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AMBIENT AQUATIC LIFE WATER QUALITY CRITERIA FOR ATRAZINE - REVISED DRAFT

# DRAFT

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# ATRAZINE - REVISED DRAFT

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# DRAFT

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U.S. ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF WATER OFFICE OF SCIENCE AND TECHNOLOGY HEALTH AND ECOLOGICAL CRITERIA DIVISION WASHINGTON D.C.

## NOTICES

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# DRAFT

### FOREWORD

Section 304(a)(1) of the Clean Water Act of 1977 (P.L. 95-217) requires the Administrator of the Environmental Protection Agency to publish water quality criteria that accurately reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare that might be expected from the presence of pollutants in any body of water, including ground water. This document is a revision of draft criteria published in 2001 based upon consideration of scientific input received from the public and new information. Criteria contained in this document replace any previously published EPA aquatic life criteria for the same pollutant.

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304(a)(1) and section 303(c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. Criteria presented in this document are such scientific assessments. If water quality criteria associated with specific stream uses are adopted by a state as water quality standards under section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that state. Water quality criteria adopted in state water quality standards could have the same numerical values or method resulting in a numerical value as criteria developed under section 304. However, in many situations states might want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as part of state water quality standards that criteria become regulatory. Guidelines to assist the states and Indian tribes in modifying the criteria presented in this document are contained in the Water Quality Standards Handbook (U.S. EPA, 1994). This handbook and additional guidance on the development of water quality standards and other waterrelated programs of this Agency have been developed by the Office of Water.

This draft document is guidance only. It does not establish or affect legal rights or obligations. It does not establish a binding norm and cannot be finally determinative of the issues addressed. Agency decisions in any particular situation will be made by applying the Clean Water Act and EPA regulations on the basis of specific facts presented and scientific information then available.

> Geoffrey H. Grubbs Director Office of Science and Technology

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## **EXECUTIVE SUMMARY**

## **Background:**

Atrazine is the most extensively used herbicide in the United Sates for control of weeds in agricultural crops and is toxic to aquatic organisms. EPA has developed ambient water quality criteria for atrazine for the protection of aquatic life through its authority under section 304(a) of the Clean Water Act (CWA). These water quality criteria are guidance for Sates and Tribes and in themselves have no binding legal effect. The criteria may for the basis for State and Tribal water quality standards and in turn become enforceable through National Pollutant Discharge Elimination System (NPDES) permits or other environmental programs.

## **Freshwater Criteria:**

For atrazine the criterion to protect freshwater aquatic life freshwater aquatic life and their uses is an Average Primary Producer Steinhaus Similarity deviation for a site less than 5% (as determined using CASM or other appropriate model and index) not exceeded more than once every three years on the average (or other appropriate return frequency sufficient to allow system recovery) and a one-hour average concentration that does not exceed 1,500 ug/L more than once every three years on the average. The 5% index for the protection of aquatic plant community should also be protective of most freshwater animals.

## Saltwater Criteria:

For atrazine, the criterion to protect saltwater aquatic life from chronic toxic effects is 17 ug/L. This criterion is implemented as a thirty-day average, not to be exceeded more than once every three years on the average. The criterion to protect saltwater aquatic life from acute toxic effects is 760 ug/L. This criterion is implemented as a one-hour average, not to be exceeded more than once every three years on the average.

The criteria for atrazine were developed by the EPA Office of water (OW) using a large aquatic toxicity data base and extensive mesocosm and mesocosm data. Adverse effect of atrazine on survival, growth, and reproduction of aquatic organisms and on plant community structure were demonstrated in numerous laboratory and field studies.

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This document provides guidance to States and Tribes authorized to establish water quality standards under the Clean Water Act (CWA) to protect aquatic life from acute and chronic effects of atrazine. Under the CWA, States and Tribes are to establish water quality criteria to protect designated uses. While this document constitutes U.S. EPA's scientific recommendations regarding ambient concentrations of atrazine, this document does not substitute for the CWA or U.S. EPA's regulations; nor is it a regulation itself. Thus, it cannot impose legally binding requirements on U.S. EPA, States, Tribes, or the regulated community, and it might not apply to a particular situation based upon the circumstances. State and Tribal decision-makers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance when appropriate. U.S. EPA may change this guidance in the future.

# DRAFT

## **INTRODUCTION1**

Atrazine is a herbicide with the empirical formula  $C_8H_{14}Cl_5N_5$  and a molecular weight of 215.7. It is a white, crystalline solid with a melting point of 173-175°C, a boiling point of 279°C, and solubility in water of 33 mg/L at 25°C (Farm Chemicals Handbook 2000; Hunter et al. 1985). Atrazine has an <u>n</u>-octanol-water partition coefficient (log P) of 2.82, a vapor pressure of 7.34 x 10<sup>-4</sup> mm Hg, a Henry's Constant of 8.32 x 10<sup>-6</sup> atm<sup>3</sup>/M, and a hydrolysis half-life in excess of 1,000 days (Hunter et al. 1985). These physico-chemical properties contribute to its environmental partitioning and degree of persistence in the aquatic environment.

Atrazine is used extensively in the United States, Canada and other countries for the control of weeds in agricultural crops, especially in crops such as corn, sorghum, wheat and soybeans. It is one of the most heavily used pesticides in North America, generally being among the top few in terms of total pounds of herbicide used (Braden et al. 1989; Burridge and Haya 1988; Ciba-Geigy 1994; Council on Environmental Quality 1984; Moxley 1989; Pike 1985; Richards and Baker 1993). Annual domestic usage during the past two decades has been in the general range of 30 to 40 million kilograms applied to approximately 70 million acres of farm land in the U.S. (U.S. EPA 2000). It is also commonly used in other countries (Bester and Huhnerfuss 1993; Bester et al. 1995; Caux and Kent 1995; Galassi et al. 1992, 1993; Lode et al. 1994). Atrazine is also used in combination with other herbicides including alachlor, ametryne, linuron, paraquat, propachlor, amitrole, and cyanazine (Farm Chemicals Handbook 2000).

With this magnitude of application, atrazine has commonly been detected in surface waters of agricultural watersheds where it has been used. Due to its relative mobility from soil, atrazine surface water concentrations are highest in field runoff, with concentration peaks generally following early major storm events that occur within a few weeks of application (Glotfelty et al. 1984; Muir et al. 1978; Triplett et al. 1978; Wauchope 1978; Wauchope and Leonard 1980). Concentrations in the low mg/L range may be encountered in edge-of-field run-off (Hall et al. 1972; Kadoum and Mock 1978; Klaine et al. 1988; Roberts et al. 1979). Field run-off is diluted upon entering a stream or lake, resulting in atrazine concentrations that are generally much lower (e.g., 1-10  $\mu$ g/L range) in such waters (Frank and Sirons 1979; Frank et al. 1979; Richards and Baker 1993; Richard et al. 1975; Roberts et al. 1979; Wu 1981). Only trace levels (i.e., <1.0-33 ng/L) were reported in a pesticide monitoring study in California

<sup>&</sup>lt;sup>1</sup>A comprehension of the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephen et al. 1985), hereafter referred to as the Guidelines, is necessary to understand the following text, tables and calculations.

(Pereira et al. 1996). However, individual maximum concentrations may be considerably higher. Elevated levels of atrazine  $2 \mu g/L$  have been documented by Frenzel et al. (1998) in the Platte River of Nebraska for greater than 60 days, and  $5 \mu g/L$  for greater than 30 days. When considered over several years, maximum concentrations reported in some creeks and rivers from midwestern agricultural areas have ranged from 5 to 70  $\mu g/L$  (Ciba-Geigy 1992a,b,c,d, 1994; Frank and Sirons 1979; Frank et al. 1979, 1982; Illinois State Water Survey 1990; Muir et al. 1978; Richards and Baker 1993; Roberts et al. 1979). Factors that strongly and positively correlate with the release of atrazine from soil include sediment organic carbon, landscape position, and tillage (Novak 1999).

Surface waters surrounded by agricultural lands may receive several pulsed doses over the growing season corresponding to rainfall events (Herman et al. 1986). Annual patterns of atrazine concentrations in Ohio streams show peak time-weighted mean concentrations of about 6  $\mu$ g/L in early June, with a rapid increase from April to June, followed by a rapid decrease from June to August (Richards and Baker 1993). Time-weighted mean concentrations between August and December are considerably lower, most frequently being less than 1.0  $\mu$ g/L. Atrazine concentrations are the lowest, and uniformly so, between January and April. Also, smaller streams were shown to have higher peak concentrations, but of shorter duration, than larger streams (Richards and Baker 1993). The annual cycle is similar in southwestern Ontario, but with the annual peak concentrations occurring at lower levels and several weeks later than in Ohio (Bodo 1991). Nonetheless, atrazine concentrations in Ontario have regularly exceeded 2  $\mu$ g/L, which is the Canadian water quality guideline for aquatic life protection (Trotter et al. 1990). Exceedances have similarly been reported in surface waters of Quebec (Caux and Kent 1995).

Among the highest surface water concentrations of atrazine are those in small reservoirs in southern Illinois. These are currently being intensively monitored (Tierney et al. 1994a). Maximum concentrations as high as 55  $\mu$ g/L have been reported from these reservoirs.

Similar seasonal trends in concentrations of atrazine to those in Ohio streams have been observed in streams in Illinois (Ciba-Geigy 1992a; Illinois State Water Survey 1990), in Iowa (Ciba-Geigy 1994), and in other midwestern states (Ciba-Geigy 1992c). In large rivers such as the Mississippi, Missouri and Ohio Rivers, peak concentrations have most commonly occurred in June, with mean levels of less than 5.0  $\mu$ g/L during the spring period (Ciba-Geigy 1992b). The maximum concentrations were generally between 2 and 8  $\mu$ g/L, with a single maximum as high as 17.25  $\mu$ g/L (Ciba-Geigy 1992b,c). Atrazine concentrations in the Mississippi River between Minneapolis, Minnesota and New Orleans, Louisiana from July to August, 1991 ranged from 0.054  $\mu$ g/L to 4.7  $\mu$ g/L (Pereira and Hostetler 1993).

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Atrazine residues in Illinois lakes tended to be lower than those in the streams (with less pronounced peak values), however, the lower concentrations were sustained for longer durations (Ciba-Geigy 1992a). It should be noted that the maximum observed atrazine concentration was less than 3.0  $\mu$ g/L at 61 percent of 42 sites monitored over 6 years between 1975 and 1988 (Ciba-Geigy 1992a).

Atrazine concentrations were considerably lower in Chesapeake Bay and its tributaries (Ciba-Geigy 1992e). Here, the maximum observed concentration in a tributary was 14.6  $\mu$ g/L, and only three out of 600 samples analyzed between 1976 and 1991 exceeded 3.0  $\mu$ g/L. The highest observed maxima in the Upper and Lower Chesapeake Bay were 1.7 and 0.38  $\mu$ g/L, respectively. Models for the Great Lakes suggest that concentrations should be quite low, not likely to exceed 0.13  $\mu$ g/L (Tierney et al. 1994b). Individual measurements from Lake Erie taken at Toledo, Ohio, have not exceeded 0.35  $\mu$ g/L, while concentrations measured from samples collected in Lake Michigan at Michigan City, Indiana, have been below 0.20  $\mu$ g/L (Ciba-Geigy 1992c).

In addition to field run-off, atrazine residues are also transported by volatilization into the atmosphere and subsequent deposition. Atrazine has been measured in fog (Glotfelty et al. 1987), and trace amounts have been shown to be transported by the wind (Elling et al. 1987). Atrazine was present year-round in rainwater samples in Maryland, with the highest concentration of  $2.2 \mu g/L$  occurring in May (Wu 1981).

Atrazine has been shown to be enriched at the microsurface layer of water (Wu 1981; Wu et al. 1980). This may be due to the presence of microsurface films which tend to concentrate certain chemicals. Wu (1981) suggested that atrazine enrichment in the microsurface layer was more likely a source of direct input rather than a result of atmospheric wet deposition, and that the main source of atrazine at the site studied in Maryland was agricultural runoff.

Studies of atrazine persistence in water have produced varying results. Huckins et al. (1986) reported the loss of atrazine from water within 4 days in a simulated prairie pond microcosm. In shallow artificial streams, a 50 percent loss of atrazine occurred in 3.2 days (Kosinski 1984; Moorhead and Kosinski 1986). Lay et al. (1984) reported an 82 percent loss in 5 days and a 95 percent loss in 55 days. The half-life of atrazine in wetland mesocosms was from 8 to 14 days (Detenbeck et al. 1996). The half-life of <sup>14</sup>C-labeled atrazine has been measured in estuarine water as 3 to 12 days, compared to 15 to 30 days in estuarine sediment and 330 to 385 days in agricultural soils (Jones et al. 1982; Kemp et al. 1982a).

These rapid losses in small artificial systems and in an estuarine environment are contrasted with reports of a 300-day half-life in a larger lake system (Yoo and Solomon 1981), surface water losses of only 33 percent in 120 days and 0 percent in 85 days in two separate 0.49 hectare pond

applications (Klaassen and Kadoum 1979), and a loss of only 40-50 percent in pond water over a period of more than 5 months (Gunkel 1983). In two months time, approximately 25-30 percent of individual 20 and 500  $\mu$ g/L atrazine applications to a 0.045 hectare Kansas pond had disappeared from the water (deNoyelles et al. 1982). Approximately 25 percent of the initial applications remained after 12 months. The half-life of atrazine was approximately 3 months in Tasmanian streams (Davies et al. 1994a).

The above information indicates that the persistence of atrazine in water is highly variable, dependent perhaps upon both the nature of the aquatic system into which it is introduced as well as the climatic conditions at the exposure site. For example, Comber (1999) determined that significant hydrolysis of atrazine occurs only at pH values of 4 or less, while photolysis was initiated only by wavelengths below 300 nm at higher pH (pH 6 to 8). Based on this author's experiments, the aquatic half-life of atrazine in sunny upland waters was predicted to be 6 days, but in low land rivers with higher pH (7 to 8.5), the half-life would be in the order of months rather than days. The opposite is true for groundwater where the half-life would be in the order of years due to exceedingly slow rates of hydrolysis.

Biodegradation is considered to be one of the most important processes governing the environmental fate of atrazine (Radosevich et al. 1996). Microbes isolated from aquatic ecosystems that are capable of degrading atrazine have been reported. Mirgain et al. (1993) isolated a *Pseudomonas putida/Xanthomonas maltophilia* pair with atrazine-degrading ability. Certain soil bacteria have also been shown to be capable of degrading atrazine both aerobically and anaerobically (Behki et al. 1993; Radosevich et al. 1995, 1996). Some soil fungi also can degrade atrazine (Donnelly et al. 1993). In a salt marsh environment, the incorporation of atrazine into the sediment appeared to be a prerequisite for its degradation (Meakins et al. 1995). Very little degradation occurred in the water column.

Seybold et al. (1999) recently examined the fate of atrazine (<sup>14</sup>C-labeled) from two undisturbed sediments over a 2-year period. The atrazine was released from the sediment into the water column primarily through diffusion from the pore water. The amount of atrazine released was affected by sediment type and temperature. More atrazine residue was released into the water column at 5°C than at 24°C. However, degradation of the atrazine in sediment was high; less than 2 percent of extractable atrazine and metabolites remained after 2 years. The authors concluded that the accumulation and later release of atrazine is greatest at cold water temperatures and in sediments with low adsorption capacity. Kruger et al. (1996) found that the mobilities of atrazine and its degradates were negatively correlated with soil organic matter content and positively correlated with sand content of Iowa soils.

The major atrazine degradate in aquatic systems is hydroxyatrazine (U.S. EPA 2000). Others include deethylatrazine, deisopropylatrazine, and diaminoatrazine. The degradation products of atrazine were found to be less toxic to algae (Stratton 1984) and submerged aquatic plants (Jones and Winchell 1984) then the parent compound. Equivalent studies of atrazine degradate toxicity to aquatic animals is sparse. Results from mammalian studies indicate that some atrazine degradates may be more toxic than parent compound (U.S. EPA 2000).

The mode of atrazine's toxic action toward plants is blockage of electron transport within the Hill reaction of photosystem II, thereby inhibiting photosynthesis (Moreland 1980). Vascular plants and algae are both affected by this mode of action. In this way, atrazine has the demonstrated capacity to reduce primary productivity in aquatic ecosystems (deNoyelles et al. 1982; Dewey 1986; Herman et al. 1986; Kosinski and Merkle 1984; Pratt et al. 1988). On the other hand, the mode of toxic action toward aquatic animals has not been documented, probably because atrazine is not considered acutely toxic to these species. Recent evidence implicates atrazine as an indirect endocrine disruptor (Dodson et al. 1999; Petit et al. 1997) that may act by stimulating the activity of the aromatase enzyme that converts testosterone to estrogen (Sanderson et al. 2000). The occurrence of abnormal gonadal development (including feminization and hermaphroditism) and reduced laryngeal muscle size in exposed *Xenopus laevis* males has been reported at levels ranging from 1 µg/L atrazine (Hayes et al. 2002) to approximately 20-21 µg/L atrazine (Carr et al. 2003; Carr and Solomon 2003; Renner 2002). Other investigators have demonstrated that atrazine causes induction of xenobiotic metabolizing systems (Miota et al. 1999), and enhances the toxicity of organophosphorous insecticides to aquatic invertebrates (Belden and Lydy 2000; Pape-Lindstrom and Lydy 1997).

Several reviews exist on atrazine and its environmental impact (CCREM 1989; deNoyelles et al. 1994; Eisler 1989; Huber 1993, 1994; Solomon et al. 1996). These reviews indicated that a few species of aquatic plants have been shown to be slightly affected by atrazine at concentrations below 10  $\mu$ g/L. The review by deNoyelles et al. (1994) stated that herbicides have little direct effects upon animals, and that they tend to produce ecosystem effects from the bottom of the food chain upward, in contrast to insecticides which act in the opposite direction. Huber (1993) and Solomon et al. (1996) stated that plants readily recovered from the inhibitory effects of atrazine once the exposure was reduced or eliminated.

A comprehension of the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985), hereafter referred to as the Guidelines, and the response to public comment concerning that document (U.S. EPA 1985) are necessary to understand the following text, tables, and calculations. Results of intermediate calculations such as recalculated LC50 values and Species Mean Acute Values are given to four significant figures to prevent roundoff error in subsequent calculations, not to reflect the precision of values. The criteria presented herein are the Agency's best estimate of maximum concentrations of the chemical of concern to protect most aquatic organisms or their uses from any unacceptable short- or long-term effects. Whenever adequately justified, a national criterion may be replaced by a site-specific criterion (U.S. EPA 1983a), which may include not only site-specific criterion concentrations (U.S. EPA 1983b), but also site-specific durations of averaging periods and site-specific frequencies of allowed excursions (U.S. EPA 1991). The latest comprehensive literature search for this document was conducted in November, 1999. Data in the files of the U.S. EPA's Office of Pesticide Programs concerning the effects of atrazine on aquatic organisms and their uses have been evaluated for use in the derivation of aquatic life criteria. Some more recent information received through the submission of public scientific views on the 2001 document and additional toxicity testing conducted since 2001 was also included.

## ACUTE TOXICITY TO FRESHWATER ANIMALS

The data that meet the requirements of the Guidelines concerning the acute toxicity of atrazine to freshwater organisms are available for 17 species (Table 1). Acute toxicity data for eight freshwater invertebrate species ranged from 3,000  $\mu$ g/L for the hydroid coelenterate, *Hydra* sp. (Brooke 1990) to 49,000  $\mu$ g/L for the cladoceran, *Daphnia magna* (Putt 1991). A stonefly (*Acroneuria* sp.) was the second most sensitive invertebrate tested, with an EC50 of 6,700  $\mu$ g/L (Brooke 1990). A cladoceran (*Ceriodaphnia dubia*) had a Species Mean Acute Value (SMAV) of >12,120  $\mu$ g/L (Jop 1991a; Oris et al. 1991), and the amphipod, *Hyalella azteca*, had an LC50 of 14,700  $\mu$ g/L (Brooke 1990). The remaining invertebrate species tested, the snails (*Physa acuta* and *Physa sp.*) and an annelid (*Lumbriculus variegatus*), had LC50 values in excess of 20,000, 34,100 and 37,100  $\mu$ g/L, respectively (Roses et al. 1999; Brooke 1990).

The rainbow trout (*Oncorhynchus mykiss*) was the most sensitive freshwater vertebrate species tested, with an LC50 of 5,300  $\mu$ g/L (Beliles and Scott 1965). The goldfish, *Carassius auratus*, was the most tolerant fish species and is11.32 times less sensitive to atrazine than rainbow trout (Table 1). The fathead minnow (*Pimephales promelas*) had a SMAV of 20,000  $\mu$ g/L (Dionne 1992), while the LC50 for the brown trout (*Salmo trutta*) was 27,000  $\mu$ g/L (Grande et al. 1994). The SMAVs for the remaining vertebrate species, all fishes, were 6,300, >10,000, >10,000, >13,856 and >18,000  $\mu$ g/L for

the brook trout, *Salvelinus fontinalis* (Macek et al. 1976); largemouth bass, *Micropterus salmoides* (Jones 1962); channel catfish, *Ictalurus punctatus* (Jones 1962); bluegill, *Lepomis macrochirus* (Beliles and Scott 1965; Macek et al. 1976); and coho salmon, *Oncorhynchus kisutch* (Lorz et al. 1979), respectively. The SMAV was based upon a flow-through test in the case of the fathead minnow, where other test results were also available.

Three species of amphibians were tested with atrazine (Table 1). The leopard frog (*Rana pipiens*), wood frog (*Rana sylvatica*) and American toad (*Bufo americanus*) each has a LC50 value of >20,000  $\mu$ g/L atrazine. Based on these values, the amphibians evaluated are relatively acutely insensitive to atrazine.

Freshwater Genus Mean Acute Values (GMAVs) were identical to the SMAVs in all cases with the exception of *Physa* and *Oncorhynchus*, where the two species tested had different SMAVs (Table 3). Two of the four most sensitive freshwater genera to atrazine are invertebrates. The freshwater Final Acute Value (FAV) for atrazine was calculated to be  $3,021 \mu g/L$  using the procedure described in the Guidelines and the GMAVs for invertebrates, fish and amphibians in Table 3. The freshwater FAV is lower than all available freshwater SMAVs except that for the Hydra, which it is less than one percent higher (Figure 1).

## ACUTE TOXICITY TO SALTWATER ANIMALS

The acute toxicity of atrazine to resident North American saltwater animals has been determined with eight species of invertebrates and two species of fish (Table 1). Although only two fish species were tested, fish appear to have a similar sensitivity to atrazine as invertebrates. The saltwater SMAVs range from 2,324  $\mu$ g/L for mysids, *Americamysis bahia* (formerly *Mysidopsis bahia*), to >30,000  $\mu$ g/L for the eastern oyster, *Crassostrea virginica*. The copepod, *Acartia tonsa*, had similar LC50 values resulting from a static unmeasured test (Ward and Ballantine 1985) and two renewal tests (Thursby et al. 1990) with measured values of 94, 91.73 and 210.1  $\mu$ g/L, respectively. An additional flow-through measured test (McNamara 1991a) with the same species yielded an LC50 of 4,300  $\mu$ g/L. It is unclear why there is such a large difference between the flow-through measured value and the other measured results. There was nothing unusual about the variability of the chemistry data from the flow-through tests to indicate a problem (coefficient of variations ranged from 2 to 15 percent). A possible explanation is that the measured values from the static renewal tests were conducted with 70 percent

technical grade atrazine, while the flow-through test used 97.1 percent atrazine. The other 30 percent may have contributed to the higher toxicity. Because there is no obvious problem with the flow-through data set for *A. tonsa*, the Guidelines state that the flow-through measured value must be used. Therefore, the SMAV for this species is 4,300  $\mu$ g/L. LC50 values for the copepod, *Eurytemora affinis*, were 500, 2,600 and 13,200  $\mu$ g/L at salinities of 5, 15 and 25 g/kg, respectively (Hall et al. 1994a,b). The resultant SMAV was 2,579  $\mu$ g/L. The opposite trend was observed for the sheepshead minnow; the LC50 values were 16,200, 2,300 and 2,000  $\mu$ g/L at salinities of 5, 15 and 25 g/kg, respectively, for larval fish (Hall et al. 1994a,b). Two other LC50 values of 13,000 and >16,000  $\mu$ g/L for sheepshead minnow was derived from the flow-through concentration measured test by Machado (1994b) and Ward and Ballantine (1985). However, because the former LC50 values were from a more sensitive life-stage, an SMAV of 4,208  $\mu$ g/L has been calculated for this species.

