-triazine) is a triazine herbicide used on food crops, residential lawns, turf grass, sod, forestry sites and Christmas tree farms for selective, non-selective, pre-emergence and early post emergence weed control (Thompson, 2002; Ware, 1991). Atrazine is the second most heavily used herbicide in the United States, with approximately 64 million pounds of active ingredient applied annually (Thelin and Gianessi, 2000). This herbicide is characterized by a ringed structure (Fig. 1) containing three nitrogen atoms and three carbon atoms (Ware, 1991), and has been used in agriculture since 1959 (Thompson, 2002).

Atrazine's mechanism of action in target weed species is inhibition of photosynthesis (Ware, 1991). Atrazine disrupts electron transport along the photosystem II pathway (reviewed by Huber, 1993). Other processes within plants which rely on the energy produced by photosynthesis may also be indirectly affected. Some of these processes include the opening of stomata, transpiration, ion transport, phytohormone balance, and ion balance. Metabolic disruptions may also result, affecting RNA, enzyme, and protein synthesis (reviewed by Huber, 1993). Photosynthetic microalgae and aquatic macrophytes are the most susceptible non-target organisms in aquatic environments (Solomon et al., 1996).

Atrazine can enter the aquatic environment via surface runoff, spray drift, irrigation, groundwater leaching, and atmospheric transport. Atrazine is frequently measured in surface waters throughout the United States at concentrations of 0.1-30 μg/L (Solomon et al., 1996). It is also the most commonly detected pesticide in ground and surface water, detected up to twenty times more frequently than any other herbicide in the United States (Belluck et al., 1991).

In 1994, the U.S. Environmental Protection Agency (U.S. EPA) began a special review of atrazine's potential to negatively impact environmental and human health, based on the large amount of scientific data generated in the decades of atrazine use (U.S. EPA, 2001a). At the end of the special review, the U.S. EPA proposed a criterion continuous concentration for atrazine of 12.35 μg/L for freshwater and 26.71 μg/L for saltwater (U.S. EPA, 2001b). These proposed criteria were challenged by a number of Clean Water Network member organizations (U.S. EPA, 2002) due to: 1.) lower effects levels documented in additional plant toxicity studies not used by the EPA, 2.) data supporting the endocrine disruption potential of atrazine, and 3.) uncharacterized toxicity of atrazine degradation products (U.S. EPA, 2002). In June 2002, the Natural Resources Defense Council filed a petition asking the EPA to take atrazine completely off the market, charging that its leading manufacturer did not properly disclose that 17 workers had developed prostate cancer and that atrazine had been linked to deformities in frogs (U.S. EPA, 2003).

Due to the ubiquitous presence of atrazine in aquatic environments, and its heavy use in both agricultural and domestic settings, there is concern for its potential impacts on aquatic organisms, human health, and ecosystem services. Ecosystem services may include aspects such as drinking water quality, commercial fish and shellfish yield, recreation and tourism. The purpose of this review is to summarize the specific occurrence of atrazine in the coastal environment and to characterize the effects of atrazine on marine and estuarine organisms. Atrazine's potential to cause endocrine disrupting effects (e.g. Brusick, 1994; Crain et al., 1997), developmental abnormalities in amphibians (e,g, Tavera-Mendoza et al. 2001; Hayes et al., 2002; Carr et al., 2003), and carcinogenic effects in mammals (e.g. Taets et al., 1998) has been investigated in a number of studies. As a consensus has not been reached regarding these effects,

they will not be included in the scope of this review. In addition to the U.S. EPA atrazine review, a number of peer-reviewed literature risk assessments have been conducted on atrazine. Most notably are the works of Huber (1993), and Solomon et al. (1996). There has not been a specific assessment of atrazine in marine and estuarine ecosystems. We will focus, therefore, on the occurrence of atrazine in the U.S. coastal zone, the toxicity values determined for marine and estuarine organisms, and the risk of environmental effects based on current proposed water quality criteria. The U.S. EPA 2003 revised saltwater quality criteria (USEPA SWQC) state that "saltwater aquatic life and their uses should not be affected unacceptably if the one-hour average concentration of atrazine does not exceed 760 µg/L more than once every three years on the average (acute criterion) and if the thirty-day average concentration of atrazine does not exceed 17 µg/L more than once every three years on the average (chronic criterion)."

Technical Review

Fate and transport of atrazine in the coastal marine environment

Atrazine is moderately water-soluble (33 mg l^{-1} at 25 °C) and is mobilized from soils into surface waters during rainfall events (Azevedo et al., 2000). Atrazine has been measured in fog (Glotfelty et al., 1987; Glotfelty et al., 1990), and trace amounts are transported by wind (Elling et al., 1987). Estuaries often serve as sinks for pollutants transported from upland areas. Given its chemical properties, atrazine is likely to be carried from freshwater areas and deposited in slower moving estuarine systems. For example, in 1991, 160 metric tons of atrazine were discharged from the Mississippi River Basin into the Gulf of Mexico (Pereira and Hostettler, 1993).

In the environment, atrazine degradation is enhanced by light and by the presence of organic matter or minerals, in particular humic and fulvic acids. Atrazine has an estimated half

life of 30 days in estuarine surface waters (Solomon et al., 1996). Using a microcosm exposure, Jones et al. (1982) determined atrazine's half-life in estuarine sediment to be 15 to 20 days.

Several processes such as microbial decay, hydrolysis and photolysis have the potential to impact atrazine degradation in estuarine systems. Its accumulation and persistence in coastal sediments contribute to the transport of atrazine and its metabolites to surface and subsurface waters. The atrazine degradation pathway proceeds through chemical transformations at low sediment pH to hydroxyatrazine (Fig. 2). Additionally, microbial N-dealkylation of the ethyl and isopropyl side chains can produce deethylatrazine (DEA) and deisopropylatrazine (DIA) (Kaufman and Kearney, 1970). The formation of hydroxyatrazine, a primary intermediate, occurs under acidic conditions in the presence of humic acids. Hydroxyatrazine is relatively polar and has the potential to bind tightly to soil organic carbon, becoming unavailable (Houout et al., 1998). The dealkylation products DEA and DIA, on the other hand, retain the chlorine atom and are considered to be phytotoxic (Houout et al., 1998; DeLorenzo et al., 1999b).

Salt marsh sediments tend to have high soil organic carbon (SOC) content, and therefore high sorption capacity for atrazine. Smalling and Aelion (2004) examined the extent of atrazine mineralization and transformation by indigenous microbes in natural coastal sediments. They found that atrazine persistence was correlated with SOC, with longer half-lives associated with lower SOC. SOC content decreased with depth and subsequent lower microbial activity related to slower degradation of atrazine. Thus in subsurface sediments, atrazine or its metabolites may be more readily transported to shallow groundwater because of long half-lives associated with low soil organic carbon content (Smalling and Aelion, 2004).

Based on results from spiked estuarine sediments, sediments with less carbon and limited binding sites showed increased formation and persistence of DEA (Smalling and Aelion, 2006).

A non-polar secondary metabolite, methylated atrazine (M-ATR) not previously documented to be derived from atrazine, was found to be chemically produced, and concentrations were an order of magnitude higher than DEA. M-ATR may have potential to be persistent in the coastal environment due to the inability of microorganisms to remove the isopropyl groups on the triazine backbone (Smalling and Aelion, 2006).

Atrazine does not have significant potential for bioaccumulation due to its fairly low octanol-water partition coefficient (log K_{OW} = 2.61) (Solomon et al., 1996). In addition, elimination mechanisms and accumulation studies in a variety of species showed that atrazine did not accumulate in mollusks, leeches, or fish (Solomon et al., 1996). In a study of atrazine uptake by 5 different algal species, maximal cellular uptake of all species was less than 2% of the total atrazine available in solution (Weiner et al., 2004). Their results indicated that smaller cells with greater surface area to volume ratios will incorporate more atrazine and, in general, will be more sensitive to atrazine exposure. Similar findings were reported by Tang et al. (1998) for freshwater green algae and diatoms.

Measured concentrations of atrazine in the coastal marine environment

Thurman et al. (1991 and 1992) found atrazine levels in freshwater streams in the Midwestern United States 3 to 10 times greater than the U.S. EPA maximum contaminant level for drinking water (3 µg/L). Their data showed that 55% of 122 river basins exceeded the maximum contaminant level during post planting sampling in 1989 and 98% of basins had detectable trace levels of atrazine and its metabolites. A more recent survey of water in the Mississippi River by the U.S. Geological Survey found atrazine in 100% of the samples collected in April, May, and June (Thurman et al., 1994). Over one-quarter of these samples, and the median concentration of all samples, exceeded the USEPA maximum contaminant level

(Thurman et al., 1994). In a national survey of pesticides in drinking water wells in the United States, atrazine was the most frequently detected pesticide, with approximately 1,570 community water system wells and 70,800 rural domestic wells contaminated (Thurman et al., 1994).

Atrazine concentrations in the U.S. coastal zone are typically lower than those in the Midwestern ("corn belt") region of the country (Table 1). During 1993, 136 estuarine surface water samples were collected from the mid-Texas coast (Corpus Christi - Port Lavaca, TX) (Pennington et al., 2001). Agricultural, tidal creek, and bay sites were sampled. Collections were made throughout the growing season (February – October, 1993) before and after periods of significant (>1.25 cm) rainfall. Atrazine was detected in 100% of the samples collected during February through July (Pennington et al., 2001; Fig. 3). Generally, the mean atrazine concentrations were approximately 9 times higher at agricultural sites than at bay sites. A clear trend existed showing a significantly greater mean atrazine concentration at edge of field sites (8.52 ± 1.79 µg/L) than tidal creek sites (1.66 ± 0.31 µg/L) and bay sites (0.92 ± 0.19 µg/L). Atrazine concentrations at all sites ranged from < 0.01 (below detection limit) to 62.5 µg/L, with an average of 3.96 ± 0.75 µg/L (Pennington et al., 2001).

A similar trend was demonstrated in South Florida agricultural canals, Florida Bay, and Biscayne Bay. While atrazine was detected in nearly all samples collected, maximum atrazine concentrations were detected in the upper reaches of the canals, with levels decreasing downstream (Scott et al., 2002; Fulton et al., 2004; Harman-Fetcho et al., 2005). A recent South Florida study by Harman-Fetcho et al. (2005), reported that atrazine had the highest measured surface water concentration (0.108 µg/L) out of all 42 chemicals analyzed.

Atrazine was also detected in 100% of the samples analyzed from Winyah Bay, an estuarine environment in South Carolina (Kucklick and Bidleman, 1994a). Atrazine was

detected at concentrations ranging from 0.005-0.848 µg/L and showed a distinct seasonal change, with highest concentrations occurring in Spring. Surface water samples from the Pee Dee River which feeds Winyah Bay also displayed this trend, with higher concentrations recorded in May than December (0.61 µg/L and 0.007 µg/L, respectively). The mean atrazine concentration in the Pee Dee River and upper Winyah Bay was 0.28 µg/L (Kucklick and Bidleman, 1994a). Atrazine concentrations in Winyah Bay were similar to those found in the Mississippi River (0.256-0.521µg/L; May and June 1998; Pereira and Rostad, 1990). Atrazine was also detected in all samples collected from North Inlet, SC, a National Estuarine Research Reserve, at concentrations ranging from 0.001µg/L to 0.104 µg/L. Atrazine levels measured in Chesapeake Bay's Rhode River ranged from 0.002 µg/L to 2 µg/L (Wu, 1981). In Maryland, atrazine was present year-round in rainwater samples, with high concentrations reaching up to 2.2 µg/L (Wu, 1981).

During the time period of 2001 to 2003, the Center for Coastal Environmental Health and Biomolecular Research laboratory collected and analyzed 1,248 coastal South Carolina surface water samples were for atrazine concentration using an immunoassay technique (RaPID Assay®, Strategic Diagnostic, Inc.). For all sites across all three years of the study, 22.6% of the samples had detectable levels of atrazine. Tidal creeks had the lowest prevalence of atrazine (10.5%), followed by shallow coastal ponds (38.5%). The highest percentage of samples with detectable levels of atrazine (46.2%) was found in shallow (<2 m) coastal wells. Atrazine concentrations in tidal creeks remained low during all three years (Figure 4). In 2001, the highest atrazine level was measured in a shallow coastal residential pond near Charleston, SC (15.8 µg/L) (Figure 4). The average atrazine concentration for ponds in 2001 was 1 µg/L (Figure 4). Mean concentrations in ponds and wells were very similar for 2002 averaging 0.079 µg/L and

0.097µg/L, respectively (Figure 4). The mean concentration measured in wells (0.030 µg/L) was somewhat lower than the mean concentration measured in ponds (0.068 µg/L) in 2003 (Figure 4). The close agreement between pond and groundwater atrazine levels may indicate a major subsurface route of exposure.