Saltwater GMAVs (Table 3) were identical to the SMAVs in all cases with the exception of *Acartia* where the two species tested had different SMAVs. Three of the four most sensitive saltwater genera to atrazine are crustaceans. The saltwater FAV for atrazine, 1,519  $\mu$ g/L, was calculated using the procedure described in the Guidelines and the GMAVs in Table 3. This saltwater FAV is lower than all available saltwater SMAVs (Figure 2).

## CHRONIC TOXICITY TO FRESHWATER ANIMALS

The data concerning the chronic toxicity of atrazine that are usable according to the Guidelines are available for 6 freshwater species (Table 2a). Eight freshwater tests have been completed with two invertebrate and four fish species.

The cladoceran, *Ceriodaphnia dubia*, was exposed to atrazine over its entire life cycle in two 7day tests (Oris et al. 1991). The end result was identical in both tests, with chronic limits of 2,500 and 5,000  $\mu$ g/L, and a calculated chronic value (geometric mean) of 3,536  $\mu$ g/L. An accompanying acute toxicity test resulted in an LC50 of >30,000  $\mu$ g/L (Oris et al. 1991). The resultant acute-chronic ratio was >8.484 (Table 2b).

In another 7-day life cycle exposure with *C. dubia* (Jop 1991b), atrazine did not affect survival at any of the test concentrations (i.e., 290, 600, 1,200, 2,500 or 4,900  $\mu$ g/L). However, reproduction was significantly reduced at the two highest treatment levels. An average of 10 young per female were produced at these two treatments compared to a mean of 23 for the pooled controls. The chronic limits in this study were 1,200 and 2,500  $\mu$ g/L, and the chronic value was 1,732  $\mu$ g/L. An accompanying

acute value of >4,900  $\mu$ g/L (Jop 1991a) resulted in an acute-chronic ratio of >2.829. Therefore, the species mean acute-chronic ratio is >4.899 (Table 3).

The midge, *Chironomus tentans*, was continuously exposed to atrazine for two generations in a life-cycle test (Macek et al. 1976). The test was initiated by exposing first generation eggs through the various larval instar stages, pupation and emergence. Eggs from first generation adults were then continuously exposed in a similar fashion. Mean measured concentrations were 110, 230, 420, 780 and 1,330  $\mu$ g/L. No significant differences between controls and the lowest exposure (110  $\mu$ g/L) were noted in hatchability, survival, pupation or emergence in first generation animals. Significant reductions in the number of adults emerging in the first generation exposure occurred at atrazine concentrations of 230 and 420  $\mu$ g/L. First generation larvae exposed to higher concentrations experienced high mortality at the early instar stages. In the second generation, hatchability was reduced at 420  $\mu$ g/L, while pupation and emergence were reduced at 230 and 420  $\mu$ g/L of atrazine. Exposure to 110  $\mu$ g/L had no effect on growth or development of the chironomid larvae. Based on these observations, the chronic limits were 110 and 230  $\mu$ g/L, and the resultant chronic value (geometric mean) was 159.1  $\mu$ g/L. A corresponding acute value of 720  $\mu$ g/L for a test that was fed (Macek et al. 1976) yielded an acute-chronic ratio of 4.525 for *C. tentans*.

Rainbow trout (*Oncorhynchus mykiss*) were exposed to atrazine in an early-life stage test (ELS) conducted in reconstituted water with a hardness of 50 mg/L as calcium carbonate (Whale et al. 1994). The ELS test was divided into 3 main stages: (I) immediately post-fertilization to hatching (30-day duration), (II) post-hatch to swim up (28-day duration), (III) post-swim up to 3 months old (28-day duration), for a total exposure of 86 days. Mean measured concentrations (mean  $\pm$  SD) were <10 (water control), <10 (solvent control),  $36 \pm 12$ ,  $130 \pm 50$ ,  $410 \pm 170$ ,  $1,100 \pm 660$ , and  $3,800 \pm 2,200 \mu g/L$ , respectively. Significant mortalities (58.8 percent) occurred in the highest atrazine exposure during stage I and II of the test although no other dose response relationships could be defined. Significant decrease in fish wet weight was observed in concentrations of 1,100 and 3,800  $\mu g/L$  compared to the solvent control, although fry exposed to 1,100  $\mu g/L$  did show signs of a recovery in wet weight toward the end of the stage III exposure. Statistical analysis of the dry weights of these same fish samples showed that a significant decrease in weight occurred only in fish exposed to 3,800  $\mu g/L$  atrazine. Because of the recovery in growth at the 1,100  $\mu g/L$  atrazine concentration, the chronic limits in this study were set at 1,100 and 3,800  $\mu g/L$ , resulting in a chronic value of 2,045  $\mu g/L$ . An accompanying acute value is not available for this species, therefore, an acute-chronic ratio cannot be calculated.

Yearling brook trout (*Salvelinus fontinalis*) and their offspring were continuously exposed to atrazine for 306 days at mean measured concentrations of 65, 120, 240, 450 and 720  $\mu$ g/L (Macek et al.

1976). At 90 days, significant reductions in weight and total length of first generation fish occurred at concentrations of 240  $\mu$ g/L and above. At 306 days, weight and total length of first generation fish were significantly less than controls at atrazine exposures of 120  $\mu$ g/L and above. Fish at these exposures also appeared lethargic in comparison to the controls and fish at 65  $\mu$ g/L. Spawning activity and hatchability of second generation fry did not appear to be affected, although considerable variability between replicates in the observed characteristics of total number of eggs spawned, number of eggs per female, percent fertilization and hatchability precluded statistical interpretation. High replicate variability was also observed in morphological development of the embryos. At 30 days of exposure, fry survival was similar for all treatments, but was significantly reduced at concentrations of 240  $\mu$ g/L and above. Based on the most sensitive measure, i.e., growth of first generation fish at 306 days, the chronic limits were 65 and 120  $\mu$ g/L, with a resultant chronic value of 88.32  $\mu$ g/L. A corresponding acute value of 6,300  $\mu$ g/L (Macek et al. 1976) yielded an acute-chronic ratio of 71.33 for brook trout (Table 2b).

A fathead minnow full life-cycle chronic test that extended for 274 days was performed, with mean measured atrazine concentrations of 0, 150, 250, 460, 990 and 2,000  $\mu$ g/L (Dionne 1992). At 30 days, first generation larval length was significantly reduced by concentrations \$990  $\mu$ g/L, whereas, at 60 days, length was reduced at concentrations \$460  $\mu$ g/L. At 274 days, survival was significantly reduced at 990 and 2,000  $\mu$ g/L of atrazine. There was no effect upon the reproductive characteristics of number of eggs per spawn, total number of eggs produced, number of spawns per female, or number of eggs per female at any treatment level. Hatching success was slightly, but significantly, reduced at concentrations of 250  $\mu$ g/L and above. Second generation larval growth (length and weight) was significantly reduced at \$460  $\mu$ g/L of atrazine. The chronic limits were reported to be 250 and 460  $\mu$ g/L, based upon first and second generation larval hatching and growth. This resulted in a chronic value of 339.1  $\mu$ g/L. An accompanying acute value of 20,000  $\mu$ g/L (Dionne 1992) yielded an acute-chronic ratio of 58.98.

Bluegills (*Lepomis macrochirus*) were continuously exposed to atrazine for 18 months starting with 7-10 cm long fish, continuing through spawning, and into a second generation for 60 days (Macek et al. 1976). Mean measured exposure concentrations were 8, 14, 25, 49 and 95  $\mu$ g/L. Survival and growth of first generation fish exposed to atrazine for 6 and 18 months were similar to the controls. Spawning activity was too sporadic to indicate any adverse effects. Percent hatchability of eggs was similar to controls at concentrations between 14 and 95  $\mu$ g/L. Low fry survival in the second generation controls for the first 30 days precluded observations on survival effects due to atrazine in this time

interval. However, between 30 and 90 days, survival was near 100 percent in the controls and all atrazine treatments. Total length of second generation fish through 90 days was considered to be unaffected by any of the atrazine exposures. From a lack of any adverse effect at concentrations as high as 95  $\mu$ g/L, the chronic limits were set at 95 and >95  $\mu$ g/L. The resultant chronic value was >95  $\mu$ g/L. A corresponding acute value of >8,000  $\mu$ g/L (Macek et al. 1976) yielded an acute-chronic ratio of >84.21.

The acute values for *C. tentans*, *S. fontinalis* and *L. macrochirus* in tests reported by Macek et al. (1976) were used in calculating acute-chronic ratios even though the acute test concentrations were not measured. This was because of close agreement between nominal and measured concentrations in the chronic tests. For six chronic tests, the overall agreement between measured and nominal concentrations was 94.4 percent. Therefore, it appeared likely that the nominal concentrations presented for acute tests were also in good agreement with actual concentrations.

## CHRONIC TOXICITY TO SALTWATER ANIMALS

The chronic toxicity of atrazine to saltwater species has been determined in three 8-day life cycle tests with the copepod, *Eurytemora affinis*, a 28-day life cycle test with the mysid, *Americamysis bahia*, and an early life-stage test (28-day) with the sheepshead minnow, *Cyprinodon variegatus* (Table 2a). Survival was the most sensitive endpoint in the 8-day chronic tests with *E. affinis*. Tests were performed at salinity levels of 5, 15 and 25 g/kg. At a salinity of 5 g/kg, survival was significantly reduced to 37 percent at the 17,500 µg/L concentration, while at the next lower concentration of 12,250 µg/L it was similar to controls at 71 percent (Hall et al. 1995). The chronic value was 14,640 µg/L. At a salinity of 15 g/kg, the chronic limits were 17,500 and 25,000 µg/L, and the chronic value was 20,920 µg/L. Sensitivity appeared greater at a salinity of 25 g/kg, with chronic limits of 4,200 and 6,000 µg/L, and a chronic value of 5,020 µg/L. Only at this highest salinity level was the acute value greater than the chronic value. The resultant Acute-Chronic Ratio of 2.629, determined at a salinity of 25 g/kg (13,200 µg/L ÷ 5,020 µg/L), was considered to be the correct ratio for this species, and was used in subsequent calculations involving the Species Mean Acute-Chronic Ratio.

Survival was the most sensitive endpoint in the mysid test (Ward and Ballantine 1985). Survival was 60, 30, and 20 percent at 190, 290 and 470  $\mu$ g/L, respectively. No statistically significant effect was observed for survival at concentrations #80  $\mu$ g/L. Reproduction did not occur at 470  $\mu$ g/L, but no adverse effects on reproduction were observed at all lower concentrations. The chronic value for mysids, is 123.3  $\mu$ g/L based upon no survival effects at 80  $\mu$ g/L and a 40 percent reduction in survival at

190  $\mu$ g/L. The acute value, as determined by the same authors, is 1,000  $\mu$ g/L and the resulting acutechronic ratio is 8.110 (Table 2b).

In the sheepshead minnow test (Ward and Ballantine 1985), juvenile survival was significantly reduced at 3,400  $\mu$ g/L, but not at #1,900  $\mu$ g/L. All fish exposed to 5,700  $\mu$ g/L died. There was no effect on either hatching success or growth in any of the concentrations with surviving fish (#5,700  $\mu$ g/L). The chronic value for sheepshead minnows, based on mortality of juveniles, is 2,542  $\mu$ g/L. The acute value for the sheepshead minnow, as determined by the same authors, is a "greater than" value (>16,000  $\mu$ g/L). Therefore, the resulting acute-chronic value is >6.294.

The range of definitive species mean acute-chronic ratios (ACRs) for both freshwater and saltwater differ by more than a factor of 10 (Table 2b - Acute-Chronic Ratios with greater than values were not used for these calculations), and are not related to rank order of acute sensitivity (Table 3). Since the available species mean ACRs do not meet Guideline requirements (Stephan et al. 1985), a Final Acute-Chronic Ration (FACR) cannot be calculated, nor can a freshwater or saltwater Final Chronic Value (FCV) based on the available aquatic animal data.

## TOXICITY TO AQUATIC PLANTS

For inclusion in Table 4, according to the Guidelines, exposures with algae must have been for a minimum of 4 days. With vascular plants, chronic exposures must have been conducted. In both cases, it is a requirement that the concentrations of atrazine were measured during the tests. A Final Plant Value can be obtained by selecting the lowest result from a test with an important aquatic species in which the concentrations of test material were measured and the endpoint was biologically important.

Two species of freshwater green algae were exposed to atrazine in studies in which the exposure duration was 4 days or longer and the atrazine concentrations were measured (Table 4). *Chlamydomonas reinhardtii* cell numbers were reduced 50 percent after 4 days of exposure to 51  $\mu$ g/L (Girling et al. 2000; Schafer et al. 1993), after 7 days of exposure to 21  $\mu$ g/L, and after 10 days of exposure to 10.2  $\mu$ g/L (Schafer et al. 1993).

Selenastrum capricornutum had a 4-day EC50 of 4  $\mu$ g/L, based upon cell numbers (University of Mississippi 1990). The EC50 values for pheophytin-*a* and chlorophyll-*a* content were 20 and 150  $\mu$ g/L, respectively. The 4-day No-Observed-Effect-Concentration (NOEC) and Lowest-Observed-Effect-Concentration (LOEC) values based on cell numbers were 0.5 and 1.0  $\mu$ g/L, respectively (University of Mississippi 1990). Using the same species and cell number as an endpoint, Gala and Giesy (1990) reported a 4-day EC50 of 128.2  $\mu$ g/L, and Hoberg (1991a) reported a 4-day EC50 of 130  $\mu$ g/L. Hoberg

(1993a) calculated a 5-day EC50 of 55  $\mu$ g/L. EC10 values at 4 and 5 days were 90 and 26  $\mu$ g/L, respectively, whereas, EC90 values at 4 and 5 days were 190 and 120  $\mu$ g/L, respectively (Hoberg 1991a, 1993a). The 4-day *S. capricornutum* NOEC and LOEC determined by Hoberg (1991a) were 76 and 130  $\mu$ g/L atrazine, respectively.

A 7-day exposure of the duckweed, *Lemna gibba*, to atrazine resulted in an EC50 of 180  $\mu$ g/L, based upon frond production (Hoberg 1991b). Two 14-day studies were also conducted with *L. gibba* (Hoberg 1993b,c). A major difference in these two studies was that, in the latter study, the effect concentrations were calculated based upon the atrazine concentrations that were measured on the last day only. This may have resulted in effect levels that appeared to be lower than in the first study, where concentrations were measured more often during the test. In the first study (Hoberg 1993b), using frond number as an endpoint, the EC10, EC50 and EC90 values were 6.2, 37, and 220  $\mu$ g/L, respectively, after 14 days of exposure. Using frond biomass, the EC10, EC50 and EC90 values were 12, 45 and 170  $\mu$ g/L, respectively. The NOEC and LOEC for frond number were <3.4 and 3.4  $\mu$ g/L atrazine, respectively. In the second study (Hoberg 1993c), the EC10, EC50 and EC90 values were 2.2, 50, and 98  $\mu$ g/L, respectively, using the frond number endpoint, while the respective values for frond biomass were 4.2, 22, and 110  $\mu$ g/L. The authors determined a NOEC of 8.3  $\mu$ g/L and a LOEC of 18  $\mu$ g/L based on frond number (Hoberg 1993c).

Exposure of a different species of duckweed, *Lemna minor*, to atrazine for 14 days resulted in a NOEC of 10  $\mu$ g/L based upon a biomass endpoint (University of Mississippi 1990). In this study, a LOEC of 100  $\mu$ g/L was obtained for the biomass endpoint. The EC50, based on biomass, was 8,700  $\mu$ g/L. Girling et al. (2000) reported a *L. minor* 28-day growth NOEC of 38  $\mu$ g/L atrazine, and the LOEC was 120  $\mu$ g/L atrazine. In another study using *L. minor* (Kirby and Sheahan 1994), 10-day exposures to atrazine yielded EC50 values that were comparable to those found for *L. gibba* by Hoberg (1993b,c). EC50 values of 56, 60 and 62  $\mu$ g/L were obtained based upon frond number, fresh weight and chlorophyll content, respectively.

Elodea (*Elodea canadensis*) was exposed to atrazine for 20 days by Girling et al. (2000), and the NOEC and LOEC values based on length were 20 and 30  $\mu$ g/L atrazine, respectively. In a study conducted by the University of Mississippi (1990), the effects of atrazine were evaluated both in the absence and presence of sediment. In the absence of sediment, LOEC values of 10 and 100  $\mu$ g/L were observed, based upon mature frond production and biomass, respectively. With sediment present, the biomass LOEC was also 100  $\mu$ g/L. Biomass EC50 values were 1,200 and 25,400  $\mu$ g/L when sediment was absent and present, respectively, in the test systems.

As stated in the Guidelines (Stephan et al. 1985), the Final Plant Value (FPV) is the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured, and the endpoint was biologically important. In this case, the freshwater FPV would be the geometric mean of the two duckweed species (*Lemna gibba* and *Lemna minor*) species mean chronic values (SMCVs) of 6.44  $\mu$ g/L (Hoberg 1993b,c) and 46.19  $\mu$ g/L (University of Mississippi 1990; Girling et al. 2000), or 17.25  $\mu$ g/L atrazine (Text Table A). Using the geometric mean of the two SMCVs for *Lemna* is consistent with the Guidelines, and is how all the SMAVs and GMAVs are calculated in the WQC documents.

Species	Acute Value (EC50)	SMAV (µg/L)	GMAV (µg/L)	NOEC - LOEC (µg/L)	Chroni c Value (µg/L)	SMCV (µg/L)	Reference
Green alga, Chlamydomonas reinhardtii	51 (4 days)	51			-		Girling et al. 2000
Green alga, Chlamydomonas reinhardtii	51 (4 days)	51	51				Schafer et al.1993
Green alga, Selenastrum capricornutum	4 (4 days)			0.5 - 1.0 (4 days - cell #)	0.7071		Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	130 (4 days)			76 - 130 (4 days - cell #)	99.398	8.384	Hoberg 1991a
Green alga, Selenastrum capricornutum	128.2 (4 days)	40.55	40.55				Gala and Giesy 1990
Duckweed, <i>Lemna gibba</i>	180 (7 days)			<3.4 - 3.4 (14 days - frond #)	3.4		Hoberg 1991b, 1993b
Duckweed, <i>Lemna gibba</i>	50 (14 davs)	94.89		8.3 - 18 (14 davs - frond #)	12.2	6.440	Hoberg 1993c
Duckweed, Lemna minor	56 (10 days)	56	72.89	10 - 100 (14 days - biomass)	31.62		Univ. of Mississippi 1990
Duckweed, Lemna minor				38 - 120 (28 days - growth)	67.5	46.19	Girling et al. 2000
Elodea, Elodea canadensis				20 - 30 (20 days - length)	24.49		Girling et al. 2000
Elodea, Elodea canadensis	1,200 (10 days)	1,200	1,200	10 - 100 (10 days - biomass)	31.62	27.83	Univ. of Mississippi 1990

Text Table A. Selected Freshwater Acute and Chronic Plant Data Taken From Table 4.

Information on the sensitivities of saltwater plants to atrazine is available for five phytoplankton species and five vascular plant species, representing nine genera (Table 4). Although the phytoplankton test results do not meet the minimum requirement of a four-day exposure, they are included here to show that their sensitivity to atrazine is similar to vascular plants. All of the plant effect concentrations were less than the acute values for aquatic animals. 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); a factor of only 3.4. Two species of estuarine submerged vascular plants, Potamogeton perfoliatus and Myriophyllum spicatum, exposed for 28-35 days to various concentrations of atrazine, had IC50 values for final biomass and photosynthesis between 25 and 117  $\mu$ g/L, with the biomass endpoint being more sensitive in both species (Kemp et al. 1982b, 1983, 1985). The sago pondweed, Potamogeton pectinatus, was tested (Hall et al. 1997) for atrazine toxicity for 28 days at three salinities (1, 6, and 12 g/kg). Dry weight was the most sensitive endpoint with chronic values (calculated as the geometric mean of the respective NOEC and LOEC values) of 21.2, 21.2 and 10.6 µg/L at salinities of 1, 6, and 12 g/kg salinity, respectively. The wild celery, Vallisneria americana exposed to atrazine for 42 days had chronic 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  $\mu$ g/L (Delistraty and Hershner 1984).

For saltwater, the FPV would be the geometric mean of the three *Potamogeton pectinatus* (Sago pondweed) measured chronic studies conducted by Hall et al. (1997) at different salinities, or 16.83  $\mu$ g/L atrazine (Text Table B). Using the geometric mean of the SMCVs for the three *P*. *pectinatus* tests is consistent with the Guidelines, and is how all the SMAVs and GMAVs are calculated in the WQC documents.

Species	Salinity (g/kg)	NOEC - LOEC (µg/L)	Chronic Value (µg/L)	SMCV (µg/L)	Reference
Redheadgrass pondweed, Potamogeton perfoliatus	9	IC50 (35 davs - biomass)	30	30	Kemp at al. 1982b, 1983, 1985
Sago pondweed, Potamogeton pectinatus	1	15 - 30 (28 days - dry wt.)	21.2		Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	6	15 - 30 (28 days - dry wt.)	21.2		Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	12	7.5 - 15 (28 days - dry wt.)	10.6	16.83	Hall et al. 1997
Eurasian water milfoil, Mvriophyllum spicatum	9	IC50 (35 davs - biomass)	25	25	Kemp at al. 1982b, 1983, 1985
Wild celery, Vallisneria americana	5	3.2 - 12 (42 days - leaf area)	6.19		Correll and Wu 1982
Wild celery, Vallisneria americana	3, 6	100 - 320 (42 days - dry wt.)	178.9	33.28	Forney and Davis 1981
Eelgrass, Zostera marina	22	LC50 (21 days)	540		Delistraty and Hershner 1984
Eelgrass, Zostera marina	20	LC50 (21 days)	100		Delistraty and Hershner 1984
Eelgrass, Zostera marina	20	LC50 (21 days)	365		Delistraty and Hershner 1984
Eelgrass, Zostera marina	19	LC50 (21 days)	367	291.6	Delistraty and Hershner 1984

## Text Table B. Selected Saltwater Acute and Chronic Plant Data Taken From Table 4.

## ECOSYSTEM EFFECTS DATA

Several aquatic ecosystem studies, either artificial laboratory microcosms or field mesocosms, have provided valuable insight into ecosystem structural and functional responses to atrazine (see Other Data, Table 6). A mixed assemblage of algal species exposed to  $10 \mu g/L$  of atrazine for periods of time ranging from 1 day to 3 weeks exhibited reductions in gross productivity between 39 and 78 percent (Kosinski and Merkle 1984; Kosinski et al. 1985). Exposure of an experimental stream periphyton community to 1,000  $\mu g/L$  for 14 days caused severe population density reductions in several species, and total destruction of the green alga, *Cladophora glomerata* (Kosinski 1984). The extreme toxicity to *C. glomerata* is notable because of the dominant role that it often plays in structuring a benthic community. Similarly, Moorhead and Kosinski (1986) observed reduced net primary productivity at

100  $\mu$ g/L in an assemblage of mixed stream algal species. By contrast, in a mixed stream community, no effects were observed upon stream macroinvertebrate community structure, periphyton production or biomass, or the community photosynthesis/respiration ratio following a 30-day exposure at 25  $\mu$ g/L (Lynch et al. 1985).

Malanchuk and Kollig (1985) observed chemical changes in an experimental stream community consisting of microscopic autotrophs and heterotrophs following the introduction of atrazine at a nominal concentration of 100  $\mu$ g/L for a 2-week exposure period, after which time the atrazine was removed from the ecosystem. They observed decreased diurnal fluctuations in pH and dissolved oxygen concentrations, as well as lower mean values for these water characteristics while atrazine treatment was on-going, but nitrate nitrogen levels increased. Following the cessation of atrazine treatment, there was a rapid recovery for each of these water characteristics back to control levels.