Atrazine effects on marine phytoplankton

Within aquatic systems, microalgae are potentially the most susceptible organisms to atrazine contamination. These photosynthetic species may be affected directly by atrazine's inhibition of Photosystem II (Solomon et al., 1996). Among microalgae, sensitivity to atrazine has been reported to vary across algal phyla (Guanzon et al., 1996). Published literature is inconsistent in reports of taxon sensitivity. Most commonly, chlorophytes have been reported as the most sensitive to atrazine, followed by cyanobacteria, cryptomonads, dinoflagellates, and euglenophytes, respectively (Abou-Waly et al., 1991; Cohen, 1996). However, one study found that abundance of cyanobacteria increased with atrazine exposure, while all other taxa decreased (DeLorenzo et al., 1999). Another study found that cyanobacteria were the least sensitive taxa to atrazine (Hamala and Kollig, 1985).

DeLorenzo et al. (2004, and in prep.) determined 96h growth rate EC50 values for several marine microalgal species ranged from 26-145 µg/L atrazine. No trends in atrazine toxicity related to microalgal taxonomic group were observed (Table 2). Species sensitivity rankings also varied with endpoint measured. For example, cellular biovolume was a significantly more sensitive test endpoint for *A. operculatum*, whereas chlorophyll *a* was a significantly more sensitive test endpoint for *Ankistrodesmus* sp., and phototrophic carbon assimilation was a significantly more sensitive test endpoint for *S. major* and *A. operculatum* (DeLorenzo et al., 2004). Algal subcellular responses to atrazine were also species dependent. Chlorophyll *a* concentration per cell decreased in the green algae, increased in *A. operculatum*

and did not change in *S. major*. Total lipids per cell increased in *S. major*, decreased in *A. operculatum* and did not change in the green algae. *Ankistrodesmus* sp. pigments were not significantly altered after atrazine exposure. However selected *A. operculatum* pigments increased per cell, and selected *D. tertiolecta* and *S. major* pigments decreased per cell in the atrazine treatments. Atrazine significantly reduced cellular biovolume in most algal species. Species with smaller biovolumes and less chlorophyll *a* per cell tended to be more sensitive to atrazine exposure based on population growth rate (DeLorenzo et al., 2004). Smaller algal species with greater cell surface area to volume ratios also incorporated more atrazine and, in general, were more sensitive to atrazine exposure (Weiner et al., 2004). Pennington et al. (2001) reported a 96h growth rate EC50 value of 147 µg/L atrazine for the marine prymnesiophyte, *Pavlova* sp.

Weiner et al. (2007) examined the physiological effects of atrazine on four marine microalgal species by quantifying changes in carbon allocation into macromolecular pools (low molecular weight (LMW) molecules, lipids, polysaccharides, and proteins). Algae were exposed to three atrazine treatments based on their previously determined 96h growth rate EC50 values: 0.5x EC50, 1x EC50, and 2x EC50. Despite decreased growth rate in all species, total carbon uptake per cell varied among species. Specifically, total carbon uptake per cell was unaffected in *Isochrysis galbana*; increased in *Synechococcus* sp.; and decreased in *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. In the chlorophyte, *D. tertiolecta*, carbon allocation into LMW molecules increased while carbon allocation into protein decreased. *D. tertiolecta* also increased carbon allocation into lipids and polysaccharides at the highest atrazine treatment. In the cyanobacterium, *Synechococcus* sp., carbon allocation into LMW molecules decreased and carbon allocation into protein increased. The diatom, *P. tricornutum*, had decreased carbon

allocation into protein at the highest atrazine concentration tested. No significant macromolecular changes were found in the prymnesiophyte, *I. galbana*. Weiner et al. (2007) concluded that alterations in the macromolecular composition of microalgal species may negatively affect higher trophic levels, as nutritionally altered algal cells may have a reduced energy per mass uptake for consumers.

The U.S. EPA has summarized additional atrazine toxicity data for salt-water phytoplankton. This information is available in the U.S EPA Ambient Aquatic Life Water Quality Criteria for Atrazine Revised Draft (U.S. EPA, 2003). Short-term (two and three day) growth tests with phytoplankton resulted in EC50 values ranging from 79 to 265 µg/L (Mayer, 1987; Walsh, 1983). EC50 values based on differing endpoints (e.g., oxygen evolution or growth) for various green algal species ranged from 37 µg/L to 600 µg/L (Gaggi et al. 1995; Hollister and Walsh, 1973; Walsh, 1972; U.S. EPA, 2003). Atrazine effects on phytoplankton doubling time were demonstrated in 7-d exposures of *Nannochloris oculata* and *Phaeodactylum tricornutum* at concentrations of 15 µg/L and 50 µg/L, respectively (Mayasich et al., 1986). Plumley and Davis (1980a) observed reduced photosynthesis in *Nitzschia sigma* and reduced chlorophyll in *Thalassiosira fluviatilis* in 7-day exposures to 220 µg/L atrazine.

Effects of atrazine on phytoplankton communities

Atrazine exposure to naturally derived estuarine microbial communities in simulated tidal creek mesocosms affected productivity, biomass and taxonomic composition. Atrazine was found to reduce chlorophyll *a*, phototrophic carbon assimilation and phototrophic biovolume in 40 and 160 µg/L treatments (DeLorenzo et al., 1999a). Significant reductions in phototrophic variables occurred within 24 hours of pesticide exposure and no recovery was detected after nine

days. Atrazine induced changes in the composition of the algal community. Most taxa were reduced in abundance by atrazine exposure; however, cyanobacterial taxa generally became more abundant. Bacterial abundance was significantly elevated in the 160 µg/L treatment 24-48 hours after dosing, but densities had returned to levels similar to the control after nine days (DeLorenzo et al., 1999a). There was no significant effect on small ciliates, however, large ciliates and small flagellates increased significantly in number after 48 hours and remained elevated after nine days. Atrazine at 160 µg/L significantly reduced large flagellate density throughout the experiment (DeLorenzo et al., 1999a). DeLorenzo et al. (1999b) exposed naturally derived estuarine microbial communities to atrazine and its primary degradation product, deethylatrazine in laboratory treatments. Chlorophyll *a*, phototrophic carbon assimilation, dissolved oxygen, and phototrophic biovolume were significantly reduced at concentrations of 50 and 250 µg/L atrazine and deethylatrazine. Cryptophyte and diatom abundance were reduced, whereas blue-green algae increased in abundance (DeLorenzo et al., 1999b).

Similar assessments using microbial communities collected from South Florida found that atrazine exposures of 20 µg/L and 200 µg/L significantly decreased the relative abundance of chlorophytes and chrysophytes and increased the number of diatom and heterotrophic protist taxa (Downing et al., 2004). Regardless of site, season or exposure time, the highest atrazine dose (200 µg/L) significantly reduced chlorophyll *a*, phototrophic carbon assimilation and bacterial biomass, but stimulated heterotrophic bacterial productivity (Downing et al., 2004).

Another estuarine mesocosm study was designed to examine changes in the microalgal community as well as addressing the effects of a long-term atrazine exposure followed by an acute pulsed exposure (Pennington, 2002). Mesocosms were dosed to maintain a long-term,

low-level exposure of approximately 5 µg/L atrazine (measured concentration after tidal mixing) during the first six weeks of the study. During week seven, treatment mesocosms were dosed at approximately 60 µg/L atrazine (measured concentration after tidal mixing). Time-weighted averages for each treatment were calculated based on the daily measured atrazine concentrations during the 7 week test so that peak values did not skew the long-term average concentrations. The microalgal community EC50 for cell density was estimated at 11.1 µg/L (95% C.I. 9.8 - 11.9 µg/L) for the entire testing period (Pennington, 2002). The exposure scenario used in this study indicated that adverse affects on estuarine microalgal communities might occur at atrazine concentrations below the proposed water quality criteria (17 µg/L).

In summary, community level atrazine experiments generally indicated that atrazine inhibition of photosynthesis resulted in decreased phytoplankton abundance, productivity and species diversity, usually followed by increased bacterial abundance and productivity. These results suggest that exposure of the estuarine microbial food web to atrazine may lead to both functional and structural changes, which could impact higher trophic levels. For example, in an estuarine mesocosm exposure with juvenile *Mercenaria mercenaria*, clams showed significant weight reductions as a result of atrazine exposure (Lawton, 2001). This was determined to be an indirect effect of significantly reduced phytoplankton abundance in the atrazine exposures. Using the time-weighted atrazine concentrations from the chronic (7-week) exposure, an EC50 (growth) value for % change in clam dry weight was calculated to be 11.9 µg/L (95% C.I. 6.6 - 12.2 µg/L).

Effects of atrazine in mixture

It is common for multiple pesticides to be detected in environmental samples. Their combination may elicit additional toxicity to aquatic organisms. A few studies have examined the toxicity of atrazine and other pesticides in mixture to estuarine phytoplankton species. Many

herbicides tend to be additive in toxicity when mixed with atrazine (e.g. irgarol, DeLorenzo and Serrano, 2006; a mixture of 18 triazine herbicides, Faust et al., 2001). The toxicity of atrazine in mixture with the pyrethroid insecticide bifenthrin was also reported to be additive (Hoagland et al., 1993). Some other pesticides, however, have been found to be more toxic in mixture (synergistic) with atrazine than when tested individually. The toxicity of atrazine in mixture with chlorothalonil (a commonly used fungicide) was approximately 2 times greater than that of the individual chemicals (DeLorenzo and Serrano, 2003). The combination of atrazine and another commonly used herbicide, alachlor, was also slightly more toxic than the individual compound to the marine phytoplankton species, *Pavlova* sp. (Pennington, 1996). The organophosphate insecticide chlorpyrifos has been reported to exhibit synergistic toxicity in mixture with atrazine (Belden and Lydy, 2000; Belden and Lydy, 2001). Atrazine (200 μg/L) increased the toxicity of chlorpyrifos to midge larvae (*Chironomus tentans*) by a factor of 4 (Belden and Lydy, 2000), with lower levels (40 μg/L) also causing a significant increase in toxicity. Researchers have suggested that the joint action may be due to the induction of cytochrome dependent monooxygenases within the organism, resulting in an increase in the amount of organophosphate activated to the oxygen analog, a more potent acetylcholinesterase inhibitor (Belden and Lydy, 2000). Key et al. (2004) tested a mixture of two insecticides and atrazine with larval grass shrimp, *Palaemonetes pugio*. Fipronil and imidacloprid were not more toxic in mixture, but the addition of non-toxic atrazine levels (26.7 μg/L) increased toxicity of the mixture to grass shrimp larvae. The mixture was 1.2 times more toxic than either of the individual compounds tested alone. Therefore, decreases in estuarine populations resulting from pesticide exposure could occur at lower than expected concentrations in aquatic systems where atrazine and other pesticides are present in mixture.

Effects of atrazine degradation products

Relatively little is known regarding the effects of atrazine's degradation products. Hydroxyatrazine is relatively polar and has the potential to bind tightly to soil organic carbon, becoming unavailable and nontoxic due to the loss of chlorine at the two-position (Houout et al., 1998). The dealkylation products deethylatrazine and deisopropylatrazine, on the other hand, retain the chlorine atom and are considered to be phytotoxic. Two studies indicated that deethylatrazine, atrazine's primary degradation product, is almost as toxic to microalgae as atrazine (DeLorenzo et al., 1999; Winkelmann and Klaine, 1991). Kotrikla et al. (1997) examined toxicity of several atrazine metabolites on microalgal growth rate. They showed that the removal of the propyl group from atrazine results in a 3.5 times less toxic derivative than the removal of the ethyl group (Kotrikla et al., 1997).

Effects of repeated exposure to atrazine

It has been suggested that given limited exposure and sufficient time between exposures, aquatic communities may not be at risk from low levels of atrazine (<20 μg/L) (Huber, 1993; Solomon *et al*., 1996). Several studies have shown evidence of recovery after short-term exposure to low levels of atrazine (Rocchio and Malanchuk, 1986; Larsen *et al*., 1986; DeNoyelles *et al*., 1982). Organisms may exhibit "recovery" after acute or chronic exposure but subsequent pulses (acute dose) may be more toxic. Growth of the marine phytoplankton species, *Pavlova* sp., was not significantly affected by chronic exposure to atrazine (20 μg/L), even over multiple generations. However, acute sensitivity to the herbicide increased (96 h growth rate EC_{50} decreased from 147 μg/L to 96 μg/L) with subsequent acute exposure (Pennington and Scott, 2001). Nelson et al. (1999) found a similar phenomenon in freshwater benthic diatoms at a chronic exposure level of 1 μg/L. Hamala and Kollig (1985) found little recovery in

phytoplankton community structure 7 days after a chronic 14 d atrazine exposure at 100 µg/L. In an estuarine mesocosm exposure, microalgal communities did not recover from a chronic (6 week) low-level (approximately 5 µg/L) atrazine exposure during the 14-day recovery period provided (Pennington, 2002). Furthermore, microalgal communities that received the chronic low-level atrazine exposure were significantly more sensitive to a subsequent high-level (approximately 200 µg/L) atrazine exposure than communities without chronic exposure (Pennington, 2002).