Biomass reductions were also noted in a stream aufwuchs community exposed to 24 or 134  $\mu$ g/L of atrazine for 12 days (Krieger et al. 1988), although a 24-hour exposure of 77.5  $\mu$ g/L had no effect upon algal cell numbers or biomass in a natural stream periphyton community (Jurgenson and Hoagland 1990). An exposure of as low as 0.5  $\mu$ g/L for 6 months resulted in an initial decrease in phytoplankton species followed by a recovery (Lakshminarayana et al. 1992). Gruessner and Watzin (1996), however, did not observe any effects of atrazine on a stream community of attached algae and benthic invertebrates at a concentration of 5  $\mu$ g/L when exposed for 14 days. Pearson and Crossland (1996) reported an inhibition of photosynthesis by the periphyton community of an artificial stream following exposure of 100  $\mu$ g/L of atrazine for 30 days.

In a static pond microcosm (1 L beaker), Brockway et al. (1984) found that a 7-day exposure to  $5.0 \mu g/L$  had no effect upon diurnal oxygen production, a measure of photosynthesis, by the various species of green and blue-green algae present. A 50  $\mu g/L$  exposure for 12 days resulted in a 25 to 30 percent reduction in diurnal oxygen production, while 7- to 12-day exposures at 100 to 5,000  $\mu g/L$  further decreased oxygen production. Berard et al. (1999) observed seasonal and species-dependent effects in a lake microcosm plankton community after 10 to 21 days of exposure to 10  $\mu g/L$  atrazine. During the experiment, growth was generally stimulated for Chryptophytes and Chrysophytes, but inhibited in *Chlorella vulgaris*.

Exposure of a freshwater microcosm to 5.1  $\mu$ g/L of atrazine for 7 weeks did not affect the species composition of phytoplankton, zooplankton or benthic macroinvertebrates, but did cause a slight decrease in photosynthetic activity (Van den Brink et al. 1995). Hamala and Kollig (1985) found an approximate 75 percent decrease in the productivity/respiration (P/R) ratio in a 14-day exposure to 100  $\mu$ g/L of a periphyton-dominated microcosm which contained 33 algal taxa. They also observed reduced algal densities, decreased species diversity, altered species composition and reduced biomass

accumulation. In a 21-day recovery period, net community productivity returned to control values within 16 days, while very little recovery occurred in community structural characteristics. This fairly rapid recovery in a functional characteristics indicated that the primary effect of atrazine at this exposure level was algistatic and not algicidal for those species involved in the recovery.

Stay et al. (1985), using a 3.7 L laboratory microcosm consisting of 10 algal species and 5 animal species (one protozoan, one rotifer, and three crustaceans), found that reduction in the ratio of <sup>14</sup>C uptake/chlorophyll-*a* was the most sensitive measure of atrazine effect. This suggested that the effectiveness of the photosynthetic system was impaired. The lowest exposure (i.e., 43.8  $\mu$ g/L over 60 days) resulted in significant reductions (approximately 60 to 90 percent) in the ratio throughout most of the study. Higher exposures (nominal concentrations of 100 to 500  $\mu$ g/L) caused further reductions in this ratio, but not as large a difference as between controls and the lowest exposure.

Peichl et al. (1984) observed changes in the population densities of zooplankton in a pond mesocosm study after 70 days of exposure to 200  $\mu$ g/L of atrazine. In a later study (Peichl et al. 1985), the authors observed changes in the phytoplankton community after 121 days of exposure to only 10  $\mu$ g/L. Experimental ponds in Kansas that were exposed for several years to single annual applications of atrazine at nominal concentrations of 20  $\mu$ g/L or more exhibited reductions in the production and biomass of phytoplankton, in macrophyte populations and in populations of benthic insect grazers, bullfrog (*Rana catesbeiana*) tadpoles, grass carp (*Ctenopharyngodon idella*) that had been introduced, and in bluegills (deNoyelles et al. 1982, 1989, 1994). Initial nominal concentrations of 20, 100, 200 and 500  $\mu$ g/L depressed phytoplankton growth within a few days in the ponds. However, after 3 weeks, phytoplankton production and biomass were similar to controls. deNoyelles and Kettle (1985) observed reduced photosynthesis of 40 percent or more in short-term (24-hour) bioassays at these same atrazine concentrations, but longer-term bioassays (20 days) and the experimental pond studies showed a recovery from this initial reduction.

Benthic insect community structure was studied in the same experimental ponds used in Kansas following two single annual treatments at 20, 100 and 500  $\mu$ g/L (Dewey 1986; Dewey and deNoyelles 1994). Significant reductions of both species richness and total abundance of emerging insects was observed at the lowest exposure of 20  $\mu$ g/L. Abundance of the herbivorous, non-predatory insects was reduced at 20  $\mu$ g/L, but not abundances of the predatory species. This indicated that the observed loss of total insects was a secondary effect due to feeding habit and loss of plant life, rather than a direct toxic effect. Loss of insect habitat, particularly in the form of macrophytes, also likely had some effect upon the insect community. These effects tended to destabilize the ecosystem (Dewey and deNoyelles 1994).

Species composition of macrophytes was altered in a pond mesocosm community following an 8-week exposure to 50  $\mu$ g/L of atrazine (Fairchild et al. 1994a). However, functional characteristics were unaffected, indicating functioning redundancy within the ecosystem. Juttner et al. (1995) did not observe any effects upon the plankton community of a pond mesocosm following a 2-month exposure to 5  $\mu$ g/L, but did observe decreased oxygen production, pH and conductivity at 10  $\mu$ g/L, and decreased phytoplankton populations at 182  $\mu$ g/L. At 318  $\mu$ g/L, reproduction was affected in *Daphnia longispina* and a population of rotifers, *Polyarthra* sp., was eliminated.

In a laboratory microcosm using a naturally derived microorganism community, Pratt et al. (1988) observed that a 21-day exposure to a mean measured concentration of 10  $\mu$ g/L of atrazine did not affect the dissolved oxygen, a measure of photosynthetic function, but that a concentration of 32.0  $\mu$ g/L caused significant reductions in this characteristics. This resulted in a calculated maximum acceptable toxicant concentration (MATC) of 17.9  $\mu$ g/L based upon this functional endpoint. Several other endpoints, such as protozoan colonization, biomass protein, chlorophyll-*a* and potassium levels, were less sensitive than dissolved oxygen, and had a calculated MATC of 193  $\mu$ g/L.

Stay et al. (1989) studied atrazine effects in 1 L laboratory microcosms containing mixed phytoand zooplankton cultured from three Oregon lakes and one pond. A 42-day exposure of approximately 15  $\mu$ g/L atrazine did not affect net primary productivity, the P/R ratio, or pH, but these characteristics were significantly reduced from controls at a mean measured concentration of approximately 84  $\mu$ g/L.

Larsen et al. (1986) measured photosynthetic <sup>14</sup>C uptake in a 3 L Taub microcosm community at different time intervals for up to 373 days after treatment with atrazine. EC50 values ranged from 24  $\mu$ g/L at 177 days to 131  $\mu$ g/L at 43 days after atrazine treatment.

A 50 m<sup>2</sup> pond community exposed to atrazine for 4 months at a concentration between 60 and 120  $\mu$ g/L eliminated a population of duckweed, *Lemna minor*, within 27 days (Gunkel 1983). Gunkel also observed a rapid succession of algal species and a reduced rate of reproduction in *Daphnia pulicaria*. Treatments of a pond mesocosm community for 2 years with 20, 100 and 300  $\mu$ g/L of atrazine caused decreases in cell numbers of green algae and of cladoceran populations, but increased numbers of cryptomonads (Neugebauer et al. 1990).

In experimental ponds treated in May and June with 20  $\mu$ g/L of atrazine for two years, there was decreased abundance of *Endochironomus nigricans* in June and of total macroinvertebrates in both May and June, followed by recovery in July (Huggins et al. 1994). Epiphytes, detritovores and generalists also exhibited initial decreases in populations, followed by a recovery. A short-term exposure (>3 hour) of pond algae to 10  $\mu$ g/L of atrazine was observed to increase the rate of fluorescence for photosystem II (Ruth 1996).

In two reports of studies conducted at the same site, a lake community was enclosed with a limnocorral (5 m x 5 m x 5 m deep) to which atrazine was added. Both studies focused on the periphyton community. In the first study (Herman et al. 1986), the limnocorrals received two nominal atrazine applications of 100  $\mu$ g/L, one on day 0 and another on day 35. After 34 days of exposure to measured concentrations ranging between 80 and 140  $\mu$ g/L, a reduction in periphyton ash-free dry weight was observed. Over a 9-week period with two atrazine applications 6 weeks apart, which resulted in measured concentrations of approximately 80 to 140  $\mu$ g/L after the first application and 110 to 190  $\mu$ g/L after the second application, reductions occurred in chlorophyll-*a*, organic matter and total periphyton algal biomass. In the second study (Hamilton et al. 1987), a 230-day exposure to a mean measured atrazine concentration of 80  $\mu$ g/L caused approximate reductions of 60 percent in biomass, 22 percent in cell numbers and 32 percent in number of species. The results were more pronounced in exposures to mean measured atrazine concentrations of 140 and 1,560  $\mu$ g/L. A shift in community structure occurred from a chlorophyte-dominated community to a diatom-dominated community.

Aquatic enclosures exposed to a nominal atrazine application of 100 µg/L on June 1 followed by a second application of the same concentration 35 days later, exhibited a gradual die-off of the phytoplankton, a long period of recovery for the green algal community, and a distinct shift in the taxonomic composition of algae (Hamilton et al. 1988). Thirteen days after the first application, significant declines occurred in populations of the green algal species *Elakatothrix gelatinosa*, *Tetraedon* minimum, Sphaerocystis schroeteri, and Oocystis lacustris, and of the dinoflagellate, Gymnodinium spp. Seventy-seven days after the second application, phytoplankton communities were still distinctly different, and total fresh weight biomass was reduced. By 323 days after the first application, the phytoplankton assemblages were again similar between control and treated enclosures. From day 1 to day 114, control enclosures had an average of five more taxa than the atrazine-treated enclosures. During the period between days 49 and 77, the green algal (*Chlorophyta*) biomass represented <7 percent of that found in the controls. By the following spring (day 323), the biomass had returned to control levels. The herbicide treatment did not affect the rotifer or crustacean communities. In the same exposures, Hamilton et al. (1989) observed that the atrazine-treated enclosures became clearer with increased Secchi disc readings, while readings of dissolved oxygen, chlorophyll, dissolved organic carbon, and particulate organic carbon decreased.

Using 1.70 m<sup>2</sup> enclosures in a moderately eutrophic lake, Lampert et al. (1989) observed decreased photosynthesis and decreased populations of certain zooplankters at atrazine concentrations of 0.1 and 1.0  $\mu$ g/L. At 0.1  $\mu$ g/L, populations of *Daphnia* sp. were severely reduced within 15 days, and oxygen concentrations were reduced after 10 days. At 1.0  $\mu$ g/L, concentrations of chlorophyll-*a* and oxygen were reduced after 18 days as were populations of *Daphnia*, *Cyclops*, and *Bosmina* species, and

nauplii larvae. At 0.1  $\mu$ g/L, there was an apparent recovery after about 25 days. The authors noted, however, that the effects of atrazine observed in their experimental plastic bag enclosures may have been exaggerated, because gas exchange and re-colonization from the surrounding medium were limited. Likewise, the enclosures may have accentuated trophic feeding dynamics of primary consumers, as fish and larger zooplankton (predators) were excluded. Genoni (1992) observed a decreased algal population density and a decreased "scope for change in ascendency" in a microcosm community exposed to 250  $\mu$ g/L. The scope for change in ascendency is a biological system response endpoint, considered to be analogous to the scope for growth endpoint for individual organisms.

Gustavson and Wangberg (1995) observed some minor changes in species composition of the phytoplankton community in a lake mesocosm community after a 20-day exposure to 20  $\mu$ g/L. EC50 values were 58 and 52  $\mu$ g/L for the phytoplankton community, and 52 and 54  $\mu$ g/L for the periphyton community. Brown and Lean (1995) found that a short-term exposure (3 hours) of lake phytoplankton to atrazine resulted in a much lower EC50 based upon photosynthetic carbon assimilation (i.e., 100  $\mu$ g/L), than when based upon phosphate or ammonium assimilation (14,000 and >33,000  $\mu$ g/L, respectively). A stream periphyton community exhibited a significant reduction in chlorophyll-*a* following a brief exposure (<4 hours) to 109  $\mu$ g/L of atrazine (Day 1993). Caux and Kent (1995) observed a reduction in green algae in Quebec streams following the spring atrazine runoff pulse, with a maximum stream concentration of approximately 40  $\mu$ g/L. Detenbeck et al. (1996) observed a decrease in the gross productivity of a wetland mesocosm community after 9 to 27 days of exposure at an atrazine concentration of 15  $\mu$ g/L. There also was an increase in the concentrations of dissolved nutrients in the water.

In the range of 10 to 100  $\mu$ g/L, it appears that atrazine changes planktonic community structure and composition (Berard et al. 1999, Peichel et al. 1984), which may recover in functional characteristics after cessation of treatment, e.g., productivity, pH, dissolved oxygen production deNoyelles et al. 1982, 1989, 1994; Malanchuk and Kollig 1985), but not necessarily structure (Hamala and Kollig 1985). Planktonic community structure effects are seasonal and species-dependent (Berard et al. 1999), with the diatom community generally less sensitive than green algae (Lakshminarayana et al. 1992).

Changes in habitat and loss of certain plant species at 20  $\mu$ g/L can lead to secondary effects higher in the food web (Dewey and DeNoyelles 1994), but even at this initial exposure level, structure and functional integrity of aquatic insect communities are generally maintained, as indicated by only very small changes in species diversity and evenness indices (Dewey 1986). Concentrations above 50  $\mu$ g/L, on the other hand, cause more severe reductions in productivity, plant biomass, and community structure, as well as indirect effects on herbivorous invertebrates and fish. Changes in species composition without loss of functionality at 50  $\mu$ g/L atrazine, however, indicates a great deal of functional redundancy within some systems (Fairchild et al. 1994a).

Rotifer and crustacean communities are generally less sensitive to direct atrazine toxicity with an LOEC of about 200  $\mu$ g/L (Peichl et al. 1984). Other benthic macroinvertebrate species can be affected at as low as 20  $\mu$ g/L, but the effects (mostly abundance) are seasonal (Huggins et al. 1994).

Studies by Berard et al. (1999), Kosinski and Merkle (1984), Kosinski et al. (1985), Lakshminarayana et al. (1992), Lampert et al. (1989), and Peichl et al. (1984, 1985) have observed effects at lower concentrations. The lowest recorded effects of atrazine occurred in experimental enclosures with natural communities (Lampert et al. 1989).

In summary, aquatic ecosystem structural and functional parameters have most frequently been observed to be adversely affected by atrazine concentrations exceeding  $10 \mu g/L$ . The lowest concentrations of atrazine that have resulted in temporary negative effects upon abundance of aquatic plants (primary effect) and animals (secondary effect) have generally occurred at 15-20  $\mu g/L$  and above. It appears that for effects at concentrations up to  $15 \mu g/L$ , the communities can recover quite rapidly following dissipation of the atrazine concentration. In a review of microcosm and mesocosm studies with atrazine, Giddings and Biever (1994) concluded that concentrations of 20  $\mu g/L$  or less typically caused minor effects, if any, on primary production and plant community composition, and recovery occurred quickly, even if atrazine remained in the system.

## IMPACTS TO PLANT COMMUNITY STRUCTURE AND FUNCTION

## **Impacts to Plant Community Structure and Function**

The Guidelines and the CWA expect that the Agency will establish a sound scientific basis for all of its water quality criteria for the protection of aquatic life. In light of this expectation and because of the unique use and chemical characteristics of atrazine, the Agency has selected an approach to deriving the chronic criterion for the protection of freshwater aquatic life as described below.

In summary, threshold concentrations were determined from realistic and complex time variable atrazine exposure profiles (chemographs) for modeled aquatic community structure changes. Methods were developed to estimate ecological community responses for monitoring data sets of interest based on their relationship to micro- and mesocosm study results, and thus to determine whether a certain exposure profile at a site may have exceeded a level-of-concern.

This required a two step process: (1) Determine the magnitude and duration of exposure of aquatic plants to atrazine that constitute LOC(s) for aquatic communities and/or ecosystems, and (2) Determine the best available method(s) to interpret monitoring data relative to these LOC(s).

## Endpoints

The initial assessment endpoint was chosen based on the reported results from 77 micro- and mesocosm studies for which atrazine was tested: change in aquatic community structure and function of primary producers. This endpoint appeared to be the most sensitive of the effect endpoints affecting aquatic plants. Further, the effect of atrazine on aquatic plants, whether direct or indirect, appeared to be more sensitive than effects on other organisms in the aquatic ecosystem, e.g., aquatic invertebrates, fish. Thus, by focusing on aquatic plant community structural changes, we would be in effect, protecting against adverse effects on the rest of the aquatic community. The measurement endpoints reported in available studies which tested atrazine were: laboratory – growth (rate) and biomass; microcosms, mesocosms and models - reduction in primary production and changes in structure of primary producer communities.

## **Community Level Studies**

Ecological responses of aquatic communities to atrazine exposures can be assessed using community level studies, such as micro- and mesocosms. The subgroup reviewed 25 different studies with 77 reported effects/no effects on aquatic plants (See Appendix 1). Twenty-four results were from tests on ponds or lakes; 20 on artificial streams; and, 33 were microcosm tests. Eight results were on macrophytes, 29 on periphyton, and 40 on phytoplankton. However, only a limited number of exposure profiles could be tested in these studies. Typically, one to three concentrations of atrazine were tested in these studies each with a single application to the test system at initiation. Atrazine concentrations were often kept constant for a variable duration period before the concentrations slowly decrease with time. Unfortunately, the variable quality of these studies and the many different study designs did not always allow a reliable association of exposure magnitude and duration to a certain community level effect, and in many cases the duration of the studies was too short to document community recovery.

To better understand the impact of exposure duration and magnitude on aquatic communities, the effects reported in these studies had to relate to specific exposure durations and magnitudes. First, the 77 study results had to be quantified as to severity of effects of atrazine on the aquatic plant community. Brock et al 2000 analyzed a majority of the study results and quantified them as follows:

Effect Scores (Brock et al 2000):

- 1 = no effect
- 2 = slight effect
- 3 = significant effect followed by return to control levels within 56 d
- 4 = significant effect without return to control levels during an observation period of less than 56 d
- 5 = significant effect without return to control levels for more than 56 d

Studies not analyzed by Brock but considered in this analysis were scored with the same methods. The distribution of the scores for the 77 study results were as follows (also see Appendix 1):

Distribution of Effect Scores:

- 15 were ranked as 1;
- 12 were ranked as 2;
- 12 were ranked as3;
- 23 were ranked as 4;
- 15 were ranked as 5

Next, the 77 effect scores representing the results from the 25 micro- and mesocosm studies for atrazine were plotted against the study specific test concentrations and exposure durations in Figure 1.





As expected, based on the mode of action of atrazine that inhibits primary production by reversibly blocking photosynthesis, the effects observed in micro- and mesocosm studies generally become more severe with increasing exposure time and magnitude.

The challenge for step two was to define an appropriate exposure concentration and duration relationship that properly defines duration specific levels of concern. For that purpose, ecological modeling was used to simulate a large number of exposure durations and magnitudes for the ecological response in a generic Midwestern 2<sup>nd</sup> to 3<sup>rd</sup> order stream. Two ecological models were initially considered: (1) the <u>Comprehensive Aquatic Systems Model</u> (CASM) (Bartell et al. 2000, Bartell et al 1999, DeAngelis et al 1989), and (2) AQUATOX<sup>2</sup>. The decision to use CASM was made after a preliminary comparison revealed that CASM could include a larger number of species in the community

<sup>&</sup>lt;sup>2</sup> See <u>http://www.epa.gov/waterscience/models/aquatox/about.html</u> and <u>http://www.myweb.cableone.net/dickpark/AQTXFacts.htm</u>

structure, which appeared to better support our assessment endpoint. In addition, CASM had a relatively uncomplicated exposure profile for a chemical such as atrazine.

## **Model Parameterization**

A large number of single-species laboratory toxicity test results on atrazine toxicity to aquatic organisms (See Giddings et al 2000), including aquatic plants (macrophytes, periphyton, and phytoplankton) were available (Figure 2). A subset of these data (CASM EC50 geometric means) was selected and used to drive the toxicity of atrazine to aquatic organisms in the CASM simulation model (See Appendix 2). The modeled toxicity profile included twenty-six producer species (10 plankton, 10 periphyton, 6 macrophytes), and 17 consumer species. Three toxicity scenarios were modeled: 10<sup>th</sup> centile, geometric mean, and 90th centile for species with more than one toxicity study. The geometric mean scenario (toxicity scenario 1) was chosen for the reported model results.


Figure B: Plant Species Sensitivity Distribution for EC10, EC50, and EC90 values overlaid with the Plant Species Sensitivity Distribution (EC50 geometric mean) used to parameterize CASM.

### **CASM Model Simulations**

CASM is an ecological food chain model. It was set-up to run simulations for exposure durations from 1 to 260 days, and concentrations from 20 to 220 : g/L atrazine. The scenarios were designed to simulate a generic  $2^{nd}$  or  $3^{rd}$  order Midwestern stream, typical for the majority of atrazine use on corn and sorghum. The CASM model provides the following results: production – modeled as biomass production (g Carbon m<sup>-2</sup>) for 1 m<sup>2</sup> surface area) (Appendix 3a), and community structure (similarity) – species population size derived from species daily biomass (Appendix 3b). Thus, the model integrates direct and indirect effects to indicate changes in community structure. The endpoint selected for the model results was percent (%) change in aquatic community structure (as determined by Steinhaus Similarity coefficient) of primary producers (phytoplankton, periphyton, macrophytes).

# **CASM Steinhaus Similarity Analysis**

Coefficients of similarity are used to determine whether the composition of two communities is similar. The Steinhaus coefficient or similarity index is based on the species abundances (in this case indicated by the species specific daily biomass) common to two communities. The index is described in the following equation:

Where  $a_{i,k}$ : abundances of species k in sample I

$$S = \frac{2 \sum_{k=1}^{n} Min(a_{1,k}, a_{2,k})}{\sum_{k=1}^{n} a_{1,k} + \sum_{k=1}^{n} a_{2,k}}$$

n

The similarity indices for each possible pair of samples per day are calculated and this results in a matrix of between (different treatments) similarities as in Figure 3.

	<b>1d1</b>	<b>1d2</b>	<b>1d3</b>	etc.	Xd260
1d1		B	B	B	B
1d2			B	B	B
1d3				B	B
etc.				B	B
Xd260					B

Figure C. Example of a matrix of similarities resulting

from Similarity Index calculations.

Similarity indices were calculated for primary producers, consumers, and fish over exposure periods from 1 to 20 days (See Appendix 3b). The results show that the changes in percent (%) change in aquatic community structure of primary producers is a more sensitive (conservative) measurement endpoint than the same for consumers or fish.

# Determining the LOC - CASM Steinhaus similarity vs. the effects of Atrazine exposure in microand mesocosm studies

A wide range of single pulses of different duration and magnitude were simulated and used to calculate community structure changes. Community structure changes were expressed as percent (%) change in the Steinhaus similarity index that was calculated based on the simulated daily biomass for each individual species and plotted over time.

Table 1: A) Maximum daily percent change<sup>a</sup> in community structure (Steinhaus similarity) of primary producers for a modeled generic 2nd-3rd order Midwestern stream.

Atrazine	Pulse du	ration [d]	]p					
conc. [:g/L]	1	3	5	10	20	60	130	260
20	0.1°	0.2	0.7	0.9	1	1.2	1.2	2.3
25	0.8	1.9	2.9	5	7.8	11.7	13	15.5
30	0.8	1.9	2.9	5	7.8	11.7	13	15.8
40	1.1	2.3	3.2	5.2	8	11.7	13.1	16.6
50	1.1	2.3	3.1	5.2	7.9	11.6	13.1	17.5
70	3.7	8	10.7	13.8	16.1	17.3	18.1	22.5
90	4.4	9.4	12.6	15.9	18.2	18.2	18.3	23.5
130	4.5	9.6	12.7	15.8	17.8	17.8	17.8	20.1
170	5.6	13.1	18.1	24.1	29.7	56.3	67.1	72.4
220	5.7	13.2	18.2	24	29.7	56.3	67.1	72.3

B) Year end percent change<sup>a</sup> in community structure (Steinhaus similarity) of primary producers for a modeled generic 2nd-3rd order Midwestern stream.

Atrazine	Pulse duration [d] <sup>b</sup>							
conc. [:g/L]	1	3	5	10	20	60	130	260
20	0 <sup>c</sup>	0	0	0.2	0.2	0.2	0.2	2.3
25	0.7	1.7	2.7	4.7	7.3	10.9	12.1	15.5
30	0.7	1.7	2.7	4.6	7.2	10.8	12.1	15.8
40	0.7	1.9	3	4.9	7.5	11	12.4	16.6
50	0.7	1.9	2.9	4.9	7.5	10.9	12.9	17.5
70	1.5	3.7	5.2	7.9	10.9	14.6	17.6	22.5
90	1.7	4.1	5.7	8.5	11.6	15.5	18.3	23.5
130	1.7	4	5.7	8.4	11.5	15.3	16.4	20.1
170	2	5.4	8.1	15.5	27.9	51.7	61.2	71.5
220	2	5.3	8.1	15.4	27.8	51.6	61.1	71.1

C) Average percent change<sup>a</sup> in community structure (Steinhaus similarity) of primary producers for a modeled generic 2nd-3rd order Midwestern stream.

Atrazine Pulse duration [d] <sup>b</sup>								
conc. [:g/L]	1	3	5	10	20	60	130	260
20	0 <sup>c</sup>	0	0.1	0.4	0.4	0.5	0.5	0.7
25	0.5	1.2	1.9	3.4	5.1	7.4	8.2	8.5
30	0.4	1.2	2	3.5	5.2	7.6	8.4	8.7
40	0.8	1.8	2.6	4.1	5.8	8.3	9.3	9.7
50	0.8	1.8	2.6	4.2	6	8.9	10.1	10.7
70	2.2	4.8	6.4	9.1	11.6	14.9	16.9	17.5
90	2.6	5.6	7.4	10.2	12.8	15.8	17.5	18
130	2.6	5.6	7.4	10.2	12.7	15.4	16.3	16.4
170	2.9	6.8	9.8	16.3	25.5	40.6	46.3	48.4
220	2.9	6.8	9.8	16.4	25.5	40.6	46.3	48.4

<sup>a</sup>Based on the mean values of 100 Monte Carlo simulations using the Comprehensive Aquatic Systems Model (CASM)

<sup>b</sup>Consecutive days of constant exposure beginning on model day 105 (April 15)

For further evaluation, the maximum daily percent (Table1 A), year-end percent, i.e. at day 260 post application (Table 1 B), and the average percent change in community structure in the primary producer community (Table 1 C) were calculated. Maximum daily deviations indicate the short-term (temporary) maximum change in community structure. The average community structure change integrates short-term changes and long-term recovery of the communities. A comparison of short- and long-term %-impact shows that for concentrations >20 : g/L, short-term changes are always between 1- to 2-fold the average response. For example, an average 5% community structure change may cause a less than or equal to 10% short-term (temporary) change in primary producer community structure. The average percent change in community structure was chosen for the reported results since it captures the short-term changes as well as recovery.

The modeling results in Table 1C were used to help define duration-specific levels of concern. Two approaches were used. First, the simulated response (or effect) had to be set in context to the microand mesocosm data. A similarity index value was estimated for each micro- and mesocosm test result by finding the average model similarity deviations (%) of a simulated exposure profile closest to the conditions used in each study (test concentration and exposure duration) (See Appendix 1 for assigned index values for each of the 77 test results). Next, the index values were plotted against the Brock et al effect scores for each micro- and mesocosm test results for comparison (See Figure 4).

There is a lot of scatter that is reflective of the diversity of this data; however, there is a clear, strong correlation of the scores with the index. An index value of 5 (vertical red line on the figure) conservatively separates the 3/4/5 from the 1-2 scores. That means that a 5% change in community structure (Steinhaus similarity) of the CASM simulations compares to a large majority of the micro- and mesocosm studies with no to slight effects (leaving only 8% potential false negatives and false positives, i.e., false negatives - 6 out of 77 studies above the effects score 3 line and to the left of the 5% line; false positives - 6 out of 77 studies blow the effects score 3 line and to the right of the 5% line).



Figure D. Correlation between the Similarity Index [CASM AVG % change in community structure for 77 atrazine micro- and mesocosm studies] and the Brock et al 2000 effect scores.

For the second approach, the CASM simulation results in Table 1C were interpolated to develop a set of concentration / duration pairs equivalent to 5% effect from CASM. The interpolated results follow:

<u>Time (days)</u>	Concentration (: g/L)
1.1	220
1.6	130
3	75
5	63
10	53
20	24.8
60	23.3
130	22.9
260	22.7

For times greater than 3 days, a linear interpolation was performed across the different concentrations at each time. For times from 60 to 260 days, the abrupt shift in response between 20 and 25:g/L made interpolation tenuous, but the best estimate would seem to be in the mid-part of the range and this did not involve much uncertainty given the narrow range. For times less than 3 days, the response did not reach 5%, but the additional points seem to be points needed at high concentrations. Thus, interpolations were performed across times at a fixed concentration instead of across concentrations at a fixed time.

Next, these concentration-duration pairs, representing the 5% index points based on interpolation, were plotted with lines connecting each point on Figure 1 (See Figure 5 below).



Figure E. Micro- and mesocosm study effect concentrations scored according to Brock et al 2000 and plotted against the study specific exposure duration. Interpolated 5% CASM Similarity Index points plotted.
The plot of the interpolated 5% Similarity index points, like Figure 4, conservatively separates the 3/4/5 from the 1-2 scores. Based on both approaches, an index of 5%, meaning a 5% change in community structure of primary producers, is a reasonable LOC for atrazine exposures in freshwater environments.

#### Discussion of Uncertainty in Selection of Data, Methods, and Decisions

Since the potential risk of atrazine to aquatic communities will be based on a set of micro- and mesocosm tests, the critical decision is which tests to include or exclude. The large set of available studies for atrazine included in this analysis (Appendix 1) have various strengths and weaknesses and use many different testing designs and methods. The key point here is that there are a large number of such studies and the subgroup decided to be relatively inclusive, rather than excluding data for various limited uncertainties or ambiguities. This approach provides a better data set for weight-of-evidence and allows for addressing "false-negatives" and "false-positives" in light of the overall frequency/magnitude of the wide range of possible exposure situations. It would not be prudent to rely on any one or two of these studies.

# Quantification of Results of Micro- and Mesocosm Tests

The effect scores in Brock et al (2000) were used to quantify the results of the micro- and mesocosm tests. The subgroup reached general agreement that the scores assigned to the 77 results were reasonable, and that scores of 2 ('slight' effect) do not constitute a level-of-concern, while scores of 3 (a pronounced 'slight effect') do. Brock et al further characterized a score of 2 as "*effects reported in terms of 'slight'; 'transient', and short-term and/or quantitatively restricted response of sensitive endpoints, and effects only observed at individual samplings.*" Scores of 3 were characterized as a "*clear response of sensitive endpoints, and effects on loss sensitive species'; 'temporary effects on several sensitive species'; 'temporary elimination of sensitive species'; 'temporary effects on less sensitive species / endpoints', and effects observed at some subsequent samplings." This last decision is perhaps the most critical risk decision here, because these scores define the actual level of protection being sought. Therefore, Appendix 1 is arranged by decreasing effects score and shows the range and nature of effects represented by the different scores.* 

Another aspect of quantification is the exposure duration that any score and concentration relate to. This might not seem to be an issue because the exposure duration is fixed and specified in any test, but for long exposures in which severe effects occur early, might not these effects be better related to a shorter duration? For example, the significant effects (scored as a 5 and described as a decrease in macrophyte coverage in the pond by 95%) in the Kettle et al. (1987) study were related to a full year's exposure (actually 300 days). However, the study also reported that there was ~60% decrease in coverage after 60 days. It was decided to stay with the 300 day test duration because (1) the exposures in the study were constant over the whole time period, (2) Brock et al as well as other authors reported the test duration as ~1 year, and (3) the most dramatic effect without testing for recovery did occur after the ~year long exposure duration. Yet, some could argue that 60% decrease in macrophyte coverage is significant and should also be scored as a 5 and included. However, the uncertainty resulting from this observation for the calculation of the time specific LOC(s) in this document is very small because, as shown in Figure 5, the concentrations causing community structure changes do not further decrease for constant exposure periods longer than 20 to 30 days, i.e. longer exposure periods do not significantly change the effect threshold. The Kettle et al study was conducted at the borderline of this threshold concentration (ca. 20 : g/L). In the weight-of-evidence approach applied here, it constitutes only one of the large numbers of such studies that also measured less severe impact at the comparable concentrations and exposure durations.

# Extrapolation of Micro- and Mesocosm Tests to Different Exposure Time Series

Another critical decision was to use an aquatic ecological community model as the extrapolation tool. It is important to emphasize that EPA is not claiming that the model accurately predicts the effects in any particular community, but rather that it is a useful means for integrating the kinetics of various processes (toxic effects on photosynthesis, plant growth dynamics, interactions among plant species across a growing season) and describing the RELATIVE effects of different exposure time series on the overall response.

#### **Parameterization of Model**

The critical data here are the plant laboratory toxicity data assigned to each species in CASM. These data are the key factor determining the concentration at which CASM predicts significant effects (slightly above 20 : g/L) and describing the "step-wise" nature of the effects versus concentration. Because of the concern about effect levels that reflect the more sensitive organisms, Figure 2 and Appendix 2 show that the decision to use the geometric mean toxicity values (EC50s) for CASM appears to adequately represent plant species sensitivity distribution. However, one consequence of the limited number of possible species in the model is that only a few species represent sensitivities below the 10<sup>th</sup> centile and above the 90<sup>th</sup> centile. Additional analyses using the 10<sup>th</sup> and the 90<sup>th</sup> centile of the EC50 instead of geometric means was conducted to test for the potential impact of the species sensitivity on the CASM results (Appendix 5). For the majority of the simulations, the lower toxicity profiles (scenario 2) did not cause significantly higher responses than the geometric mean scenario. It was also observed that the higher and lower toxicity scenarios did not necessarily bracket the geometric mean scenario. This can partly be explained by the complex nature of the food-chain interactions in the

ecological model. The impact of slightly different species sensitivity distributions used to parameterize the model is therefore probably low, when compared to relative importance of the species composition in the food-chain model.

EPA recognizes that different species have different relative importance in CASM results and this varies seasonally. Even if each CASM species is linked to the most relevant laboratory species, the original selection of CASM species and the assignment of the laboratory data represent a major uncertainty and further evaluation using model parameterizations representing different generic aquatic communities are recommended.

#### Selection of Model Variable to Relate to Micro- and Mesocosm Results

The selection of this endpoint is a critical decision, even if model results are calibrated to the micro- and mesocosm data, because different endpoints have different time-dependencies. These differences will affect the relative level of concern for different exposure series. While EPA believes that the average similarity index is a reasonable choice, we also recognize that its meaning is somewhat uncertain. The critical point is the time trajectory of the index when the effect on the average community structure is less than that at the end of the year. EPA recognizes that the recommended average index combines direct toxic effects and consequent shifts in later seasonal plant succession. However, it is important to note that this index can have different time dependence than an endpoint such as overall primary productivity, and thus is a key decision.

### ENDOCRINE DISRUPTION EFFECTS DATA

Atrazine has been reported in a number of studies as an endocrine disruptor. Researchers at the University of California at Berkeley (Hayes et al. 2002) have reported that frogs (*Xenopus laevis*) exposed to atrazine in the water at concentrations  $\#1 \ \mu g/L$  suffered abnormalities in gonadal development, including feminization and hermaphroditism, which could render male frogs sterile. In addition, these same exposures resulted in a reduction in the size of the laryngeal muscle in male frogs, an important muscle used for the mating call of the frog. Studies conducted by Carr et al. (2003) and Carr and Solomon (2003) designed to replicate the Hayes et al. (2002) experiments observed these same effects at approximately 20-21  $\mu$ g/L atrazine. A third study conducted by Sullivan et al. (2003) with *Xenopus laevis* looking at the same end-points yielded an effect level of 20  $\mu$ g/L atrazine (the lowest concentration tested). Although the atrazine concentrations reported in this latter study were nominal, measurements of actual atrazine levels in a more recent experiment by the same authors (unpublished study) of the same design and methodology showed good agreement between nominal and measured concentrations. As stated by Sullivan et al. (2003), "these results allow us to confidently indicate actual atrazine concentrations are likely to have occurred in this study."

Until this issue is resolved, justification and defense of a freshwater chronic criterion based on the endocrine disrupting effects of atrazine on amphibians is difficult. A recently convened Scientific Advisory Panel (SAP) reviewed EPA's (2003) evaluation of 17 laboratory and field studies concerning the potential developmental effects of atrazine on amphibians. The SAP agreed with EPA's conclusion that additional studies are warranted to reduce the scientific uncertainty regarding whether atrazine causes replicable effects on amphibians (Scientific Advisory Panel 2003). Substantial additional research to resolve this issue is currently underway, or planned for the immediate future. Once additional data are available that conclusively demonstrate a significant reproductive effect (or other endpoint that significantly impairs the populations ability to survive long term) to aquatic species, then derivation of the freshwater chronic criterion will be reexamined.

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Species	Method <sup>a</sup>	Chemical	Exposure Medium	Effect (metamorphosis completed)	Effect Level (µg/L) <sup>b</sup>	References
African clawed frog (larval), <i>Xenopus laevis</i>	R,M	-	10% Holtfreter's solution	abnormalities in gonadal development, including feminization and hermaphroditism	#1	Hayes et al. 2002a,b
African clawed frog (larval), Xenopus laevis	R,M	-	10% Holtfreter's solution	reduction in the size of the laryngeal muscle in male frogs	1	Hayes et al. 2002a,b
African clawed frog (larval), <i>Xenopus laevis</i>	R,M	98.6%	FETAX solution	increased incidence of intersex animals (based on assessment of gonadal morphology)	21.3	Carr et al. 2003
African clawed frog (larval), <i>Xenopus laevis</i>	R,M	98.6%	FETAX solution	reduction in the size of the laryngeal muscle in male frogs	>21.3	Carr et al. 2003
African clawed frog (9-11 days old), <i>Xenopus laevis</i>	R,U	99%	Moderately Hard Reconstituted Laboratory Water	mean weight at metamorphosis	20	Sullivan and Spence 2003

Text Table C. Summary of Endocrine Disruption Effects of Atrazine to Freshwater Organisms

<sup>a</sup> S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.

<sup>b</sup> Results are expressed as atrazine, not as the chemical.

## BIOACCUMULATION

The data available according to the Guidelines concerning the bioaccumulation of atrazine are included in Table 5. Only freshwater data are available. Macek et al. (1976) analyzed muscle tissue or the eviscerated carcasses of fish at the end of extended exposure periods. Brook trout exposed to atrazine at 740  $\mu$ g/L for 308 days contained less than 200  $\mu$ g/kg of atrazine in muscle tissue, resulting in a bioconcentration factor (BCF) of <0.27. Fathead minnows exposed to atrazine at 210  $\mu$ g/L for 301 days had less than 1,700  $\mu$ g/kg of atrazine in pooled samples of eviscerated carcasses, for a BCF of

<8.1. Bluegills exposed to 94  $\mu$ g/L for 546 days also contained less than 200  $\mu$ g/kg in their muscle tissue, for a BCF of <2.1.

Dionne (1992) exposed fathead minnows to atrazine for up to 274 days using <sup>14</sup>C-labeled atrazine and measuring the radiolabel in fish tissue. The values obtained represent maximum possible BCFs. Regardless of the life-stage or exposure duration, maximum BCFs were less than or equal to 8.5 in all cases.

There is no U.S. Food and Drug Administration action level or any other established maximum allowable concentration of chemical residues in tissue available for atrazine. Therefore, a Final Residue Value cannot be determined.

### **OTHER DATA**

Many tests with atrazine and various freshwater or saltwater organisms have been conducted either for a different duration or by different protocols than those specified in the Guidelines for inclusion in Tables 1, 2, 4 and 5. These test results are presented in Table 6. For example, plant tests were included in Table 6 rather than Table 4 if the test duration was less than 4 days or the exposure concentrations were not measured (an exception was the saltwater species phytoplankton data that was included in Table 4 for comparison purposes). Tests with animals were included in Table 6 for a number of reasons, including considerations of test duration, type of test, and test endpoints other than those of toxicity or bioaccumulation. Below is a summary of their results.

At the lowest levels of biological organization, mixed nitrifying bacteria were unaffected regarding ammonium oxidation at 28-day exposures up to 2,000  $\mu$ g/L of atrazine (Gadkari 1988), and cell growth in the bacterium, *Pseudomonas putida*, was not inhibited following a 16-hour exposure at 10,000  $\mu$ g/L (Bringmann and Kuhn 1976, 1977). Progressing phylogenetically, Rohwer and Fluckiger (1979) obtained a 14-day growth LOEC of 2,160  $\mu$ g/L for *Anabaena cylindrica*, while Stratton (1984) obtained a 12 to 14-day EC50 of 1,200  $\mu$ g/L in terms of cell number. The latter EC50 value was approximately 5 to 7 times higher than the 24-hour EC50 values based on <sup>14</sup>C uptake of 253, 178 and 182  $\mu$ g/L as reported by Larsen et al. (1986) for this same species (Table 6). The other species of cyanobacteria tested by Stratton (1984), *Anabaena inaequalis* and *Anabaena variabilis*, had highly different EC50 values of 30 and 4,000  $\mu$ g/L after 14 days. *A. inaequalis* and *Pseudoanabaena* sp. exhibited reduced photosynthetic uptake of <sup>14</sup>C in the amounts of 65 and 91 percent, respectively, following a 22-hour exposure to 2,667  $\mu$ g/L of atrazine (Peterson et al. 1994).

A number of tests have been performed with the cyanobacterium, *Anabaena flos-aquae*. Hughes (1986) and Hughes et al. (1986, 1988) reported an EC50 based on cell number of  $230 \mu g/L$  following a

5-day exposure. A concentration of 40  $\mu$ g/L non-radiolabeled atrazine reduced <sup>14</sup>C uptake by approximately 50 percent after 1 to 3 days of exposure, after which the reduction was less (Abou-Waly et al. 1991a). At this concentration of atrazine, chlorophyll-*a* content was initially reduced but recovered with time. Using this characteristic, the 3-day EC50 was 58  $\mu$ g/L, while the 7-day EC50 was 766  $\mu$ g/L (Abou-Waly 1991b). *A. flos-aquae* had a 4-day EC50 based on chlorophyll-*a* that exceeded 3,000  $\mu$ g/L in a study by Fairchild et al. (1998).

The cyanobacterium *Microcystis aeruginosa* exhibited the onset of cell growth inhibition at a concentration of 3  $\mu$ g/L in an 8-day exposure (Bringmann and Kuhn 1976, 1978a,b). After 5 days of exposure, cell numbers were significantly reduced at 108  $\mu$ g/L, and the minimum algistatic concentration was 440  $\mu$ g/L (Parrish 1978). Kallqvist and Romstad (1994) obtained a 6-day EC50 of 630  $\mu$ g/L with *M. aeruginosa*, while Peterson et al. (1994) reported that photosynthetic <sup>14</sup>C uptake was highly reduced (84-96 percent) in *M. aeruginosa* following a 22-hour exposure to 2,667  $\mu$ g/L of atrazine. A 4-day EC50 of 90  $\mu$ g/L was reported for an unidentified species of *Microcystis* based on biomass (Fairchild et al. 1998).

Toxicity studies of atrazine toward several other species of cyanobacteria have been reported. Peterson et al. (1994) found that *Aphanizomenon flos-aquae* and *Oscillatoria* sp. exhibited highly reduced photosynthetic uptake of <sup>14</sup>C (97 and 87 percent, respectively) from a 22-hour exposure to 2,667  $\mu$ g/L of atrazine. The latter is consistent with the lowest complete inhibition of growth reported for *Oscillatoria* cf. *chalybea* after 6 days of exposure to 2,160  $\mu$ g/L atrazine (Schrader et al. 1997). A 31day exposure of *Plectonema boryanum* to 10,000  $\mu$ g/L of atrazine resulted in a 69 percent decrease in cell numbers (Mallison and Cannon 1984), whereas, 5-day exposures of *Synechococcus leopolensis* yielded an EC50 of 130  $\mu$ g/L (Kallqvist and Romstad 1994).

The green alga, *Ankistrodesmus braunii*, had an 11-day EC50 of 60  $\mu$ g/L (Burrell et al. 1985). Similarly, <sup>14</sup>C uptake EC50 values of 72 and 61  $\mu$ g/L resulted from 24-hour exposures of *Ankistrodesmus* sp. to atrazine (Larsen et al. 1986). The green alga, *Chlamydomonas geitleri* Ettl, had a slightly higher EC50 of 311  $\mu$ g/L based on CO<sub>2</sub> fixation after a 1-hour exposure (Francois and Robinson 1990). Similarly, a growth-based EC50 of 330  $\mu$ g/L was obtained for *Chlamydomonas noctigama* after 3 days of atrazine exposure (Kallqvist and Romstad 1994).

The green alga, *Chlamydomonas reinhardtii*, appears more sensitive to atrazine, exhibiting approximately a 32 percent inhibition of photosynthesis in an 8-hour exposure to 10  $\mu$ g/L (Valentine and Bingham 1976), and EC50 values based on reduction in photosynthetic activity (<sup>14</sup>C uptake) in 24-hour exposures of 19 to 48  $\mu$ g/L of atrazine (Larsen et al. 1986). Atrazine-sensitive and atrazine-resistant strains of *C. reinhardtii* responded to 2-minute exposures by a difference of approximately an order of magnitude in their respective EC50 values of 45 and 484  $\mu$ g/L (Hersh and Crumpton 1989). A 65-hour

exposure to 49.6  $\mu$ g/L resulted in a 13 percent reduction of chlorophyll (Hiranpradit and Foy 1992), and Fairchild et al. (1998) obtained a 96-hour chlorophyll-based EC50 of 176  $\mu$ g/L for this same species.

Foy and Hiranpradit (1977) exposed an unknown *Chlamydomonas* sp. to various concentrations of atrazine for 72 to 96 hours. Concentrations of 50 to 52  $\mu$ g/L inhibited growth by 84.9 percent and reduced chlorophyll by 12.8 percent. Slight additional increases in growth inhibition were observed with increased atrazine concentrations up to 832  $\mu$ g/L. Fairchild et al. (1994a) obtained a 4-day EC50 based on biomass of 176  $\mu$ g/L to a different species of *Chlamydomonas*.

*Chlorella fusca* cell reproduction was reduced and an EC50 of  $26 \ \mu g/L$  was calculated following a 24-hour exposure to atrazine (Altenburger et al. 1990). Similarly, Faust et al. (1993) obtained a 24hour EC50 of 15  $\mu g/L$  for this species, and Kotrikla et al. (1997) report 14-day EC50 values based on growth inhibition of 53.91 (exponential growth phase) and 75.73  $\mu g/L$  (stationary growth phase). In contrast, *Chlorella kessleri* exhibited 30 percent growth inhibition following a 72-hour exposure at a concentration of 1,078  $\mu g/L$  (El-Sheekh et al. 1994), while *Chlorella pyrenoidosa* had 70 to 95 percent reduced growth following 2-week exposures to atrazine concentrations ranging from 500 to 10,000  $\mu g/L$ (Virmani et al. 1975). Photosynthesis in this species was inhibited by approximately 64 percent following an 8-hour exposure to 100  $\mu g/L$  atrazine (Valentine and Bingham 1976). Stratton (1984) obtained an EC50 of 300  $\mu g/L$  following a 12- to 14-day exposure. A 30 percent reduction in growth and 40 percent reduction in chlorophyll-*a* was observed in a 10-day exposure to 53.9  $\mu g/L$  (Gonzalez-Murua et al. 1985), while a 110-hour exposure to 49.6  $\mu g/L$  reduced chlorophyll by 39 percent (Hiranpradit and Foy 1992). Photosynthetic CO<sub>2</sub> uptake was inhibited by more than 80 percent in *C. pyrenoidosa* following a less than 50-minute exposure to 125  $\mu g/L$  (Hannan 1995).