Atrazine effects on marine macrophytes

Two species of estuarine submerged vascular plants, *Potamogeton perfoliatus* and *Myriophyllum spicatum*, exposed for 28-35 days to atrazine at various salinities, had 50% inhibition concentrations (IC50 values) for final biomass and photosynthesis between 25 and 117 µg/L, with the biomass endpoint being more sensitive in both species (U.S. EPA, 2003; Hall et al. 1997). The wild celery, *Vallisneria americana,* exposed to atrazine for 42 days had EC50 values of 6.19 µg/L for leaf area (Correll and Wu, 1982) and 178.9 µg/L for dry weight (Forney and Davis, 1981). Four separate 21-day exposures of the seagrass, *Zostera marina*, resulted in LC50 values ranging from 100 to 540 µg/L (Delistraty and Hershner, 1984). Other studies with *Z. marina* reported reduced oxygen evolution at 100 µg/L, and complete inhibition of photosynthesis and growth at 1,000 µg/L and 1,900 µg/L of atrazine (U.S. EPA, 2003).

The red alga, *Porphyridium cruentum*, had an EC50 based on oxygen evolution of 79 µg/L when exposed for 90 minutes (Hollister and Walsh, 1973), and the kelp, *Laminaria hyperborea*, had a 24-hour LOEC value for respiration of >1,000 µg/L (Hopkins and Kain, 1971). The 28-day LOEC for this species based on growth of new sporophytes was 10 µg/L (Hopkin and Kain 1978). With another species of kelp, *L. saccharina*, a 2-day exposure to 72.2

µg/L of atrazine significantly reduced sexual reproduction, while no effect was detected at 33.2 µg/L (U.S. EPA, 2003).

Walsh et al. (1982) reported a 40-hour EC50 of 320 µg/L for the turtlegrass, *Thalassia testudinum*. The emergent salt-marsh rush, *Juncus roemerianus*, exhibited effects indicative of stress after a 35-day exposure to 30 µg/L, while the salt-marsh grass, *Spartina alterniflora*, only exhibited enhanced peroxidase activity at a concentration as high as 3,100 µg/L for the same length of time (Lytle and Lytle 1998). Photosynthesis IC50 values ranged from 77 to 120 µg/L for vascular plants (*Potamogeton perfoliatus, Zannichellia palustris, Ruppia maritime, Myriophyllum spicatum*, respectively) in short-term (2- to 4-hour) exposures to atrazine (Jones and Winchell 1984).

The U.S. EPA 2003 revised chronic water quality criteria for atrazine in saltwater (17 µg/L) is based on the Final Plant Value (FPV) determined for *Potamogeton pectinatus* (the Sago pondweed). The FPV was calculated as the geometric mean of the three (Sago pondweed) chronic values determined by Hall et al. (1997) at different salinities.

Atrazine effects on marine animals

The acute toxicity of atrazine to saltwater animals has been determined with several species of invertebrates and two species of fish. For larval sheepshead minnow (*Cyprinodon variegatus*), LC50 values were 16,200, 2,300 and 2,000 µg/L at salinities of 5, 15 and 25 g/kg, respectively (Hall et al. 1994a,b). Two other LC50 values of 13,000 and >16,000 µg/L for sheepshead minnow were derived from the flow-through concentration measured test by Machado (1994b, as cited in U.S. EPA, 2003) and Ward and Ballantine (1985). Ward and Ballantine (1985) found that juvenile survival was significantly reduced an early life-stage test (28-

day) at 3,400 µg/L. All fish exposed to 5,700 µg/L died. There was no effect on either hatching success or growth in any of the concentrations with surviving fish (<5,700 µg/L). A 28d chronic value for sheepshead minnows, based on mortality of juveniles, was 2,542 µg/L (U.S. EPA, 2003). The 48-hour LC50 determined for the juvenile spot, *Leiostomas santhurus*, was >1,000 µg/L (Butler 1964; Mayer 1987).

The marine copepod, *Acartia tonsa*, had 24h, 48h, and 96h LC50 values of 15,000, 8,100, and 6,100 µg/L, respectively (U.S. EPA, 2003). The effect of salinity on atrazine toxicity was tested with the copepod, *Eurytemora affinis*, with LC50 values of 500, 2,600, and 13,200 µg/L at salinities of 5, 15, and 25 g/kg, respectively (Hall et al., 1994a,b). Eight-day chronic tests with *E. affinis* were also performed at salinity levels of 5, 15 and 25 g/kg, yielding LC50 values of 14,640, 20,920, and 5,020 µg/L, respectively (Hall et al. 1995). Only at the highest salinity level was the acute value greater than the chronic value, thus an acute to chronic ratio of 2.6 was calculated using the values from the 15 ‰ salinity exposure (U.S. EPA, 2003).

Sublethal testing with various copepod species has revealed greater sensitivity to atrazine. For example, a 10-day chronic atrazine exposure with the estuarine copepod *Eurytemora affinis* resulted in delayed development at concentrations of 25 µg/L (Forget-Leray et al. 2005). Bejarano and Chandler (2003) demonstrated multigenerational effects of atrazine on reproduction in the estuarine meiobenthic copepod (*Amphiascus tenuiremis*). Although atrazine did not affect copepod survival or development rate, atrazine concentrations as low as 2.5 µg/L caused an increase in reproductive failure and reduced offspring production in the F1 generation. Another study characterized the effects of atrazine on the abundance and composition of indigenous meiobenthic copepod and nematode assemblages in a 28 day estuarine mesocosm exposure (Bejarano et al., 2005). Copepod densities were reduced approximately 70% in the

atrazine treatment of 26 µg/L, compared to control densities. Nematode densities were not significantly affected, but the nematode to copepod ratio was significantly higher in the atrazine treatment.