The green alga, *Chlorella vulgaris*, had 24-hour EC50 values of 325, 305 and 293  $\mu$ g/L in three separate tests based upon <sup>14</sup>C uptake (Larsen et al. 1986). Similarly, a 30-minute EC50 value of 305  $\mu$ g/L based on decreased oxygen evolution was obtained for the same species by Van der Heever and Grobbelaar (1997). Following 7 days of exposure to 250 to 5,000  $\mu$ g/L (only 2.3 to 4.7 percent remained on day 7), dry weights of *C. vulgaris* were reduced from 31 to 62 percent (Veber et al. 1981). This same species had an EC50 of 94  $\mu$ g/L based upon chlorophyll concentration after a 96-hour exposure (Fairchild et al. 1998). Reduced growth was initially observed for *C. vulgaris* exposed for 12 days to 10  $\mu$ g/L, although signs of recovery were evident by the end of the exposure (Berard et al. 1999).

In an undefined species of *Chlorella*, a 72- to 96-hour atrazine exposure at 52  $\mu$ g/L resulted in a 31 percent inhibition of growth and a 39 percent reduction in chlorophyll (Foy and Hiranpradit 1977). In that same study, higher exposures generally resulted in greater adverse effects. More recently, a 2- to 3-day atrazine exposure of 21.6  $\mu$ g/L reduced the growth rate of one *Chlorella* sp. by 55 percent (Hersh

and Crumpton 1987), and another study using *Chlorella* sp. exhibited very rapid responses to atrazine with EC50 values of 35 to 41  $\mu$ g/L based upon photosynthetic oxygen evolution following a 2-minute atrazine exposure (Hersh and Crumpton 1989). Fairchild et al. (1994a) reported a 4-day biomass-based EC50 of 92  $\mu$ g/L in yet another study using an unidentified species of the genus *Chlorella*.

Virmani et al. (1975) observed 75 and 92 percent reductions in growth of a much less sensitive species of green algae, *Chlorococcum hypnosporum*, following 2-week exposures to 5,000 and 10,000  $\mu$ g/L atrazine, respectively. Similarly, a high test concentration (2,157  $\mu$ g/L) was necessary to inhibit calcification in *Gloetaenium loitlesbergarianum* in a 96-hour test (Prasad and Chowdary 1981). Short exposures (2 minutes) to *Franceia* sp. yielded EC50 values between 430 and 774  $\mu$ g/L, measured as photosynthetic oxygen evolution (Hersh and Crumpton 1989).

In three tests with the green alga, *Scenedesmus obliquus*, the 24-hour EC50 values for <sup>14</sup>C uptake were between 38 and 57  $\mu$ g/L (Larsen et al. 1986). The green alga, *Scenedesmus quadricauda*, exhibited photosynthesis inhibition of approximately 42 percent after 8 hours at an atrazine exposure of 10  $\mu$ g/L (Valentine and Bingham 1976). Bringmann and Kuhn (1977, 1978a,b) found that 30  $\mu$ g/L caused the onset of cell multiplication inhibition after 8 days of atrazine exposure to this species. *S. quadricauda* exhibited a 12- to 14-day EC50 of 100  $\mu$ g/L based on cell number (Stratton 1984). Bogacka et al. (1990) studied photosynthesis reductions in *S. quadricauda* at various concentrations after 8 days of atrazine exposure. These authors observed a gradation from 4.5 percent reduction at 4  $\mu$ g/L to a 99.3 percent reduction at 337  $\mu$ g/L. Similarly, photosynthetic <sup>14</sup>C uptake was highly inhibited (96 percent) after 22 hours at 2,667  $\mu$ g/L of atrazine (Peterson et al. 1994). This species had a 96-hour EC50 of 169  $\mu$ g/L, based upon chlorophyll concentration (Fairchild et al. 1998).

In this same genera of algae, *Scenedesmus subspicatus* had a 4-day EC50 of 110  $\mu$ g/L (Geyer et al. 1985), and Schafer et al. (1994) found that 37  $\mu$ g/L of atrazine inhibited the effective photosynthetic rate of this species by 57.4 percent within 24 hours. This latter apparent effect concentration was corroborated by Kirby and Sheahan (1994) who reported a 2-day EC50 of 21  $\mu$ g/L based on cell numbers, as well as Zagorc-Koncan (1996) who reported a 24-hour EC50 value of 25  $\mu$ g/L based on net assimilation and inhibition. Reinhold et al. (1994) observed a 50 percent reduction in dry mass at 21.5  $\mu$ g/L within 24 hours, and Behra et al. (1999) reported a 60-day NOEC based on growth and photosynthetic oxygen evolution for this species of 20  $\mu$ g/L.

Exposure of an unidentified species of *Scenedesmus* for 72 to 96 hours at 50  $\mu$ g/L resulted in 60.2 percent growth inhibition (Foy and Hiranpradit 1977), and increased concentrations resulted in increased growth inhibition. Fairchild et al. (1994a) obtained a 4-day EC50 based on biomass of 169  $\mu$ g/L.

The green alga, *Selenastrum capricornutum*, exhibited a significant reduction in cell numbers following a 5-day exposure to 54  $\mu$ g/L of atrazine (Parrish 1978). In this study, chlorophyll-*a* reduction increased as concentrations increased from 32 and 200  $\mu$ g/L. The minimum algistatic concentration was determined to be 200  $\mu$ g/L. A similar 5-day LOEC for *S. capricornutum* growth of 220  $\mu$ g/L was recently reported by Schrader et al. (1998). Interestingly, a 7-day exposure at 100  $\mu$ g/L resulted in a 13.8 percent increase in biomass, whereas 1,000  $\mu$ g/L resulted in decreases (Johnson 1986). The lowest complete inhibition concentration of growth after a 6-day exposure was 2,160  $\mu$ g/L (Schrader et al. 1997).

There are a number of additional EC50 values from exposures of S. capricornutum to atrazine (Table 6). Larsen et al. (1986) obtained 24-hour EC50 values of 53, 34 and 42  $\mu$ g/L based upon <sup>14</sup>C uptake. In a couple of 21-day exposures (Turbak et al. 1986), biomass-based EC50 values of 58.7 and 410 µg/L were obtained using algal assay media and creek water for test media, respectively. Likewise, EC50 values were 69.7 and 854 µg/L, respectively, using these two media in 24-hour tests that measured photosynthetic oxygen evolution. Roberts et al. (1990) reported 5-day EC50 values of 100 and 95 µg/L based on cell numbers, and an EC50 of 50 µg/L based on cell numbers was reported in a 4-day exposure by Versteeg (1990). Similarly, El Jay et al. (1997) found the 4-day IC50 values based on chlorophyll-a content to be 80  $\mu$ g/L. Reductions in chlorophyll content and in <sup>14</sup>C uptake occurred at 130  $\mu$ g/L in 1- to 7-day exposures (Abou-Waly et al. 1991a). EC50 values were 283, 218 and 214 µg/L for chlorophyll-a content at 3, 5, and 7 days, respectively (Abou-Waly et al. 1991b). Fairchild et al. (1994a, 1998) reported a 4-day EC50 of 117 µg/L for chlorophyll content, while Kallqvist and Romstad (1994) obtained 3-day growth-based EC50 values of 200 and 110 µg/L. Photosynthetic <sup>14</sup>C uptake was almost completely inhibited (99 percent) within 22 hours at an exposure of  $2,667 \mu g/L$  (Peterson et al. 1994). A 96-hour EC50 of 147 µg/L was reported by Gaggi et al. (1995) for chlorophyll-a content. Additional cell number-based EC50 values reported for 72- to 96-hour exposures include 118.2 µg/L (Radetski et al. 1995), 359 µg/L (Van der Heever and Grobbelaar 1996), 200 and 220 µg/L (Abdel-Hamid 1996), and  $26 \,\mu\text{g/L}$  (Caux et al. 1996). Van der Heever and Grobbelaar (1997, 1998) expanded on their 1996 study and reported a 30-minute EC50 value based on decreased oxygen evolution of 222 µg/L (1997) and a 4hour EC50 value based on chlorophyll-a fluorescence of  $232 \mu g/L$  (1998). Benhra et al. (1997) reported an EC50 of 164.3 µg/L based on growth inhibition and Fairchild et al. (1997) reported a biomass-based EC50 of 235 µg/L.

Two tests with *Stigeoclonium tenue* yielded 24-hour EC50 values based on <sup>14</sup>C uptake of 127 and 224  $\mu$ g/L, while a test with *Ulothrix subconstricta* yielded an EC50 of only 88  $\mu$ g/L (Larsen et al. 1986).

Several diatom species have been tested for their sensitivities to atrazine. Chlorophyll-*a* content in the benthic diatom, *Craticula cuspidata*, was significantly reduced after 12 days exposure to  $83 \mu g/L$ 

atrazine immediately following 67 days in 1  $\mu$ g/L atrazine (Nelson et al. 1999). *Cyclotella meneghiniana* yielded 7-minute EC50 values based upon photosynthesis between 99 and 243  $\mu$ g/L (Millie and Hersh 1987), while a 22-hour exposure to 2,667  $\mu$ g/L of atrazine inhibited photosynthetic <sup>14</sup>C uptake by 97 percent (Peterson et al. 1994). A 6-day growth-based EC50 of 430  $\mu$ g/L was obtained for an unidentified species of *Cyclotella* by Kallqvist and Romstad (1994). Hughes (1986) and Hughes et al. (1986, 1988) determined several endpoints in 5-day exposures of *Navicula pelliculosa* to atrazine, including a 5-day EC50 of 60  $\mu$ g/L based on cell numbers. Using a 9-day recovery period following the 5-day exposure, they determined algistatic and algicidal concentrations of 1,710 and >3,200  $\mu$ g/L, respectively. Likewise, photosynthesis was almost completely inhibited (99 percent) in *Nitzschia* sp. by a 22-hour exposure to 2,667  $\mu$ g/L of atrazine (Peterson et al. 1994). The cryptomonad, *Cryptomonas pyrinoidifera*, which also appears to be somewhat less sensitive to atrazine, had a 6-day EC50 based on growth of 500  $\mu$ g/L (Kallqvist and Romstad 1994).

The duckweed, *Lemna minor*, when exposed to 20 µg/L of atrazine for 20 days, did not exhibit any adverse effects, but reduced growth occurred at concentrations of 50 to 250 µg/L (Beaumont et al. 1976,a,b, 1978). Peterson et al. (1994), on the other hand, observed that growth was inhibited 95 percent by a 7-day exposure to 2,667 µg/L. Four-day EC50 values for *L. minor* based on biomass and frond production were 153 and 92 µg/L, respectively (Fairchild et al. 1997, 1998). Biochemical and ultrastructural changes in the chloroplasts of *Lemna minor* were observed in 15-day exposures of 100 and 1000 µg/L of atrazine (Grenier et al. 1979) as well as an exposure of 248 µg/L (Grenier et al. 1987, 1989; Simard et al. 1990) for 15, 10 and 2 days, respectively. This is very close to the EC50 of 170 µg/L for frond production obtained when Hughes (1986) and Hughes et al. (1986, 1988) exposed a different species of duckweed, *Lemna gibba*, to atrazine for 5 days. Using a 9-day recovery period, the phytostatic and phytocidal concentrations were 1,720 and >3,200 µg/L, respectively.

Exposure of wild rice, *Zizania aquatica*, to 50  $\mu$ g/L of atrazine for 83 days resulted in a visible state of senescence and a 75 percent reduction in chlorophyll-*a* in the leaves (Detenbeck et al. 1996). Wild celery, *Vallisneria americana*, exhibited reduced leaf growth and whole plant biomass at an exposure of 8  $\mu$ g/L and reduced over-wintering success of tubers at 4  $\mu$ g/L (Cohn 1985). A 42-day test using this species resulted in an EC50 based on total leaf length of 163  $\mu$ g/L (Davis 1981; Forney and Davis 1981). A 14-day EC50 based on wet weight of 22  $\mu$ g/L was reported for coontail, *Ceratophyllum* sp. (Fairchild et al. 1998), and reduced stem elongation occurred within 6 to 8 days at 50  $\mu$ g/L (Detenbeck et al. 1996). These authors also found that cattails, *Typha latifolia*, were unaffected at 25  $\mu$ g/L atrazine after 19 days. The Eurasian watermilfoil, *Myriophyllum heterophyllum*, had a 14-day wet weight-based EC50 of 132  $\mu$ g/L (Fairchild et al. 1998) while *Myriophyllum spicatum* had a 28-day EC50 based on length of 1,104  $\mu$ g/L (Davis 1981; Forney and Davis 1981). This species also exhibited a 50 percent reduction in branch number at 3,700  $\mu$ g/L after 5 days (Bird 1993). Sago pondweed, *Potamogeton pectinatus*, on the other hand, had reduced biomass after 28 days at 100  $\mu$ g/L (Fleming et al. 1991), and bushy pondweed, *Najas* sp., had a 14-day wet weight-based EC50 of 24  $\mu$ g/L (Fairchild et al. 1998). A 14-day biomass-based EC50 of <38  $\mu$ g/L was reported for *Egeria* sp. (Fairchild et al. 1994a).

The exposure of *Elodea canadensis* to atrazine for 21 and 28 days resulted in EC50 values based on length of 109 and 80  $\mu$ g/L, respectively (Davis 1981; Forney and Davis 1981), and Detenbeck et al. (1996) reported that growth was unaffected after 19 days at 75  $\mu$ g/L. Fairchild et al. (1998) reported a 14-day EC50 of 21  $\mu$ g/L for *E. canadensis* based upon wet weight.

Three species of water moss (*Fontinalis antipyretica, Fontinalis hypnoides* and *Fontinalis squamosa*) were tested by Hoffman and Winkler (1990). While *F. squamosa* and *F. antipyretica* were affected in their photosynthetic production at 10  $\mu$ g/L after 24 hours and 20 days, respectively, *F. hypnoides* exhibited a much greater reduction (90 percent) in net photosynthesis within 24-hours at an exposure of only 2  $\mu$ g/L. Conversely, Johnson (1986) found that 10  $\mu$ g/L stimulated growth of mixed macrophytes, *Ceratophyllum* sp. and *Elodea* sp., but that 100 and 1,000  $\mu$ g/L decreased plant biomass after 30 days.

The protozoan, *Acanthamoeba castellanii*, had population decreases of from 5 to 40 percent when exposed for 6 days to atrazine at concentrations from 100 to 10,000  $\mu$ g/L (Prescott et al. 1977). Photosynthesis was inhibited by about 11 percent in *Euglena gracilis* at 10  $\mu$ g/L after 8 hours, and exhibited increasingly greater inhibition at higher concentrations (Valentine and Bingham 1976). Two species of protozoans, *Colpidium campylum* and *Tetrahymena pyriformis*, had 24-hour EC50 values of >50,000 (Roberts et al. 1990) and 118,500  $\mu$ g/L (Huber et al. 1991), respectively. Schafer et al. (1994) reported a 48-hour EC50 of 96,000  $\mu$ g/L for *T. pyriformis*.

Relatively high concentrations were required to produce notably adverse responses in representatives from higher animal phyla. A concentration of 5,000  $\mu$ g/L reduced the budding rate in *Hydra viridis* after 21 days (Benson and Boush 1983). The rotifer, *Brachionus calyciflorus*, had a 24-hour LC50 of 7,840  $\mu$ g/L (Crisinel et al. 1994). Two species of leeches, *Glossiphonia complanata* and *Helobdella stagnalis*, had LC50 values of 6,300 and 9,900  $\mu$ g/L, respectively, after a 27- to 28-day exposure (Streit and Peter 1978). After 21 weeks, snail (*Lymnaea palustris*) growth, fecundity and tissue glycogen content were unaffected at concentrations up to 125  $\mu$ g/L (Baturo et al. 1995), but the activities of benzo[a]pyrene and glutathione-<u>s</u>-transferase enzymes were inhibited at 5  $\mu$ g/L (Baturo and Lagadic 1996). The 24- and 48-hour LC50 values were greater than 60,000  $\mu$ g/L for both larval and juvenile mussels, *Anadonta imbecilis* (Johnson et al. 1993).

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The anostracan crustacean, Streptocephalus texanus, had a 24-hour LC50 of >30,000 µg/L (Crisinel et al. 1994). The cladoceran, Ceriodaphnia dubia, exhibited maximum acceptable toxicant concentrations (MATCs) of 7,100 and 14,100 µg/L in two 4-day tests (Oris et al. 1991). A 26-hour LC50 of 3,600 µg/L was reported for *Daphnia magna* (Frear and Boyd 1967). In 48-hour exposures of Daphnia magna to a nominal atrazine concentration of  $10 \mu g/L$ , whole body residues were only 4.4 and 2.2 times greater than the nominal concentration in water (Ellgehausen et al. 1980). Young production was reduced in *D. magna* after 21 days at 2,000 µg/L (Kaushik et al. 1985). After 96 hours of exposure, Bogacka et al. (1990) observed a 30 percent mortality in D. magna at 16,900 µg/L, and a 60 percent mortality at 48,300 µg/L. Johnson et al. (1993) reported a 48-hour LC50 of 9,400 µg/L, but the animals were fed at 24 hours. Crisinel et al. (1994) obtained a 24-hour EC50 of >30,000 µg/L, while Detenbeck et al. (1996) observed a significant decrease in the survival of these invertebrates after 48 hours of exposure at 25  $\mu$ g/L, but not at 50  $\mu$ g/L. Nishiuchi and Hashimoto (1967, 1969) found the 3-hour LC50 to be greater than 40,000 µg/L for *Daphnia pulex*. Exposures of *D. pulex* for 28 to approximately 70 days resulted in decreased survival and reproduction at concentrations ranging from 1,000 and 20,000 µg/L atrazine, with reproduction affected more than survival (Schober and Lampert 1977). Food consumption was reduced by 10 percent at 350 µg/L and by 50 percent at 1,600 µg/L after 10 minutes (Pott 1980). Bowman et al. (1981) reported an 18-hour LC50 for D. pulex of approximately 700 µg/L. Conversely, the 3-hour LC50 was in excess of 40,000 µg/L for the cladoceran, Moina macrocopa (Nishiuchi and Hashimoto 1967, 1969), and a concentration of  $1,000 \,\mu$ g/L was shown to cause 40 percent mortality and reduced population growth after 4 to 6 weeks (Shcherban 1972a,b).

The amphipod, *Gammarus fasciatus*, had a 48-hour LC50 of 5,700 µg/L (Macek et al. 1976). Similarly, exposure of *Hyalella azteca* for 18 hours resulted in an LC50 of 2,000 µg/L (Bowman et al. 1981). For the midge, *Chironomus riparius*, a 10-day exposure to atrazine yielded an LC50 of 18,900 µg/L (Taylor et al. 1991), while a 96-hour exposure of *C. tentans* in a fed test had less than 50 percent mortality at the high concentration of 28,000 µg/L (McNamara 1991b). Macek et al. (1976) reported a LC50 of 720 µg/L for a 48-hour *C. tentans* midge test initiated with first instar animals, which did not adhere to the 2<sup>nd</sup> or 3<sup>rd</sup> instar life stages requirement specified by the Guidelines. Pape-Lindstrom and Lydy (1997) and Jin-Clark et al (2002) likewise used 4<sup>th</sup> instar larvae to initiate *C. tentans* acute tests that yielded LC50 values of >20,000 and >1,000 µg/L atrazine, respectively. The 18-hour LC50 for the white dotted mosquito, *Culex restuans*, is considerably higher at approximately 60,000 µg/L (Bowman et al. 1981).

Rainbow trout, *Oncorhynchus mykiss*, embryos and sac fry exposed continuously for 23 (embryos at hatching) and 27 (sac fry, 4 days post-hatch) days had LC50 values between 696 and 888  $\mu$ g/L (Birge et al. 1979). Water hardness did not have any appreciable effect. A concentration of 4,020

 $\mu$ g/L was required to produce over 60 percent teratic larvae. Pluta (1989) reported a 48-hour LC50 of 5,660  $\mu$ g/L. Changes in the ultrastructure of trout renal corpuscles and tubules were observed following 28-day exposures to 5 to 10  $\mu$ g/L of atrazine (Fischer-Scherl et al. 1991). Similarly, 28-day exposures resulted in slight ultrastructural changes in trout renal corpuscles at 5  $\mu$ g/L, slight histopathological changes in the liver and increased ultrastructural changes in renal corpuscles at 10  $\mu$ g/L, and in further changes in renal corpuscles and liver cells at 20  $\mu$ g/L (Schwaiger et al. 1991). A 14-day exposure to 10  $\mu$ g/L of atrazine did not affect survival, body weight, liver weight or liver enzyme activity (Egaas et al. 1993). Exposure to concentrations of 3.0 and 50  $\mu$ g/L for 10 days were reported to reduce plasma protein in rainbow trout, but no effects were observed at 10  $\mu$ g/L (Davies et al. 1994b). Oulmi et al. (1995) observed kidney changes at the cellular level within 5 weeks in *O. mykiss* in the proximal tubules at 12.4  $\mu$ g/L, and in both the proximal and distal tubules at 24.0  $\mu$ g/L.

The 48-hour LC50 for the goldfish, *Carassius auratus*, was >10,000 µg/L (Nishiuchi and Hashimoto 1967, 1969), although Saglio and Trijasse (1998) observed reduced burst swimming performance in goldfish after a 24-hour exposure to 50 µg/L. The 48-hour LC50 for the common carp, *Cyprinus carpio*, was also >10,000 µg/L (Nishiuchi and Hashimoto 1967, 1969). Short-term exposures of from 4 to 24 hours to lesser concentrations between 100 and 500 µg/L resulted in increased serum cortisol and serum glucose (Hanke et al. 1983). Serum acetylcholinesterase first increased and then decreased with time of exposure. Changes were also noted in gill ATPase activity. Longer exposures of 72-hour duration to 1,000 µg/L and 100 µg/L of atrazine also yielded decreased liver glycogen (Hanke et al. 1983), and decreased liver and muscle glycogen as well as serum protein and cholesterol (Gluth and Hanke 1984, 1985), respectively. Juvenile carp yielded a 48-hour LC50 of 16,100 µg/L (Pluta 1989), and a 96-hour LC50, in which the fish were fed, of 18,800 µg/L (Neskovic et al. 1993). It was noted in the latter study that biochemical changes in the serum, heart, liver and kidneys of carp were observed after 14 days of exposure to 1,500 µg/L, as well as hyperplasia of gill epithelial cells (Neskovic et al. 1993). Conversely, no effects on gill, liver, and histopathology were observed at this same concentration (1,500 µg/L) in a study by Poleksic et al. (1997).

Jop (1991c) reported the "no observed effect concentration" (NOEC) to be in excess of 4,900  $\mu$ g/L for fathead minnows, *P. promelas*, exposed to atrazine for 7 days. Also, survival and growth were shown to be unaffected in fathead minnows exposed to 75  $\mu$ g/L for 13 days (Detenbeck et al. 1996). On the other hand, channel catfish (*Ictalurus punctatus*) embryos and sac fry had LC50 values between 176 and 272  $\mu$ g/L after exposures of either 4.5 (embryos at hatch) or 8.5 (sac fry, 4 days post-hatch) days (Birge et al. 1979). Concentrations of approximately 340  $\mu$ g/L caused an incidence of 13 to 16 percent teratic larvae, while concentrations of approximately 3,850  $\mu$ g/L resulted in 47 to 69 percent teratic larvae.

Mosquitofish (*Gambusia affinis*) survival was unaffected in a 48-hour exposure to 10,000  $\mu$ g/L (Darwazeh and Mulla 1974), and LC50 values as high as 38,200 and 31,600  $\mu$ g/L were reported for the guppy (*Poecilia reticulata*) after exposures of 48 and 72 hours, respectively (Tscheu-Schluter 1976). These data are consistent with results reported by Bogacka et al. (1990), in which the authors reported mortalities of 40 and 53.2 percent after exposing guppies for 96 hours to 28,600 and 37,200  $\mu$ g/L, respectively.

Exposure of the Mozambique tilapia, *Tilapia mossambica*, to 1,100 µg/L of atrazine for 30 to 90 days affected blood composition, oxygen consumption, water content, and the biochemistry of the brain and liver (Prasad et al. 1991a,b; Srinivas et al. 1991). A 90-day exposure also resulted in increased serum sodium and potassium, and decreased serum calcium, magnesium and bicarbonate (Prasad and Reddy 1994).

The embryo and larval stages of several amphibian species were exposed to atrazine (Birge et al. 1980), the results of which are quite different between species (Table 6). LC50 values for continuous exposure of embryos and larvae through 4 days post-hatch were 410  $\mu$ g/L for the bullfrog (*Rana catesbeiana*), 7,680  $\mu$ g/L for the leopard frog (*Rana pipiens*), 17,960  $\mu$ g/L for the pickerel frog (*Rana palustris*), and >48,000  $\mu$ g/L for the American toad (*Bufo americanus*). In most of these species, concentrations of atrazine in excess of 5,000  $\mu$ g/L were required to cause an incidence of teratic larvae in excess of 7 percent. Survival and growth of *R. pipiens* tadpoles were unaffected after 41 days of exposure to 25  $\mu$ g/L (Detenbeck et al. 1996). A 96-hour exposure of the African clawed frog (*Xenopus laevis*) embryos to 8,000  $\mu$ g/L resulted in 100 percent abnormal embryos (Morgan et al. 1996). The lowest observed effect concentration (LOEC; teratogenesis) in the study was 1,100  $\mu$ g/L. This concentration is more than an order of magnitude higher than that which delayed development and retarded the growth in the tiger salamander, *Ambystoma tigrinum*, after 86 days of exposure (Larson et al. 1998).

In summary, cyanobacteria had EC50 values for various exposure durations of 30  $\mu$ g/L or greater, while EC50 values for green algae, diatoms and cryptomonads were \$15  $\mu$ g/L. Among macrophytes, duckweed had a minimal 4-day EC50 of 92  $\mu$ g/L. Wild rice was affected at 50  $\mu$ g/L, and wild celery had reduced growth at 8  $\mu$ g/L. Several rooted vascular plants (i.e., coontail, bushy pondweed, egeria, and elodea) had 14-day EC50 values between 21 and <38  $\mu$ g/L, while that for a water milfoil was 132  $\mu$ g/L. Two species of water moss (*Fontinalis* sp.) exhibited reduced photosynthetic activity at 10  $\mu$ g/L, and one species was affected at 2  $\mu$ g/L. EC50/LC50 values for protozoans, coelenterates, annelids, molluscs and rotifers were \$6,300  $\mu$ g/L. Various crustaceans had LC50 values \$5,700  $\mu$ g/L. The most sensitive endpoints among fish were rainbow trout plasma protein and kidney ultrastructural changes at atrazine exposures of 3 and 3.5  $\mu$ g/L, respectively. The lowest LC50 values in

fish were 176-272  $\mu$ g/L for 4.5 to 8.5-day exposures with early life-stages of channel catfish. Frog embryo and tadpole life-stages had LC50 values \$410  $\mu$ g/L. As noted in the ecosystem effects data section in this document, most reductions in algal or vascular plant biomass were observed at concentrations \$15  $\mu$ g/L. This commonly resulted in the reduction of herbivore populations, as well. One exception reported effects at much lower concentrations (as low as 0.1  $\mu$ g/L). From these freshwater Other Data, most of the effect levels of possible biological significance appear to be \$15  $\mu$ g/L. This concentration is greater than the freshwater Final Chronic Value based on ecosystem effects data (10  $\mu$ g/L), and therefore does not determine the Criterion Continuous Concentration.

Additional data are available for saltwater algae, kelp, submerged vascular plants, emergent vascular plants, and aquatic animals (Table 6). EC50 values based on differing endpoints (e.g., oxygen evolution or growth) for various green algal species ranged from 37  $\mu$ g/L to 600  $\mu$ g/L (Gaggi et al. 1995; Hollister and Walsh 1973; Hughes 1986; Hughes et al. 1986, 1988; Samson and Popovic 1988; Walsh 1972). A 48-hour exposure of the green alga, *Dunaliella bioculata*, to 216  $\mu$ g/L of atrazine resulted in a growth reduction of approximately 35 percent (Felix et al. 1988). Seven-day growth tests with the green alga, *Nannochloris oculata*, at concentrations of 50 and 100  $\mu$ g/L suggested that atrazine toxicity was dependent on light and temperature (Karlander et al. 1983; Mayasich et al. 1986), although the effect was not dramatic. A concentration of 15  $\mu$ g/L changed the doubling time in *N. oculata* (Mayasich et al. 1987).

Diatom species were similar to green algae in terms of their sensitivities to atrazine. EC50 values for exposures of various durations were generally between 20 and 460  $\mu$ g/L (Hollister and Walsh 1973; Walsh 1972; Walsh et al. 1988). Plumley and Davis (1980) observed reduced photosynthesis in *Nitzschia sigma* and reduced chlorophyll in *Thalassiosira fluviatilis* in 7-day exposures to 220  $\mu$ g/L. Mayasich et al. (1987) reported a limited effect on doubling time to *Phaeodactylum tricornutum* in a 7-day exposure to 50  $\mu$ g/L of atrazine.

The red alga, *Porphyridium cruentum*, had an EC50 based on oxygen evolution of 79  $\mu$ g/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  $\mu$ g/L (Hopkins and Kain 1971). The 28-day LOEC for this species based on growth of new sporophytes was 10  $\mu$ g/L. It was shown in another species of kelp, *Laminaria saccharina*, that a 2-day exposure to \$72.2  $\mu$ g/L of atrazine was sufficient to significantly reduce sexual reproduction, but no effect was detected at 33.2  $\mu$ g/L (Thursby and Tagliabue 1990).

Inhibition concentrations of 77 to  $120 \ \mu g/L$  for a 50 percent effect on photosynthesis by vascular plants in short-term (2- to 4-hour) exposures to atrazine (Jones and Winchell 1984; Jones et al. 1986) were similar to the effects upon growth and photosynthesis in longer exposures with several other species (Table 4). Studies involving *Vallisneria americana* at low salinities for 42 to 47 days resulted in

reduced leaf production in terms of length, leaf area, and dry weight for concentrations ranging from 12 to 320  $\mu$ g/L of atrazine (Correll and Wu 1982; Forney 1980; Forney and Davis 1981). Eelgrass, *Zostera marina*, had reduced oxygen evolution at 100  $\mu$ g/L, and complete inhibition of photosynthesis and growth at 1,000 (Kemp et al. 1982a) and 1,900  $\mu$ g/L of atrazine (Schwarzschild et al. 1994). Walsh et al. (1982) report a 40-hour EC50 of 320  $\mu$ g/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  $\mu$ g/L, while the salt-marsh grass, *Spartina alterniflora*, only exhibited enhanced peroxidase activity at a concentration as high as 3,100  $\mu$ g/L for the same length of time (Lytle and Lytle 1998).

The three LC50 values for the copepod, *Acartia tonsa*, at 24, 48 and 72 hours showed that the sensitivity to atrazine increased with increasing duration of exposure (McNamara 1991b; also see Table 1). The 96-hour EC50 in the juvenile Eastern oyster, *Crassostrea virginica*, as well as the 48-hour LC50 for the juvenile spot, *Leiostomas santhurus*, were both \$1,000  $\mu$ g/L, while the brown shrimp, *Penaeus aztecus*, had a 48-hour EC50 of 1,000  $\mu$ g/L (Butler 1964; Mayer 1987). Adult fiddler crabs, *Uca pugnax*, were not very sensitive to one-time applications of atrazine either in field or laboratory exposures (Plumley et al. 1980). However, there was a seasonal effect on the sensitivity of this species even when the laboratory conditions were the same. Animals collected in the summer were more sensitive to atrazine than those collected in either the spring or fall. Two other species of crabs, *Sesarma cinereum* and *Panopeus* sp., were also insensitive to very high levels of atrazine (Plumley et al. 1980).

The acute and chronic effects of atrazine on an estuarine microbial community were recently examined by DeLorenzo et al. (1999a,b). Exposure for 9 days to 40  $\mu$ g/L of atrazine in dilute seawater (7-25 g/kg) inhibited the phototrophic component - chlorophyll-*a*, carbon assimilation, biovolume, and caused changes in species composition (DeLorenzo et al. 1999a). The same effects were observed in full strength seawater at an atrazine concentration of 47  $\mu$ g/L, but within 24 hours (DeLorenzo et al. 1999b).

#### **UNUSED DATA**

Data from some studies were not used in this document, as they did not meet the criteria for inclusion as specified in the Guidelines (Stephan et al. 1985). The reader is referred to the Guidelines for further information regarding these criteria.

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#### Studies Were Conducted with Species That Are Not Resident in North America

Alazemi et al. (1996)	Gzhetotskii et al. (1977)	Nagel (1992)
Biagianti-Risbourg and Bastide (1995)	Hussein et al. (1996)	Pantani et al. (1997)
Diaz et al. (1998)	Juhnke and Luedemann (1978)	Portmann (1972)
Forget et al. (1998)	Kirby et al. (1998)	Prasad et al. (1990, 1995)
Görge and Nagel 1990	Lewis et al. (1993	Ralph (2000)
Gunkel and Kausch (1976)	L'Haridon et al. (1993)	Steinberg et al. (1995)

Results were not used if the duration of the exposure was not specified or was unclear (e.g., Hopkins and Kain 1968; Portmann 1972; Rojickova-Padrtova and Marsalek 1999; Tellenbach et al. 1983), or if the procedures or test materials were not adequately described or translated (e.g. Braginskii and Migal 1973; Delistraty 1999; Kross et al. 1992; Moore and Lower 2001; Moore and Waring 1998; Shcherban 1973; Tang et al. 1998a,b; Wenzel et al. 1997).

Acute toxicity data were not used if an insufficient number of test organisms (Bathe et al. 1973, 1975), or exposure concentrations were used (Allran et al. 2000; Bouilly et al. 2003). Data were also not used if there was a lack of a dose response (Bester et al. 1995; Britson and Threlkeld 2000). High control moralities occurred in tests reported by Dodson et al. (1999), as well as in chronic studies with *Daphnia magna, Gammarus fasciatus* and fathead minnows (Macek et al. 1976). Studies published only as abstracts of presentations were not used (e.g., Fairchild et al. 1994b; Palmstrom and Krieger 1983; Zora and Paladino 1986). Secondary observations reported in a review were not used (e.g., Giddings and Hall 1998; Hurlbert 1975; Hutchinson et al. 1998; Lange et al. 1998; Mercurio 1998). Similarly, papers by Birge et al. (1983), Fairchild et al. (Manuscript), Mark and Solbe (1998), and Pratt et al. (1993, 1997) were not used since data from several algal taxa were grouped in the reporting of results. Stratton and Giles (1990) expressed toxicity on the basis of cell numbers.

#### Atrazine Was a Formulation or Emulsifiable Concentrate (and comprised <80% of its weight)

Antychowicz et al. (1979) Carder and Hoagland (1998) Clements et al. (1997) deNoyelles et al. (1982) Hartman and Martin (1985) Hiltibran (1967) Hofmann and Winkler (1990) Howe et al. (1998) Lin et al. (1999) Kettle et al. (1987) Pavlov (1976) Rojickova-Padrtova & Marsalek 1999

Semov and Iosifov (1973) Sreenivas and Rana (1991, 1994) Torres and O'Flaherty (1976) Walker (1964)

#### Atrazine Was a Component of a Drilling Mud, Effluent, Mixture, Sediment or Sludge

Berard et al. 1999 Britson and Threlkeld (1998) Crain et al. (1998) Goodbred et al. 1997 Guasch and Sabater (1998) Guasch et al. (1997, 1998) Hartgers et al. (1998) Lowcock et al. (1997) Ort et al. (1994) Pollehne et al. (1999) Putt (2003) Reeder et al. (1998) Vanderpoorten (1999)

Toxicity data from laboratory tests were generally not used if atrazine was dosed in the diet (e.g., Cossarini-Dunier et al. 1988), or if the concentration of solvent used in atrazine stock preparation exceeded 0.5 ml/L (e.g., Cheney et al. 1997; Crain et al. 1997, 1999; Messaad et al. 2000; Pennington and Scott 2001; Schafer et al. 1994; Tang et al. 1997); the latter representing a value below which neither acetone nor ethanol are toxic to algae (e.g., El Jay 1996; Stratton and Corke 1981), but where DMSO and atrazine may interact additively (El Jay 1996).

The results from Langan and Hoagland (1996) were not used because the tests were conducted in distilled water without addition of the appropriate salts. Toxicity tests by Schmitz et al. (1994) and Tubbing et al. (1993) were not used because the tests were performed in river water which was likely contaminated with various other chemicals. Similarly, a cytopathological study of fish exposed to a spill of atrazine plus other pesticides was not used (e.g., Spazier et al. 1992). Effects data were not used if the atrazine exposure was part of a soil mixture (e.g., Johnson et al. 1999; Jones and Estes 1984; Lytle and Lytle 1998; Miller and Doxtader 1995; Ruth 1997). McBride and Richards (1975) exposed excised tissue, and Petit et al. (1997) exposed cell cultures.

A study of atrazine accumulation by Bohm and Muller (1976) was not used due to expression of results on a volume basis rather than a weight basis. A bioconcentration study by Walsh and Ribelin (1973) was not used due to the use of nominal atrazine concentrations in the exposure water rather than measured concentrations. Data were not used if the exposure was to radiolabeled atrazine (e.g., Davis et al. 1979; Jones et al. 1982; McEnerney and Davis 1979; Neumann et al. 1987; Nikkila et al. 2001; Pillai et al. 1977, 1979; Weete et al. 1980), or atrazine was not detected in tissue (e.g., Harris et al. 1998). Uptake and accumulation from exposures in flasks or microcosms were not used if <sup>14</sup>C only was measured and not atrazine itself (e.g., Huckins et al. 1986; Isensee 1976, 1987; Kearney et al. 1977; Mailhot 1987).

Biochemical studies of resistant strains of mutated algae (e.g., Boura-Halfon et al. 1997; Forster et al. 1997; Ottmeier et al. 1991) and results from *in vitro* genotoxicity and mutagenicity tests (e.g., Ruiz and Marzin 1997) were not used. A study of atrazine effects upon promutagen activation by

*Selenastrum capricornutum* (e.g., Sauser and Klaine 1990) and alteration in allele and genotype frequencies of the oyster, *Crassostrea gigas* (e.g., Moraga and Tanguy 2000) were also not used.

#### SUMMARY

Atrazine is not highly toxic to aquatic animals on an acute basis. SMAVs for eight freshwater invertebrate species ranged from 3,000 µg/L for a hydra, *Hydra sp.*, to 49,000 for *Daphnia magna*. SMAVs for nine fish species ranged from 5,300 µg/L for the rainbow trout, *Oncorhynchus mykiss*, to 60,000 µg/L for the goldfish, *Carassius auratus* (Figure 1). The three amphibian species evaluated each has a LC50 value of >20,000 µg/L atrazine. GMAVs for atrazine are available for nine genera of saltwater animals and range from 2,324 to >30,000 µg/L; a factor of approximately 12.9 (Figure 2). GMAVs for the four most sensitive genera (three species of crustaceans and one fish) differed by a factor of approximately 2.5.

Chronic effects of atrazine exposure to aquatic animals have been studied with six freshwater species, two of which are invertebrates and four of which are fish (Figure 3). In three tests with *Ceriodaphnia dubia*, chronic values were 3,536, 3,536, and 1,732  $\mu$ g/L. The growth of a midge, *Chironomus tentans*, was retarded at 230  $\mu$ g/L of atrazine, but not at 110  $\mu$ g/L. A chronic value of 159.1  $\mu$ g/L was calculated, and a corresponding acute-chronic ratio of 4.525 was derived.

Brook trout, *Salvelinus fontinalis*, had reduced growth at 120 µg/L, but not at 65 µg/L, in a chronic exposure. A chronic value of 88.32 µg/L and an acute-chronic ratio of 71.33 were calculated. In a life-cycle test with the fathead minnow, *Pimephales promelas*, the chronic limits were set at 250 and 460 µg/L, based upon growth of larval fish, resulting in a chronic value of 339.1 µg/L and an acute-chronic ratio of 58.98. Bluegills, *Lepomis macrochirus*, were unaffected in a chronic exposure to 95 µg/L, thereby setting the chronic limits at 95 and >95 µg/L, with a chronic value of >95 µg/L. Since the acute value was a "greater than" value, the acute-chronic ratio was >84.21.

Chronic values are available for three species of saltwater organisms. The chronic values for *Eurytemora affinis* ranged from 5,020 to 20,920  $\mu$ g/L, based on survival. The chronic value for *Americamysis bahia* was 123.3  $\mu$ g/L, also based on survival. The chronic value for *Cyprinodon variegatus* was 2,542  $\mu$ g/L, based on mortality of juveniles. The resultant acute-chronic ratio for *E. affinis* was 2.629, while the acute-chronic ratios for *A. bahia* and *C. variegatus* were 8.110 and >6.294, respectively.

Effect concentrations for freshwater and saltwater plants are lower than the acute and chronic values for aquatic animals (Figures 4 and 5). Atrazine toxicity to aquatic plants, both algae and macrophytes, commonly occurs at concentrations of  $10 \mu g/L$  and above, with several reports of toxicity

to specific plant taxa at concentrations below 10  $\mu$ g/L (primarily freshwater plant species). Effects are thought to be algistatic rather than algicidal at these lower concentrations, with recovery occurring once the atrazine is removed. The lowest EC50 values for freshwater green algae with exposure durations of 4 days or longer were 10.2 and 4  $\mu$ g/L for *Chlamydomonas reinhardtii* and *Selenastrum capricornutum*, respectively. Mean EC50 values for these species would be considerably higher. The lowest reported EC50 value for a freshwater vascular plant species, *Lemna gibba*, was 37  $\mu$ g/L in a 14-day exposure, using wet weight as an endpoint (Figure 4). As stated in the Guidelines (Stephen et al. 1985), the Final Plant Value (FPV) is the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured, and the endpoint was biologically important. In this case, the freshwater FPV is 17.25  $\mu$ g/L atrazine, which is the geometric mean of the two duckweed species (*Lemna gibba* and *Lemna minor*) species mean chronic values (SMCVs) of 6.44  $\mu$ g/L (Hoberg 1993b,c) and 46.19  $\mu$ g/L (Text Table A: University of Mississippi 1990; Girling et al. 2000). Using the geometric mean of the two SMCVs for *Lemna* is consistent with the Guidelines, and is how all the SMAVs and GMAVs are calculated in the WQC documents.

Conversely, the lowest EC50 based on growth for a saltwater green algae species, *Neochloris* sp., was 82  $\mu$ g/L, while the equivalent value for a saltwater vascular plant species, *Myriophyllum spicatum*, was 25  $\mu$ g/L. For saltwater, the FPV would be the geometric mean of the three *Potamogeton pectinatus* (Sago pondweed) measured chronic studies conducted by Hall et al. (1997) at different salinities, or 16.83  $\mu$ g/L atrazine (Text Table B). Using the geometric mean of the SMCVs for the three *Potamogeton pectinatus* tests is consistent with the Guidelines, and is how all the SMAVs and GMAVs are calculated in the WQC documents.

Aquatic ecosystem structural and functional parameters have most frequently been observed to be adversely affected by atrazine concentrations of 10  $\mu$ g/L and above (Figures 4 and 5). Ecosystem effects have been shown to occur at atrazine concentrations less than 5-10  $\mu$ g/L, but data are limited. Several microcosm and mesocosm studies ranging from 7 days to 2 months report no effect of atrazine on community structure, composition and functionality at atrazine as low as 5  $\mu$ g/L (Gruessner and Watzin 1996, Brockway et al. 1984, Van den Brink 1995, Juttner et al. 1995). The ecosystem effects that do occur below 5  $\mu$ g/L are generally transient and not well established. Recovery is quite rapid and functionality is generally not compromised until much higher concentrations are reached. It appears that for effects at concentrations up to 15  $\mu$ g/L, the communities can recover quite rapidly following dissipation of the atrazine concentration. The median LOEC from 65 community studies using multiple endpoints, excluding those studies where recovery was known to occur, is 60  $\mu$ g/L, and the 5<sup>th</sup> percentile LOEC is 10  $\mu$ g/L (Figure 6). The observed effects have been on both the plant and animal communities, with the effects upon the animal community being secondary in nature, mainly a result of decreased availability of shelter and plant matter for food. Thus, permanent ecosystem effects should only occur at atrazine concentrations greater than  $10 \,\mu g/L$ .

Atrazine has been reported in a number of studies as an endocrine disruptor. Laboratory exposures of 1  $\mu$ g/L atrazine have been reported to cause abnormalities in frog (*Xenopus laevis*) gonadal development (feminization and hermaphroditism - which could render male frogs sterile) and reduction in the size of the laryngeal muscle in male frogs, an important muscle used for the mating call of the frog (Hayes et al. 2002; Text Table C). However, studies conducted by Carr et al. (2003) and Carr and Salomon (2003) designed to replicate the Hayes et al. (2002) experiments observed these same gonadal development effects at approximately 20-21 µg/L atrazine. A third study conducted by Sullivan et al. (2003) with *Xenopus laevis* looking at the same end-points yielded an effect level of  $20 \,\mu$ g/L atrazine (the lowest concentration tested). Until this issue is resolved, justification and defense of a freshwater chronic criterion based on the endocrine disrupting effects of atrazine on amphibians is not possible. A recently convened Scientific Advisory Panel agreed with EPA's conclusion that additional studies are warranted to reduce the scientific uncertainty regarding whether atrazine causes replicable effects on amphibians (Scientific Advisory Panel 2003). Substantial additional research to resolve this issue is currently underway, or planned for the immediate future. Once additional data are available that conclusively demonstrate a significant reproductive effect (or other endpoint that significantly impairs the populations ability to survive long term) to aquatic species, then derivation of the freshwater chronic criterion will be reevaluated.

Atrazine has a limited tendency to accumulate in tissues of aquatic animals. BCFs ranged from <0.27 to a maximum of 8.5 in three species of freshwater fish. There are no BCFs available for saltwater species.

The national criteria are determined on the basis of atrazine toxicity to aquatic animals (acute criteria), ecosystem effects (freshwater chronic criterion), and toxicity to plants (saltwater chronic criterion). The Criterion Maximum Concentrations (CMC) for fresh water (1,511  $\mu$ g/L) and salt water (759.5  $\mu$ g/L) are one-half of the respective Final Acute Values (3,021 and 1,519  $\mu$ g/L, respectively). These values are based on Table 1 acute toxicity values for all invertebrate and vertebrate species. The Criterion Continuous Concentration (CCC) for freshwater is based on the ecosystem effects of atrazine to aquatic plants. The saltwater CCC of 16.83  $\mu$ g/L is based on the Final Plant Value determined for the Sago pondweed.

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## NATIONAL CRITERIA

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic life and their uses should not be directly affected unacceptably if the Average Primary Producer Steinhaus Similarity deviation for a site is less than 5% (as determined using Comprehensive Aquatic Systems Model (CASM)<sup>3</sup> or other appropriate model and index) and is not exceeded more than once every three years (or other appropriate return frequency sufficient to allow system recovery) and if the one-hour average concentration does not exceed 1,500 ug/L more than once every three years on the average. The 5% index for the protection of aquatic plant community should also be protective of most freshwater animals.

The procedures described in the Guidelines indicate that, except possibly where a locally important species is very sensitive, saltwater aquatic organisms and their uses should not be affected unacceptably if the thirty-day average concentration of atrazine does not exceed 17  $\mu$ g/L more than once every three years on the average, and if the one-hour average concentration does not exceed 760  $\mu$ g/L more than once every three years on the average.

#### **IMPLEMENTATION**

As discussed in the Water Quality Standards Regulation (U.S. EPA 1983a) and the Foreword to this document, a water quality criterion for aquatic life has regulatory impact only when it has been adopted in a State water quality standard. Such a standard specifies a criterion for a pollutant that is consistent with a particular designated use. With the concurrence of the U.S. EPA, States designate one or more uses for each body of water, or segment thereof, and adopt criteria that are consistent with the use(s) (U.S. EPA 1983a,b, 1987, 1994). Water quality criteria adopted in State water quality standards could have the same numerical values as criteria developed under Section 304, of the Clean Water Act. However, in many situations States might want to adjust water quality criteria developed under Section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, States may use different data and assumptions than the U.S. EPA in deriving numeric criteria that are scientifically

<sup>&</sup>lt;sup>3</sup>CASM is an aquatic ecological food chain model, specifically, the <u>Comprehensive Aquatic Systems Model</u> (Bartell et al. 2000, Bartell et al 1999, DeAngelis et al 1989).

Bartell, S.M., K.R. Campbell, C.M. Lovelock, S.K. Nair, and J.L. Shaw. 2000. Characterizing aquatic ecological risk from pesticides using a diquat dibromide case study III. Ecological Process Models. Environ. Toxicol. Chem. 19(5):1441-1453.