Survival was the most sensitive endpoint in the mysid shrimp test (Ward and Ballantine 1985). Survival was 60, 30, and 20 percent at 190, 290, and 470 µg/L atrazine, respectively. Shrimp reproduction was completely inhibited at 470 µg/L, but adverse effects on reproduction were not observed at any lower concentrations. Atrazine was also tested with grass shrimp (*Palaemonetes pugio*). Eisler (1989) reported a 96-hour LC50 of 9,000 µg/L for adult *P. pugio*. No mortality was observed in larval *P. pugio* exposed to atrazine concentrations as high as 10,000 µg/L (Key et al., unpublished data). The brown shrimp, *Penaeus aztecus*, had a 48-hour EC50 of 1,000 µg/L (Butler 1964; Mayer 1987).

A 96-hour growth rate EC50 determined for the juvenile Eastern oyster, *Crassostrea virginica*, was greater than 1,000 µg/L (Butler, 1964; Mayer, 1987). The effects of atrazine on the juvenile (~1000 µm) hardshell clam, *Mercenaria mercenaria*, were evaluated in aqueous and sediment laboratory assays (Lawton, in press). An acute aqueous 96-hour LC50 of 5608 µg/L was determined. An aqueous chronic (10-day) bioassay indicated sublethal (reduced shell growth) effects (NOEC of 500 µg/L, LOEC of 1000 µg/L, and MATC of 707 µg/L). There were no significant effects of atrazine in a chronic (10-day) sediment exposure (Lawton, in press). Downs et al. (2001) conducted a study using molecular biomarkers to assess the effect of atrazine on the marine mud snail *Ilyanassa obsoleta*. Only one of the various biomarkers tested, heat shock protein (Hsp26), responded significantly with elevated levels under atrazine exposure.

Adult fiddler crabs, *Uca pugnax*, were not very sensitive to one-time applications of atrazine either in field or laboratory exposures (Plumley et al. 1980b). It was noted that animals

collected in the summer were more sensitive to atrazine than those collected in either the Spring or Fall. A 96-hour LC50 for *U. pugnax* of >29,000 µg/L was reported by Eisler (1989). Two other species of crabs, *Sesarma cinereum* and *Panopeus* sp., were also insensitive to very high levels of atrazine (Plumley et al. 1980b). The mud crab, *Neopanope texana*, had a 96-hour LC50 of 1,000,000 µg/L (Eisler, 1989).

A summary of the toxicity data available for atrazine and estuarine organisms is provided in Table 3. Photosynthetic plants and algae are the most sensitive taxa, followed by copepods and mysid shrimp. Based on the available data, bivalves, fish and crabs are not sensitive to atrazine at concentrations < 1,000 µg/L.

Conclusions

In systems chronically contaminated by atrazine, there is potential for altered algal community composition, production, and biomass (Guanzon et al., 1996). Because of its potential environmental impacts and health hazards, atrazine has been completely banned in a number of European countries, including Switzerland and Denmark (Muller et al., 1997).

Monitoring data indicate that atrazine is chronically present in U.S. coastal waters at relatively low concentrations. The concentrations detected have typically been below acute biological effects levels, and below the U.S. EPA proposed water quality criteria for atrazine. However, given the widespread use and relative persistence of this herbicide in coastal ecosystems, there is still reason to consider possible impacts to coastal resources. An example of a coastal resource that could potentially be adversely affected by atrazine is shellfish harvesting. Chronic atrazine contamination in estuarine waters could potentially impair shellfish production,

based on the community level study by Lawton (2001). In addition, uncertainty remains regarding the effects of long-term low levels of atrazine in mixture with other contaminants.

Increasing vegetative buffer zones between areas of atrazine application (agricultural fields, residential lawns, golf courses) and estuarine and marine waters, would limit direct runoff of atrazine into these sensitive ecosystems (Syversen and Bechmann, 2004). Other potential mitigation actions include increasing public awareness about nonpoint source runoff, implementing alternatives to pesticide application, and educating homeowners about proper pesticide application.

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Table 1. Atrazine coastal monitoring data (NA=data not available).

Location	Study Description	Concentration range (µg/L)	Mean/Median concentration (µg/L)	# of samples	% prevalence of detection	Reference
Chesapeake Bay	NA	0.09-46.0	NA	NA	NA	Huber 1993
Chesapeake Bay	NA	NA	<10 (mean)	NA	NA	Pait et al. 1992
Chesapeake Bay	Rhodes River	0.006-0.190	NA	NA	NA	Wu 1981
Chesapeake Bay	Wye River	<3-300	NA	NA	NA	Glotfelty et al. 1984
Maryland	River	0.1-30	NA	NA	NA	DeNoyelles et al. 1982
Gulf of Mexico	NOAA Sustainable Seas Expedition	<0.025-0.083	NA	42	2.3%	Pennington (unpub.)
Mid-Texas Coast	1993	<0.01-62.5	3.96	136	95.6%	Pennington et al. 2001
North Inlet, SC	0.5 m subsurface and microlayer	0.001-0.104	0.026 (mean)	7	100%	Kucklick & Bidleman, 1994a
SC Coast	2001-2003, estuaries, coastal ponds, canals, and wells	<0.025-15.8	0.069	1246	12.3%	Pennington (unpub.)
Winyah Bay, SC	0.5 m subsurface and microlayer	0.005-0.848	0.286 (mean)	39	100%	Kucklick & Bidleman, 1994a
Mississippi River	USGS survey	NA	>3 (median)	NA	100% (April-June)	Thurman et al. 1994
USA	Sites all over USA (Freshwater and Marine)	0-120	0.026 (median)	NA	NA	USGS 2001
USA	Sites all over USA (Freshwater and Marine)	0-4.2	NA	NA	NA	USGS 2001

Table 2. Comparison of 96h growth rate-EC_{50} and 95% confidence interval (CI) values (µg/L) determined for brackish-marine algal species exposed to the herbicide atrazine (salinity 20 ppt).