Bartell, S.M., G. Lefebvre, G. aminski, M. Carreau, and K.R. Campbell. 1999. An ecosystem model for assessing ecological risks in Quebec rivers, lakes, and reservoirs. Ecol. Model. 124:43-67.

defensible and protective of designated uses. State water quality standards include both numeric and narrative criteria. A State may adopt a numeric criterion within its water quality standards and apply it either state-wide to all waters for the use the criterion is designed to protect or to a specific site. A State may use an indicator characteristic or the national criterion, supplemented with other relevant information, to interpret its narrative criteria within its water quality standards when developing NPDES effluent limitations under 40 CRF 122.44(d)(1)(vi).2.

Site-specific criteria may include not only site-specific criterion concentrations (U.S. EPA 1994), but also site-specific, and possibly pollutant-specific, durations of averaging periods and frequencies of allowed excursions (U.S. EPA 1991). The averaging periods of "one hour" and "four days" were selected by the U.S. EPA on the basis of data concerning how rapidly some aquatic species react to increases in the concentrations of some aquatic pollutants, and "three years" is the Agency's best scientific judgment of the average amount of time aquatic ecosystems should be provided between excursions (Stephan et al. 1985; U.S. EPA 1991). However, various species and ecosystems react and recover at greatly differing rates. Therefore, if adequate justification is provided, site-specific and/or pollutant-specific concentrations, durations, and frequencies may be higher or lower than those given in national water quality criteria for aquatic life.

Use of criteria, which have been adopted in State water quality standards, for developing water quality-based permit limits and for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Although dynamic models are preferred for the application of these criteria (U.S. EPA 1991), limited data or other considerations might require the use of a steady-state model (U.S. EPA 1986). Guidance on mixing zones and the design of monitoring programs is also available (U.S. EPA 1987, 1991).





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# **Ranked Summary of Test Values for Freshwater Plants**




Figure 6. Range of Reported Atrazine Lowest Observed Effect Concentrations (LOECs) and No Observed Effect Concentrations (NOECs) Excluding Those LOECs Where Recovery Was Reported to Occur.



<u>Species</u>	<u>Method<sup>a</sup></u>	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	LC50 or EC50 <u>(µg/L)<sup>b</sup></u>	Species Mean Acute Value (µg/L)	<u>References</u>
			FRESHWA1	TER SPECIES		
Hydra, <i>Hydra</i> sp.	R,M	\$98.5%	48.9	<u>3,000</u>	3,000	Brooke 1990
Annelid, Lumbriculus variegatus	F,M	\$98.5%	67.3	<u>&gt;37,100</u>	>37,100	Brooke 1990
Snail, <i>Physa</i> acuta	S,M	-	-	<u>&gt;20,000</u>	>20,000	Rosés et al. 1999
Snail (adult), <i>Physa</i> sp.	R,M	\$98.5%	48.9	<u>&gt;34,100</u>	>34,100	Brooke 1990
Cladoceran (<24 hr), Ceriodaphnia dubia	S,M	97%	52	<u>&gt;4,900</u>		Jop 1991a
Cladoceran (<12 hr), Ceriodaphnia dubia	S,M	>99%	57.1	<u>&gt;30,000</u>	>12,120	Oris et al. 1991
Cladoceran (<24 hr), Daphnia magna	S,U	94%	-	6,900	-	Macek et al. 1976
Cladoceran (<24 hr), Daphnia magna	S,U	\$96%	250	>39,000	-	Marchini et al. 1988
Cladoceran, Daphnia magna	F,M	79.6%	170	<u>49,000</u>	49,000	Putt 1991
Amphipod (14-21 d), Hyalella azteca	S,M	\$98%	-	>10,000	-	Anderson and Lydy 2002
Amphipod, Hyalella azteca	F,M	\$98.5%	67.4	<u>14,700</u>	14,700	Brooke 1990
Stonefly (nymph), Acroneuria sp.	F,M	\$98.5%	67.4	<u>6,700</u>	6,700	Brooke 1990
Coho salmon (yearling), Oncorhynchus kisutch	R,M	\$80%	101	<u>&gt;18,000</u>	>18,000	Lorz et al. 1979
Rainbow trout (juvenile), Oncorhynchus mykiss	S,U	98.8%	43	<u>5,300</u>	5,300	Beliles and Scott 1965
Brown trout, Salmo trutta	R,U	-	11	<u>27,000</u>	27,000	Grande et al. 1994
Brook trout (juvenile), Salvelinus fontinalis	F,U	94%	-	<u>6,300</u>	6,300	Macek et al. 1976
Goldfish (juvenile), Carassius auratus	S,U	98.8%	43	<u>60,000</u>	60,000	Beliles and Scott 1965
Fathead minnow Pimephales promelas	R,U	94%	-	15,000	-	Macek et al. 1976
Fathead minnow (juvenile), Pimephales promelas	S,M	97%	52	>4,900	-	Jop 1991d

## Table 1. Acute Toxicity of Atrazine to Aquatic Animals

Species	<u>Method<sup>a</sup></u>	<u>Chemical</u>	Hardness (mg/L as CaCO <sub>3</sub> )	LC50 or EC50 <u>(µg/L)</u> <sup>b</sup>	Species Mean Acute Value (µg/L)	<u>References</u>
Fathead minnow, Pimephales promelas	F,M	97.1%	20-40	<u>20,000</u>	20,000	Dionne 1992
Channel catfish (sac fry), Ictalurus punctatus	S,U	80%	78	<u>&gt;10,000</u>	>10,000	Jones 1962
Bluegill (juvenile), Lepomis macrochirus	S,U	98.8%	43	<u>24,000</u>		Beliles and Scott 1965
Bluegill (juvenile), <i>Lepomis macrochirus</i>	F,U	94%	-	<u>&gt;8,000</u>	>13,856	Macek et al. 1976
Largemouth bass (fry), Micropterus salmoides	S,U	80%	78	<u>&gt;10,000</u>	>10,000	Jones 1962
Leopard frog, Rana pipiens	R,M	99%	290	<u>&gt;20,000</u>	>20,000	Allran and Karasov 2001
Wood frog, Rana sylvatica	R,M	99%	290	<u>&gt;20,000</u>	>20,000	Allran and Karasov 2001
American toad, Bufo americanus	R,M	99%	290	<u>&gt;20,000</u>	>20,000	Allran and Karasov 2001

Species	<u>Method</u> ª	<u>Chemical</u>	Salinity (g/kg)	LC50 or EC50 <u>(µg/L)</u> <sup>b</sup>	Species Mean Acute Value (µg/L)	<u>References</u>
			SALTWA1	TER SPECIES		
Eastern oyster (embryo/larval), Crassostrea virginica	S,U	97.4%	16	<u>&gt;30,000</u>	>30,000	Ward and Ballantine 1985
Copepod (nauplius), Eurytemora affinis	S,M	97.1%	5	<u>500</u>	-	Hall et al. 1994a,b
Copepod (nauplius), Eurytemora affinis	S,M	97.1%	15	<u>2,600</u>	-	Hall et al. 1994a,b
Copepod (nauplius), Eurytemora affinis	S,M	97.1%	25	<u>13,200</u>	2,579	Hall et al. 1994a,b
Copepod (adult), Acartia clausii	R,U	70%	6	<u>7,925</u>	7,925	Thursby et al. 1990
Copepod, Acartia tonsa <sup>c</sup>	S,U	97.4%	20	94	-	Ward and Ballantine 1985
Copepod (adult), Acartia tonsa	R,M	70%	31-32	210.1	-	Thursby et al. 1990
Copepod (adult), Acartia tonsa	R,M	70%	31	91.73	-	Thursby et al. 1990
Copepod (adult), Acartia tonsa	F,M	97.1%	30-34	<u>4,300</u>	4,300	McNamara 1991a

### SALTWATER SPECIES

Species	<u>Method</u> ª	<u>Chemical</u>	Salinity (g/kg)	LC50 or EC50 <u>(µg/L)</u> <sup>b</sup>	Species Mean Acute Value <u>(µg/L)</u>	References
Mysid, Americamysis bahia	F,M	97.4%	20	<u>1,000</u>	-	Ward and Ballantine 1985
Mysid, Americamysis bahia	F,M	97.1%	32	<u>5,400</u>	2,324	Machado 1994
Pink shrimp, Penaeus duorarum <sup>c</sup>	S,U	97.4%	26	<u>6,900</u>	6,900	Ward and Ballantine 1985
Grass shrimp, Palaemonetes pugio <sup>c</sup>	S,U	97.4%	26	<u>9,000</u>	9,000	Ward and Ballantine 1985
Fiddler crab, Uca pugilator <sup>e</sup>	S,U	97.4%	26	<u>&gt;29,000</u>	>29,000	Ward and Ballantine 1985
Sheepshead minnow (larva), <i>Cyprinodon variegatus</i>	S,M	97.1%	5	<u>16,200</u>		Hall et al. 1994a,b
Sheepshead minnow (larva), <i>Cyprinodon variegatus</i>	S,M	97.1%	15	<u>2,300</u>	-	Hall et al. 1994a,b
Sheepshead minnow (larva), <i>Cyprinodon variegatus</i>	S,M	97.1%	25	<u>2,000</u>	4,208	Hall et al. 1994a,b
Sheepshead minnow, Cyprinodon variegatus	F,M	97.4%	13	>16,000 <sup>d</sup>	-	Ward and Ballantine 1985
Sheepshead minnow, Cyprinodon variegatus	F,M	97.1%	32	13,000 <sup>d</sup>	-	Machado 1994b
Spot, Leiostomus xanthurus <sup>c</sup>	S,U	97.4%	12	<u>8,500</u>	8,500	Ward and Ballantine 1985

<sup>a</sup> S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.
<sup>b</sup> Results are expressed as atrazine, not as the chemical. Each Species Mean Acute Value was calculated from the associated underlined number(s) in the preceding column.
<sup>c</sup> Test organisms collected from the field.
<sup>d</sup> Not used in calculations because data are available for a more sensitive life stage.

Table 2a.	<b>Chronic Toxicity</b>	of Atrazine to Aquatic Animals
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Species	<u>Test<sup>a</sup></u>	<u>Chemical</u>	Hardness (mg/L as CaCO <sub>3</sub> )	Chronic Limits <u>(µg/L)<sup>b</sup></u>	Chronic Value (µg/L)	<u>References</u>
			FRESHW	ATER SPECIES		
Cladoceran, Ceriodaphnia dubia	LC	>99%	57.1	2,500-5,000	3,536	Oris et al. 1991
Cladoceran, Ceriodaphnia dubia	LC	>99%	57.1	2,500-5,000	3,536	Oris et al. 1991
Cladoceran, Ceriodaphnia dubia	LC	97%	52	1,200-2,500	1,732	Jop 1991b
Midge, Chironomus tentans	LC	94%	43.0	110-230	159.1	Macek et al. 1976
Rainbow trout Oncorhynchus mykiss	ELS	Technical	50.0	1,100-3,800	2,045	Whale et al. 1994
Brook trout, Salvelinus fontinalis	LC	94%	35.7	65-120	88.32	Macek et al. 1976
Fathead minnow, Pimephales promelas	LC	97.1%	24-36	250-460	339.1	Dionne 1992
Bluegill,	LC	94%	33.9	95->95	>95	Macek et al. 1976

Lepomis macrochirus

<u>Species</u>	<u>Test</u> <sup>a</sup>	<u>Chemical</u>	Salinity (g/kg)	Chronic Limits <u>(µg/L)<sup>b</sup></u>	Chronic Value (µg/L)	<u>References</u>
			SALTW	VATER SPECIES		
Copepod, Eurytemora affinis	LC	97.1%	5	12,250-17,500	14,640	Hall et al. 1995
Copepod, Eurytemora affinis	LC	97.1%	15	17,500-25,000	20,920	Hall et al. 1995
Copepod, Eurytemora affinis	LC	97.1%	25	4,200-6,000	5,020	Hall et al. 1995
Mysid, Americamysis bahia	LC	97.4%	20	80-190	123.3	Ward and Ballantine 1985
Sheepshead minnow, Cyprinodon variegatus	ELS	97.4%	13	1,900-3,400	2,542	Ward and Ballantine 1985

<sup>a</sup> LC = Life-cycle or partial life-cycle; ELS = early life-stage. <sup>b</sup> Results are based on measured concentrations of atrazine.

### Table 2b. Acute-Chronic Ratios

<u>Species</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Acute Value <u>(μg/L)</u> <sup>a</sup>	Chronic Value <u>(µg/L)</u>	<u>Ratio</u>	<u>Reference</u>
Cladoceran, Ceriodaphnia dubia	57.1	>30,000	3,536	>8.484	Oris et al. 1991
Cladoceran, Ceriodaphnia dubia	52	>4,900	1,732	>2.829	Jop 1991a,b
Midge, Chironomus tentans	43.0	720 <sup>b</sup>	159.1	4.525	Macek et al. 1976
Brook trout, Salvelinus fontinalis	35.7	6,300	88.32	71.33	Macek et al. 1976
Fathead minnow, Pimephales promelas	24-36	20,000	339.1	58.98	Dionne 1992
Bluegill, Lepomis macrochirus	33.9	>8,000	>95	>84.21	Macek et al. 1976
Copepod, Eurytemora affinis	5°	-500	14,640	0.0342	Hall et al. 1994a,b; 1995
Copepod, Eurytemora affinis	15°	2,600	20,920	0.1243	Hall et al. 1994a,b; 1995
Copepod, Eurytemora affinis	25°	13,200	5,020	2.629	Hall et al. 1994a,b; 1995
Mysid, Americamysis bahia	20 <sup>c</sup>	1,000	123.3	8.110	Ward and Ballantine 1985
Sheepshead minnow, Cyprinodon variegatus	13°	>16,000	2,542	>6.294	Ward and Ballantine 1985

<sup>a</sup> From Table 1.

<sup>b</sup> From Table 6.

° Salinity expressed as g/kg.

<u>Rank</u> ª	Genus Mean Acute Value <u>(µg/L)</u>	Species	Species Mean Acute Value <u>(µg/L)</u> <sup>b</sup>	Species Mean Acute-Chronic <u>Ratio<sup>c</sup></u>
		FRESHWATER SPECIES		
17	60,000	Goldfish, Carassius auratus	60,000	-
16	49,000	Cladoceran, Daphnia magna	49,000	
15	>37,100	Annelid, <i>Lumbriculus variegatus</i>	>37,100	
14	27,000	Brown trout, Salmo trutta	27,000	
13	>26,115	Snail, Physa acuta	->20,000	
	-	Snail, <i>Physa</i> sp.	>34,100	-
12	>20,000	Leopard frog, Rana pipiens	>20,000	-
	-	Wood frog, Rana sylvatica	>20,000	-
11	>20,000	American toad, Bufo americanus	>20,000	-
10	20,000	Fathead minnow, Pimephales promelas	20,000	58.98
9	14,700	Amphipod, Hyalella azteca	14,700	-
8	>13,856	Bluegill, Lepomis macrochirus	>13, 856	>84.21
7	>12,120	Cladoceran Ceriodaphnia dubia	>12,120	>4.899
6	>10,000	Channel catfish, Ictalurus punctatus	>10,000	-
5	>10,000	Largemouth bass, Micropterus salmoides	>10,000	-
4	9,767	Coho salmon, Oncorhynchus kisutch	>18,000	-
	-	Rainbow Trout, Oncorhynchus mykiss	5,300	-
3	6,700	Stonefly, Acroneuria sp.	6,700	-
2	6,300	Brook trout, Salvelinus fontinalis	6,300	71.33
1	3,000	Hydra, <i>Hydra</i> sp.	3,000	-

# Table 3. Ranked Genus Mean Acute Values with Species Mean Acute-Chronic Ratios

#### Table 3 (continued)

<u>Rank</u> ª	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L) <sup>b</sup>	Species Mean Acute-Chronic <u>Ratio<sup>c</sup></u>
		SALTWATER SPECIES		
9	>30,000	Eastern oyster, Crassostrea virginica	>30,000	-
8	>29,000	Fiddler crab, Uca pugilator	>29,000	
7	9,000	Grass shrimp, Palaemonetes pugio	9,000	
6	8,500	Spot, Leiostomus xanthurus	8,500	-
5	6,900	Pink shrimp, Penaeus duorarum	6,900	·
4	5,838	Copepod, Acartia clausii	7,925	-
	-	Copepod, Acartia tonsa	4,300	-
3	4,208	Sheepshead minnow, Cyprinodon variegatus	4,208	>6.294
2	2,579	Copepod, Eurytemora affinis	2,579	2.629
1	2,324	Mysid, Americamysis bahia	2,324	8.110

Ranked from most resistant to most sensitive based on Genus Mean Acute Value. Inclusion of "greater than" value does not necessarily imply a true ranking, but does allow use of all genera for which data are available so that the Final Acute Value is not unnecessarily lowered. а

<sup>b</sup> From Table 1.
<sup>c</sup> From Table 2b.

Table 3 (continued)

### Freshwater

Final Acute Value =  $3,021 \mu g/L$ 

Criterion Maximum Concentration =  $(3,021 : g/L)/2 = 1,511 \mu g/L$ 



Saltwater

Final Acute Value =  $1,519 \mu g/L$ 

Criterion Maximum Concentration =  $(1,519 \ \mu g/L)/2 = 759.5 \ \mu g/L$ 

Final Chronic Value =  $16.83 \mu g/L$  (Final Plant Value - see text)

## Table 4. Toxicity of Atrazine to Aquatic Plants

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration (days)	Effect	Concentration (µg/L) <sup>a</sup>	<u>Reference</u>
		<u>FR</u>	ESHWATE	<u>R SPECIES</u>		
Green alga, Chlamydomonas reinhardtii	-	-	4	EC50 (cell number)	51	Schafer et al. 1993
Green alga, Chlamydomonas reinhardtii	-	-	4	EC50 (cell number)	51	Girling et al. 2000
Green alga, Chlamydomonas reinhardtii	-	-	7	EC50 (cell number)	21	Schafer et al. 1993
Green alga, Chlamydomonas reinhardtii	-	-	10	EC50 (cell number)	10.2	Schafer et al. 1993
Green alga, Chlamydomonas reinhardtii			4	NOEC (growth inhibition)	3.4	Schafer et al. 1994
Green alga, Chlamydomonas reinhardtii	•		7	NOEC (growth inhibition)	5.1	Schafer et al. 1994
Green alga, Chlamydomonas reinhardtii	-		10	NOEC (growth inhibition)	3.7	Schafer et al. 1994
Green alga, Selenastrum capricornutum	-	-	4	NOEC (cell number, biomass)	0.5	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	-	-	4	NOEC (chlorophyll-a, phaeophytin-a)	10	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	-	-	4	LOEC (cell density, biomass)	1.0	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	-	-	4	LOEC (chlorophyll, phaeophytin-a)	100	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	-	-	4	EC50 (cell number)	4	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	-	-	4	EC50 (phaeophytin-a)	20	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	-	-	4	EC50 (chlorophyll-a)	150	Univ. of Mississippi 1990
Green alga, Selenastrum capricornutum	99.1%	-	4	EC50 (cell number)	128.2	Gala and Giesy 1990
Green alga, Selenastrum capricornutum	97.0%	-	4	NOEC (cell number)	76	Hoberg 1991a
Green alga, Selenastrum capricornutum	97.0%	-	4	LOEC (cell number)	130	Hoberg 1991a
Green alga, Selenastrum capricornutum	97.0%	-	4	EC10 (cell number)	90	Hoberg 1991a

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration (days)	<u>Effect</u>	Concentration (µg/L) <sup>a</sup>	<u>Reference</u>
Green alga, Selenastrum capricornutum	97.0%	-	4	EC50 (cell number)	130	Hoberg 1991a
Green alga, Selenastrum capricornutum	97.0%	-	4	EC90 (cell number)	190	Hoberg 1991a
Green alga, Selenastrum capricornutum	97.1%	-	5	NOEC (cell number)	16	Hoberg 1993a
Green alga, Selenastrum capricornutum	97.1%	-	5	EC10 (cell number)	26	Hoberg 1993a
Green alga, Selenastrum capricornutum	97.1%	-	5	LOEC (cell number)	31	Hoberg 1993a
Green alga, Selenastrum capricornutum	97.1%		5	EC50 (cell number)	55	Hoberg 1993a
Green alga, Selenastrum capricornutum	97.1%		5	EC90 (cell number)	120	Hoberg 1993a
Duckweed, Lemna gibba	97%		7	EC50 (frond production)	180	Hoberg 1991b
Duckweed, Lemna gibba	97.1%		14	NOEC (frond number)	<3.4	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	LOEC (frond number)	3.4	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	EC10 (frond number)	6.2	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	NOEC (frond biomass)	7.7	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	EC10 (frond biomass)	12	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	LOEC (frond biomass)	17	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	EC50 (frond number)	37	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	EC50 (frond biomass)	45	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	EC90 (frond biomass)	170	Hoberg 1993b
Duckweed, Lemna gibba	97.1%	-	14	EC90 (frond number)	220	Hoberg 1993b
Duckweed, Lemna gibba	97.4%	-	14	EC10 (frond number)	2.2 <sup>b</sup>	Hoberg 1993c

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration (days)	Effect	Concentration (µg/L) <sup>a</sup>	<u>Reference</u>
Duckweed, Lemna gibba	97.4%	-	14	EC10 (frond biomass)	4.2 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna gibba	97.4%	-	14	NOEC (frond number & biomass)	8.3 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna gibba	97.4%	-	14	LOEC (frond number & biomass)	18 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna gibba	97.4%	-	14	LOEC (frond biomass)	22 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna gibba	97.4%		14	EC50 (frond number)	50 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna gibba	97.4%	·	14	EC90 (frond number)	98 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna gibba	97.4%		14	EC90 (frond biomass)	110 <sup>b</sup>	Hoberg 1993c
Duckweed, Lemna minor	_		14	NOEC (biomass)	10	Univ. of Mississippi 1990
Duckweed, Lemna minor	-	-	14	LOEC (mature frond production)	10	Univ. of Mississippi 1990
Duckweed, Lemna minor	-	-	14	LOEC (biomass)	100	Univ. of Mississippi 1990
Duckweed, Lemna minor	-	-	14	EC50 (biomass)	8,700	Univ. of Mississippi 1990
Duckweed, Lemna minor	98%	-	10	EC50 (frond number)	56	Kirby and Sheahan 1994
Duckweed, Lemna minor	98%	-	10	EC50 (fresh weight)	60	Kirby and Sheahan 1994
Duckweed, Lemna minor	98%	-	10	EC50 (chlorophyll)	62	Kirby and Sheahan 1994
Duckweed, Lemna minor	-	-	28	NOEC (growth)	38	Girling et al. 2000
Duckweed, Lemna minor	-	-	28	LOEC (growth)	120	Girling et al. 2000
Elodea, Elodea canadensis	-	-	10	NOEC (biomass)	10 <sup>c</sup>	Univ. of Mississippi 1990
Elodea, Elodea canadensis	-	-	10	LOEC (biomass)	100 <sup>c</sup>	Univ. of Mississippi 1990

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration (days)	<u>Effect</u>	Concentration (µg/L) <sup>a</sup>	<u>Reference</u>
Elodea, Elodea canadensis	-	-	10	LOEC (mature frond production)	10 <sup>c</sup>	Univ. of Mississippi 1990
Elodea, Elodea canadensis	-	-	10	EC50 (biomass)	1,200 <sup>c</sup>	Univ. of Mississippi 1990
Elodea, Elodea canadensis	-	-	10	LOEC (biomass)	100 <sup>d</sup>	Univ. of Mississippi 1990
Elodea, Elodea canadensis	-	-	10	EC50 (biomass)	25,400 <sup>d</sup>	Univ. of Mississippi 1990
Elodea, <i>Elodea canadensis</i>	-	-	20	NOEC (length)	20	Girling et al. 2000
Elodea, Elodea canadensis			20	LOEC (length)	30	Girling et al. 2000
<u>Species</u>	<u>Chemical</u>	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L) <sup>a</sup>	<u>Reference</u>
		<u>s</u>	ALTWATER	SPECIES		
Diatom, Skeletonema costatum	-	30	2	EC50 (growth)	265	Walsh 1983
Green alga, <i>Chlorella</i> sp.	99.7%	30	3	EC50 (growth)	140	Mayer 1987
Green alga, <i>Neochloris</i> sp.	99.7%	30	3	EC50 (growth)	82	Mayer 1987
Green alga, <i>Platymonas</i> sp.	99.7%	30	3	EC50 (growth)	100	Mayer 1987
Red alga, Porphyridium cruentum	99.7%	30	3	EC50 (growth)	79	Mayer 1987
Redheadgrass pondweed, Potamogeton perfoliatus	96.4%	9	28	IC50 (photosynthesis)	55	Kemp et al. 1982b, 1983; Kemp et al. 1985
Redheadgrass pondweed, Potamogeton perfoliatus	96.4%	9	35	IC50 (final biomass)	30	Kemp et al. 1982b, 1983; Kemp et al. 1985
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	NOEC (dry weight)	15	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	NOEC (wet weight)	15	Hall et al. 1997

### SALTWATER SPECIES

Species	<u>Chemical</u>	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L) <sup>a</sup>	Reference
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	NOEC (rhizome tip mass)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	LOEC (dry weight)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	LOEC (wet weight)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	LOEC (rhizome tip mass)	300	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	Chronic value (dry weight)	21.2	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	Chronic value (wet weight)	21.1	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1	28	Chronic value (rhizome tip mass)	94.9	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	NOEC (dry weight)	15	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	NOEC (wet weight)	15	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	NOEC (rhizome tip mass)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	LOEC (dry weight)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	LOEC (wet weight)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	LOEC (rhizome tip mass)	300	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	Chronic value (dry weight)	21.2	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	Chronic value (wet weight)	21.2	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	6	28	Chronic value (rhizome tip mass)	94.9	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	NOEC (dry weight)	7.5	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	NOEC (wet weight)	15	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	NOEC (rhizome tip mass)	30	Hall et al. 1997

#### SALTWATER SPECIES

Species	Chemical	Salinity (g/kg)	Duration (days)	Effect	Concentration (µg/L) <sup>a</sup>	<u>Reference</u>
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	LOEC (dry weight)	15	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	LOEC (wet weight)	30	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	LOEC (rhizome tip mass)	300	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	Chronic value (dry weight)	10.6	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	12	28	Chronic value (wet weight)	21.2	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	-12	28	Chronic value (rhizome tip mass)	94.9	Hall et al. 1997
Sago pondweed, Potamogeton pectinatus	97.1%	1-12	28	Chronic value (dry weight)	5.3	Hall et al. 1997
Eurasian water milfoil, Myriophyllum spicatum	96.4%	9	28	IC50 (photosynthesis)	117	Kemp et al. 1982b. 1983; Kemp et al. 1985
Eurasian water milfoil, Myriophyllum spicatum	96.4%	9	35	IC50 (final biomass)	25	Kemp et al. 1982b, 1983; Kemp et al. 1985
Wild celery, Vallisneria americana	-	3 & 6	42	NOEC (dry weight)	100	Forney and Davis 1981
Wild celery, Vallisneria americana	-	3 & 6	42	LOEC (dry weight)	320	Forney and Davis 1981
Wild celery, Vallisneria americana	-	5	42	NOEC (leaf area)	3.2	Correll and Wu 1982
Wild celery, Vallisneria americana	-	5	42	LOEC (leaf area)	12	Correll and Wu 1982
Eelgrass, Zostera marina	-	22	21	LC50	540	Delistraty and Hershner 1984
Eelgrass, Zostera marina	-	20	21	LC50	100	Delistraty and Hershner, 1984
Eelgrass, Zostera marina	-	20	21	LC50	365	Delistraty and Hershner, 1984
Eelgrass, Zostera marina	-	19	21	LC50	367	Delistraty and Hershner, 1984

<sup>a</sup> Effect concentrations are based upon measured concentrations of atrazine during the exposure period.
<sup>b</sup> Effect concentration is based upon measured concentration of atrazine on the last day of exposure only.
<sup>c</sup> No sediment present.
<sup>d</sup> Sediment present.