Species	**Taxonomic Division (common name)**	**96h EC_{50} value (95% CI)**	**Reference**
Heterosigma akashiwo	Raphidophyte (brown)	26 (16.7-28.8)	DeLorenzo et al, in prep.
Ankistrodesmus sp.	Chlorophyte (green)	37.87 (34.21-48.24)	DeLorenzo et al., 2004
Synechococcus sp.	Cyanophyte (blue-green)	46.46 (33.03-45.68)	Weiner et al., 2004
Storeatula major	Prymnesiophyte (golden-brown)	49.16 (37.82-68.09)	DeLorenzo et al., 2004
Dunaliella tertiolecta	Chlorophyte (green)	69.44 (66.44-71.99)	DeLorenzo et al., 2004
Phaeodactylum tricornutum	Bascillariophyte (diatom)	69.44 (49.65-69.10)	Weiner et al., 2004
Amphidinium operculatum	Dinophyte (dinoflagellate)	74.18 (67.88-80.87)	DeLorenzo et al., 2004
Isochrysis gabana	Prymnesiophyte (golden-brown)	91.1 (75.26-100.9)	Weiner et al., 2004
Fibrocapsa japonica	Raphidophyte (brown)	109 (55.8-124.6)*	DeLorenzo et al., in prep.
Chatonella subsalsa	Raphidophyte (brown)	145 (111.5-179.2)*	DeLorenzo et al., in prep.
Pavlova sp.	Prymnesiophyte (golden-brown)	147 (116.4-178.7)	Pennington et al., 2001

Table 3. Summary of toxicity values (µg/L) determined for estuarine taxa exposed to the herbicide atrazine.

Taxa	Lethal endpoint reported	Range of values	Sublethal endpoint reported	Range of values
phytoplankton	none	none	2-7 d growth rate EC50	15 - 600 µg/L
macrophytes	21 d LC50	100 - 1,900 µg/L	28 - 35 d biomass IC50 reproduction	25 - 117 µg/L 10 - 72.2 µg/L
copepods	24 - 72h LC50	94 - 13,200 µg/L	F_1 population growth LOEC	2.5 µg/L
shrimp	24 - 96h LC50	~190 - >10,000 µg/L	reproduction LOEC	470 µg/L
bivalves	96h LC50	5,608 µg/L	96h EC50 / 10d shell growth	1,000 µg/L
fish	48 - 96h LC50	1,000 - 16,200µg/L	hatching success / growth	>5,700 µg/L
crabs	96h LC50	29,000 - 1,000,000 µg/L	none	none

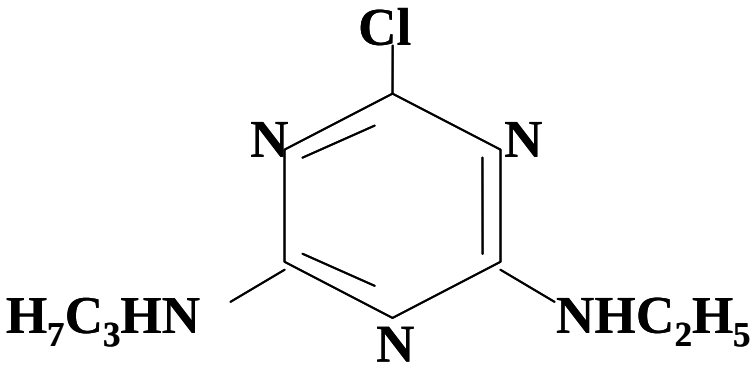

Figure 1. Molecular structure of the s-triazine herbicide, atrazine

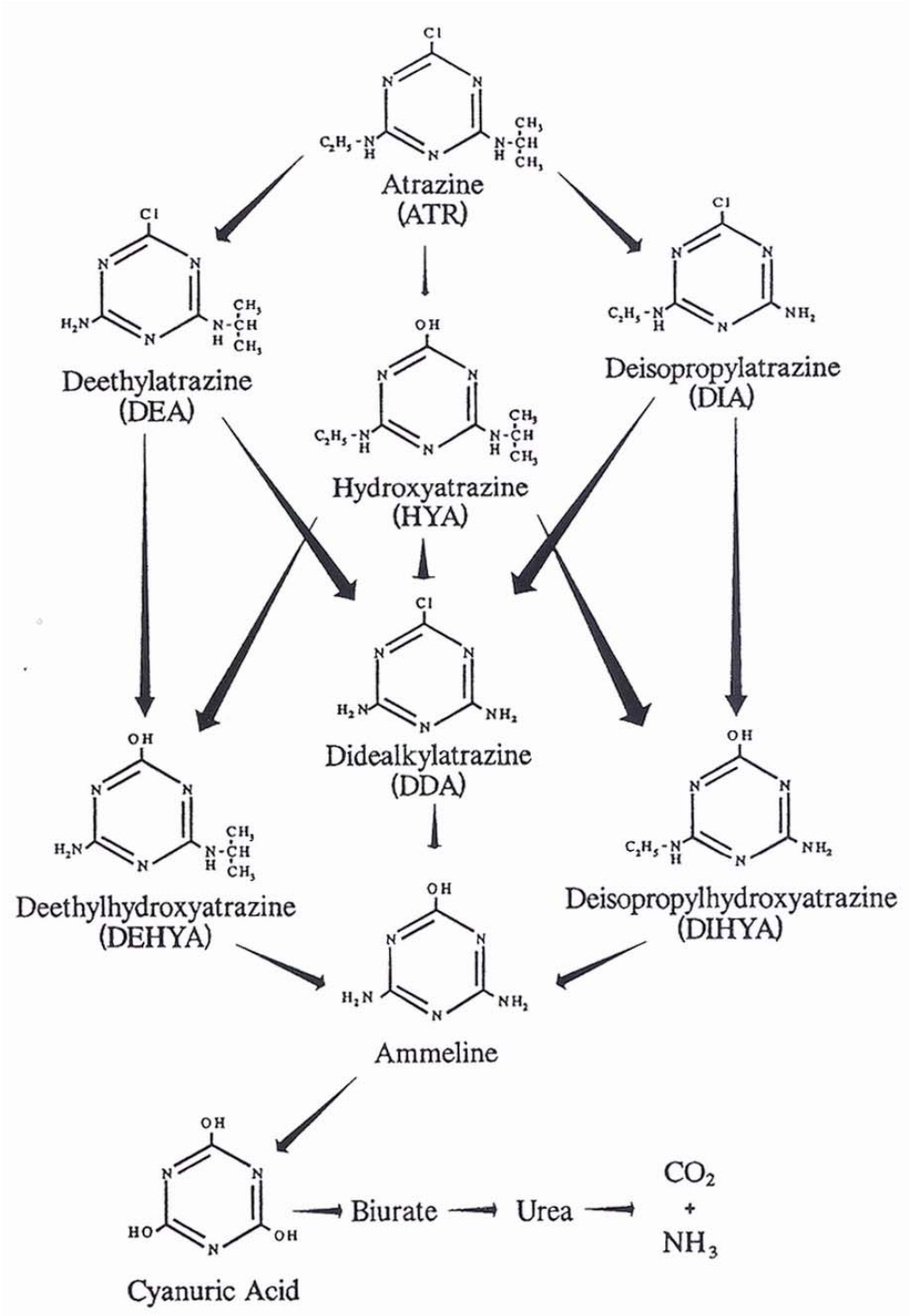

Figure 2. Degradation pathway of atrazine in soil (Kruger et al., 1993).

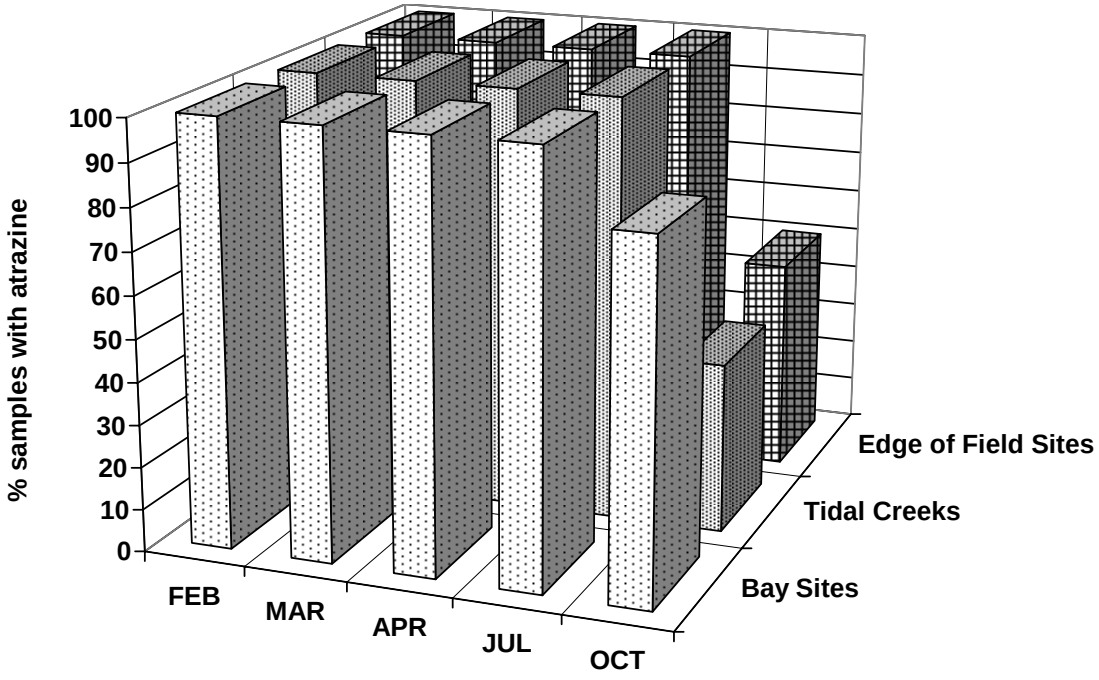

Figure 3. Percent of samples (N=136) with detectable concentrations of atrazine by station type along the mid-Texas coastline, February - October 1993 (Pennington et al., 2001).

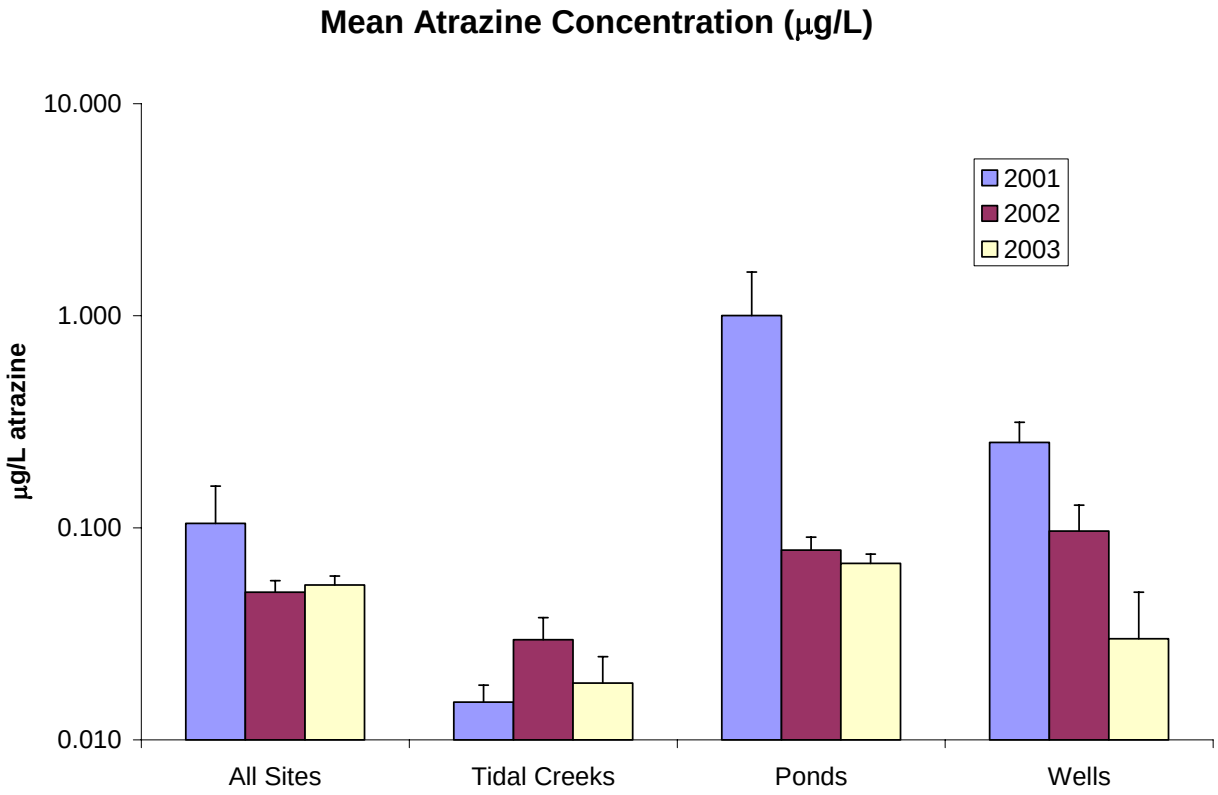

Figure 4. Mean atrazine concentrations by year from stations along coastal South Carolina (sample values less than detection limit were averaged as zeros; total N = 1,248; tidal creeks N = 746, ponds N = 456, wells N = 46).

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