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as (CaCo <sub>3</sub> )	Concentration in Water <u>(µg/L)</u>	Duration (days)	<u>Tissue</u>	BCF or <u>BAF</u>	<u>Reference</u>		
FRESHWATER SPECIES									
Brook trout, Salvelinus fontinalis	94%	35.7	740	308	Muscle	<0.27	Macek et al. 1976		
Bluegill, Lepomis macrochirus	94%	33.9	94	546	Muscle	<2.1	Macek et al. 1976		
Fathead minnow, Pimephales promelas	94%	36.2	210	301	Eviscerated carcass	<8.1	Macek et al. 1976		
Fathead minnow (F <sub>o</sub> larvae), <i>Pimphales promelas</i>	97.1%	24-36	2,000	60	Whole body	6.5ª	Dionne 1992		
Fathead minnow (adult males), Pimephales promelas	97.1%	24-36	2,000	274	Whole body	8.5ª	Dionne 1992		
Fathead minnow (adult females), Pimephales promelas	97.1%	24-36	2,000	274	Whole body	8.5ª	Dionne 1992		
Fathead minnow (F <sub>1</sub> embryos), <i>Pimephales promelas</i>	97.1%	24-36	2,000	3	Whole body composite sample	4.6ª	Dionne 1992		
Fathead minnow (14-day old larvae), Pimephales promelas	97.1%	24-36	2,000	14	Whole body	3.3ª	Dionne 1992		
Fathead minnow (30-day old larvae), Pimephales promelas	97.1%	24-36	2,000	30	Whole body	6.0ª	Dionne 1992		

# Table 5. Bioaccumulation of Atrazine by Aquatic Organisms

 $^{\mathrm{a}}$  Based on  $^{14}\mathrm{C}$  measurements, and therefore, represents a maximum possible bioconcentration factor.

## Table 6. Other Data on Effects of Atrazine on Aquatic Organisms

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<u>Reference</u>
			FRESHWAT	TER SPECIES		
Mixed nitrifying bacteria	-	-	28 days	Increased nitrite oxidation; ammonium oxidation unaffected	1,000	Gadkari 1988
Mixed nitrifying bacteria	-	-	28 days	Ammonium oxidation unaffected	2,000	Gadkari 1988
Bacterium, Pseudomonas putida	-	214	16 hr	Incipient inhibition	>10,000	Bringmann and Kuhn 1976, 1977
Cyanobacterium, Anabaena cylindrica	-	-	14 days	LOEC (growth)	2,160	Rohwer and Fluckiger 1979
Cyanobacterium, Anabaena cylindrica	-	-	19 hr	LOEC (nitrogenase activity)	2,160	Rohwer and Fluckiger 1979
Cyanobacterium, Anabaena cylindrica	-	-	1 hr	LOEC (O <sub>2</sub> production)	21,600	Rohwer and Fluckiger 1979
Cyanobacterium, Anabaena cylindrica	>95%	-	12-14 days	EC50 (cell number)	1,200	Stratton 1984
Cyanobacterium, Anabaena cylindrica	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	253 <sup>a</sup>	Larsen et al. 1986
Cyanobacterium, Anabaena cylindrica	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	178 <sup>a</sup>	Larsen et al. 1986
Cyanobacterium, Anabaena cylindrica	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	182 <sup>b</sup>	Larsen et al. 1986
Cyanobacterium, Anabaena flos-aquae	97%	-	5 days	EC50 (cell number)	230	Hughes 1986; Hughes et al. 1986, 1988
Cyanobacterium, Anabaena flos-aquae	97%	-	5 day exposure, 9 day recovery	NOEC (cell number)	<100	Hughes 1986; Hughes et al. 1986, 1988
Cyanobacterium, Anabaena flos-aquae	97%	-	5 day exposure, 9 day recovery	Algistatic concentration	4,970	Hughes 1986; Hughes et al. 1986, 1988
Cyanobacterium, Anabaena flos-aquae	97%	-	5 day exposure, 9 day recovery	Algicidal concentration	>3,200	Hughes 1986; Hughes et al. 1986, 1988
Cyanobacterium, Anabaena flos-aquae	99.9%	-	1 day	56.2% reduction in <sup>14</sup> C uptake	40	Abou-Waly et al. 1991a
Cyanobacterium, Anabaena flos-aquae	99.9%	-	3 days	50.0% reduction in <sup>14</sup> C uptake	40	Abou-Waly et al. 1991a

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA'</u>	<u>FER SPECIES</u>		
Cyanobacterium, Anabaena flos-aquae	99.9%	-	5 days	9.5% reduction in <sup>14</sup> C uptake	40	Abou-Waly et al. 1991a
Cyanobacterium, Anabaena flos-aquae	99.9%	-	1 day	49.0% reduction in chlorophyll	100	Abou-Waly et al. 1991a
Cyanobacterium, Anabaena flos-aquae	99.9%	-	3 days	2.0% reduction in chlorophyll	100	Abou-Waly et al. 1991a
Cyanobacterium, Anabaena flos-aquae	99.9%	-	5 days	21.8% reduction in chlorophyll	100	Abou-Waly et al. 1991a
Cyanobacterium, Anabaena flos-aquae	99.9%	-	7 days	29.9% reduction in chlorophyll	100	Abou-Waly et al. 1991a
Cyanobacterium, Anabaena flos-aquae	99.9%	-	3 days	EC50 (chlorophyll-a)	58	Abou-Waly et al. 1991b
Cyanobacterium, Anabaena flos-aquae	99.9%	-	5 days	EC50 (chlorophyll-a)	469	Abou-Waly et al. 1991b
Cyanobacterium, Anabaena flos-aquae	99.9%	-	7 days	EC50 (chlorophyll-a)	766	Abou-Waly et al. 1991b
Cyanobacterium, Anabaena flos-aquae	92.2%	-	4 days	EC50 (chlorophyll-a)	>3,000	Fairchild et al. 1998
Cyanobacterium, Anabaena inaequalis	>95%	-	12-14 days	EC50 (cell number)	30	Stratton 1984
Cyanobacterium, Anabaena inaequalis	Technical or analytical	-	22 hr	65% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Cyanobacterium, Anabaena variabilis	>95%	-	12-14 days	EC50 (cell number)	4,000	Stratton 1984
Cyanobacterium, Aphanizomenon flos-aquae	Technical or analytical	-	22 hr	97% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Cyanobacterium, Microcystis aeruginosa	-	214	8 days	Incipient inhibition	3	Bringmann and Kuhn 1976, 1978a,b
Cyanobacterium, Microcystis aeruginosa	97.4%	-	5 days	Reduced cell numbers	108	Parrish 1978
Cyanobacterium, Microcystis aeruginosa	97.4%	-	5 days	Minimum algistatic con- centration	440	Parrish 1978
Cyanobacterium, Microcystis aeruginosa	-	-	6 days	EC50 (growth)	630	Kallqvist and Romstad 1994
Cyanobacterium, Microcystis aeruginosa	-	-	6 days	EC50 (microplate method)	630	Kallqvist and Romstad 1994

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	<u>Effect</u>	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	TER SPECIES		
Cyanobacterium, Microcystis aeruginosa	Technical or analytical	-	22 hr	96% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Cyanobacterium, Microcystis aeruginosa	Technical or analytical	-	22 hr	84% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Cyanobacterium, Microcystis sp.	Technical or analytical	-	4 days	EC50 (biomass)	90	Fairchild et al. 1998
Cyanobacterium, Oscillatoria cf. chalybea	99.7%	-	6 days	Lowest complete inhibition conc.	2160	Schrader et al. 1997
Cyanobacterium, Oscillatoria cf. chalybea	99.7%	-	5 days	LOEC (growth)	220	Schrader at al 1998
Cyanobacterium, Oscillatoria sp.	Technical or analytical	-	22 hr	87% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Cyanobacterium, Plectonema boryanum	-	-	31 days	69% decrease in cell number	10,000	Mallison and Cannon 1984
Cyanobacterium, <i>Pseudoanabaena</i> sp.	Technical or analytical	-	22 hr	91% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Cyanobacterium, Synechococcus leopolensis	-	-	5 days	EC50 (growth)	130	Kallqvist and Romstad 1994
Cyanobacterium, Synechococcus leopolensis	-	-	5 days	EC50 (microplate method)	130	Kallqvist and Romstad 1994
Green alga, Ankistrodesmus braunii	99.9%	-	11 days	EC50 (cell number)	60	Burrell et al. 1985
Green alga, Ankistrodesmus sp.	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	72 <sup>a</sup>	Larsen et al. 1986
Green alga, Ankistrodesmus sp.	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	61 <sup>a</sup>	Larsen et al. 1986
Green alga, <i>Chlamydomonas geitleri</i> Ettl	96.4%	-	1 hr	EC50 ( $CO_2$ fixation)	311	Francois and Robinson 1990
Green alga, <i>Chlamydomonas geitleri</i> Ettl	96.4%	-	1 hr	EC50 (CO <sub>2</sub> fixation)	194°	Francois and Robinson 1990
Green alga, Chlamydomonas moewssi	95%	-	14 days	EC50 (growth inhibition)	1384 (exponential growth phase)	Kotrikla et al. 1997

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Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	TER SPECIES		
Green alga, Chlamydomonas moewssi	95%	-	14 days	EC50 (growth inhibition)	1181 (stationary growth phase)	Kotrikla et al. 1997
Green alga, Chlamydomonas noctigama	-	-	72 hr	EC50 (growth)	330	Kallqvist and Romstad 1994
Green alga, Chlamydomonas reinhardtii	-	-	8 hr	! 32% inhibition of photosynthesis	10	Valentine and Bingham 1976
Green alga, Chlamydomonas reinhardtii	-	-	8 hr	! 74% inhibition of photosynthesis	100	Valentine and Bingham 1976
Green alga, Chlamydomonas reinhardtii	-	-	8 hr	97% inhibition of photosynthesis	1,000	Valentine and Bingham 1976
Green alga, Chlamydomonas reinhardtii	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	48 <sup>a</sup>	Larsen et al. 1986
Green alga, Chlamydomonas reinhardtii	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	19 <sup>a</sup>	Larsen et al. 1986
Green alga, Chlamydomonas reinhardtii	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	44 <sup>a</sup>	Larsen et al. 1986
Green alga, Chlamydomonas reinhardtii <sup>d</sup>	-	-	1-2 days	Growth rate reduced by 100%	216	Hersh and Crumpton 1987
Green alga, Chlamydomonas reinhardtii <sup>e</sup>	-	-	1-2 days	Growth rate reduced by 13%	21.6	Hersh and Crumpton 1987
Green alga, Chlamydononas reinhardtii <sup>d</sup>	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	45	Hersh and Crumpton 1989
Green alga, Chlamydomonas reinhardtii <sup>e</sup>	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	484	Hersh and Crumpton 1989
Green alga, Chlamydomonas reinhardtii	-	-	65 hr	13% reduction in chlorophyll	49.6	Hiranpradit and Foy 1992
Green alga, Chlamydomonas reinhardtii	92.2%	-	96 hr	EC50 (chlorophyll)	176	Fairchild et al. 1998

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<u>Reference</u>
			<u>FRESHWA</u>	TER SPECIES		
Green alga, <i>Chlamydomonas</i> sp.	-	-	72-96 hr	36.2% <sup>f</sup> and 84.9% <sup>g</sup> growth inhibition; 12.8% reduction in chlorophyll	50-52	Foy and Hiranpradit 1977
Green alga, <i>Chlamydomonas</i> sp.	-	-	72-96 hr	64.1% <sup>f</sup> and 93.3% <sup>g</sup> growth inhibition; 32.4% reduction in chlorophyll	100-104	Foy and Hiranpradit 1977
Green alga, <i>Chlamydomonas</i> sp.	-	-	72-96 hr	77.5% <sup>f</sup> and 96.6% <sup>g</sup> growth inhibition; 49.9% reduction in chlorophyll	200-208	Foy and Hiranpradit 1977
Green alga, <i>Chlamydomonas</i> sp.	-	-	72-96 hr	76.6% <sup>f</sup> and 100% <sup>g</sup> growth inhibition; 84.2% reduction in chlorophyll	400-416	Foy and Hiranpradit 1977
Green alga, <i>Chlamydomonas</i> sp.	-	-	72-96 hr	78.6% growth inhibition <sup>f</sup> ; 90.5% reduction in chlorophyll	832	Foy and Hiranpradit 1977
Green alga, <i>Chlamydomonas</i> sp.	-	-	4 days	EC50 (biomass)	176	Fairchild et al. 1994a
Green alga, Chlorella fusca	99%	-	15 min	EC50 (photosynthesis)	141	Altenburger et al. 1990
Green alga, Chlorella fusca	99%	-	14 hr	EC50 (cell volume growth)	36	Altenburger et al. 1990
Green alga, Chlorella fusca	99%	-	24 hr	EC50 (cell reproduction)	26	Altenburger et al. 1990
Green alga, Chlorella fusca	\$98%	-	24 hr	EC50 (cell number)	15	Faust et al. 1993
Green alga, Chlorella fusca	95%	-	14 days	EC50 (growth inhibition)	53.91 (exponential growth phase)	Kotrikla et al. 1997
Green alga, Chlorella fusca	95%	-	14 days	EC50 (growth inhibition)	75.73 (stationary growth phase)	Kotrikla et al. 1997
Green alga, <i>Chlorella kessleri</i>	-	-	72 hr	30% growth inhibition and photosynthetic $O_2$ evolution; 6.7% reduction in protein synthesis; effects upon lipids	1,078	El-Sheekh et al. 1994

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWA	TER SPECIES		
Green alga, Chlorella pyrenoidosa	-	-	2 wk	70% reduced growth	500	Virmani et al. 1975
Green alga, Chlorella pyrenoidosa	-	-	2 wk	95% reduced growth	2,500	Virmani et al. 1975
Green alga, Chlorella pyrenoidosa	-	-	2 wk	92% reduced growth	10,000	Virmani et al. 1975
Green alga, Chlorella pyrenoidosa	-	-	8 hr	! 64% inhibition of photosynthesis	100	Valentine and Bingham 1976
Green alga, Chlorella pyrenoidosa	-	-	8 hr	96% inhibition of photosynthesis	1,000	Valentine and Bingham 1976
Green alga, Chlorella pyrenoidosa	>95%	-	12-14	EC50 (cell number)	300	Stratton 1984
Green alga, Chlorella pyrenoidosa	-	-	10 days	30% growth inhibition; 40% reduction in chlorophyll-a	53.9	Gonzalez-Murua et al. 1985
Green alga, Chlorella pyrenoidosa	-	-	10 days	65% growth inhibition; 70% reduction in chlorophyll-a	107.8	Gonzalez-Murua et al. 1985
Green alga, Chlorella pyrenoidosa	-	-	110 hr	39% reduction in chlorophyll	49.6	Hiranpradit and Foy 1992
Green alga, Chlorella pyrenoidosa	Analytical	-	<50 min	>80% inhibition of photosynthetic CO <sub>2</sub> uptake	125	Hannan 1995
Green alga, Chlorella pyrenoidosa	Analytical	-	<50 min	100% inhibition of photosynthetic CO <sub>2</sub> uptake	1,250	Hannan 1995
Green alga, Chlorella vulgaris	-	-	7 days	31.0% reduction in dry wt.	250 <sup>h</sup>	Veber et al. 1981
Green alga, Chlorella vulgaris	-	-	7 days	43.6% reduction in dry wt.	500 <sup>h</sup>	Veber et al. 1981
Green alga, Chlorella vulgaris	-	-	7 days	56.4% reduction in dry wt.	2,500 <sup>h</sup>	Veber et al. 1981
Green alga, Chlorella vulgaris	-	-	7 days	61.8% reduction in dry wt.	5,000 <sup>h</sup>	Veber et al. 1981
Green alga, Chlorella vulgaris	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	325 <sup>a</sup>	Larsen et al. 1986
Green alga, Chlorella vulgaris	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	305 <sup>a</sup>	Larsen et al. 1986

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	TER SPECIES		
Green alga, Chlorella vulgaris	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	293 <sup>b</sup>	Larsen et al. 1986
Green alga, Chlorella vulgaris	-	-	30 min	EC50 (Decrease in oxygen evolution)	305	Van der Heever and Grobbelaar 1997
Green alga, Chlorella vulgaris	92.2%	-	96 hr	EC50 (chlorophyll)	94	Fairchild et al. 1998
Green alga, Chlorella vulgaris	98%	-	12 days	Reduced growth, but showed signs of recovery	10	Berard et al 1999
Green alga, Chlorella vulgaris	98%	-	96 hr	EC50	172	Seguin et al 2000
Green alga, <i>Chlorella</i> sp.	-	-	72-96 hr	31.0% growth inhibition <sup>f</sup> ; 38.8% reduction in chlorophyll	52	Foy and Hiranpradit 1977
Green alga, <i>Chlorella</i> sp.	-	-	72-96 hr	45.3% growth inhibition <sup>f</sup> ; 30.3% reduction in chlorophyll	104	Foy and Hiranpradit 1977
Green alga, <i>Chlorella</i> sp.	-	-	72-96 hr	52.3% growth inhibition <sup>f</sup> ; 83.7% reduction in chlorophyll	208	Foy and Hiranpradit 1977
Green alga, <i>Chlorella</i> sp.	-	-	72-96 hr	59.2% growth inhibition <sup>f</sup> ; 93.5% reduction in chlorophyll	416	Foy and Hiranpradit 1977
Green alga, <i>Chlorella</i> sp.	-	-	72-96 hr	53.7% growth inhibition <sup>f</sup> ; 95.4% reduction in chlorophyll	832	Foy and Hiranpradit 1977
Green alga, <i>Chlorella</i> sp.	-	-	1-2 days	Growth rate reduced by 86%	216	Hersh and Crumpton 1987
Green alga, <i>Chlorella</i> sp.	-	-	2-3 days	Growth rate reduced by 55%	21.6	Hersh and Crumpton 1987
Green alga, <i>Chlorella</i> sp.	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	36	Hersh and Crumpton 1989
Green alga, Chlorella sp. <sup>e</sup>	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	41	Hersh and Crumpton 1989

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	Reference	
			<u>FRESHWA</u>	TER SPECIES			
Green alga, <i>Clorella</i> sp.°	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	35	Hersh and Crumpton 1989	
Green alga, <i>Chlorella</i> sp.	-	-	4 days	EC50 (biomass)	92	Fairchild et al. 1994a	
Green alga, Chlorococcum hypnosporum	-	-	2 wk	75% reduced growth	5,000	Virmani et al. 1975	
Green alga, Chlorococcum hypnosporum	-	-	2 wk	92% reduced growth	10,000	Vermani et al. 1975	
Green alga, Franceia sp. <sup>f</sup>	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	466	Hersh and Crumpton 1989	
Green alga, Franceia sp.	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	774	Hersh and Crumpton 1989	
Green alga, Franceia sp.	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	710	Hersh and Crumpton 1989	
Green alga, Franceia sp.	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	430	Hersh and Crumpton 1989	
Green alga, <i>Franceia</i> sp.	94%	-	2 min	EC50 (photosynthetic oxygen evolution)	720	Hersh and Crumpton 1989	
Green alga, Gloetaenium loitlesbergarianum	-	-	96 hr	inhibition of calcification	2,157	Prasad and Chowdary 1981	
Green alga, Pseudokirchnierella subcapitata	98%	-	96 hr	EC50	118	Seguin et al. 2001	
Green alga, Scenedesmus acutus	98%	-	96 hr	EC50	45	Seguin et al. 2001	
Green alga, Scenedesmus obliquus	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	38	Larsen et al. 1986	
Green alga, Scenedesmus obliquus	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	57	Larsen et al. 1986	
Green alga, Scenedesmus obliquus	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	49	Larsen et al. 1986	

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<u>Reference</u>
			FRESHWAT	FER SPECIES		
Green alga, Scenedesmus quadricauda	-	-	8 hr	! 42% inhibition of photosynthesis	10	Valentine and Bingham 1976
Green alga, Scenedesmus quadricauda	-	-	8 hr	! 84% inhibition of photosynthesis	100	Valentine and Bingham 1976
Green alga, Scenedesmus quadricauda	-	-	8 hr	98% inhibition of photosynthesis	1,000	Valentine and Bingham 1976
Green alga, Scenedesmus quadricauda	-	214	8 days	Incipient inhibition	30	Bringmann and Kuhn 1977, 1978a,b
Green alga, Scenedesmus quadricauda	>95%	-	12-14 days	EC50 (cell number)	100	Stratton 1984
Green alga, Scenedesmus quadricauda	-	-	8 days	4.5% reduction in photosynthesis	4	Bogacka et al. 1990
Green alga, Scenedesmus quadricauda	-	-	8 days	9.9% reduction in photosynthesis	9	Bogacka et al. 1990
Green alga, Scenedesmus quadricauda	-	-	8 days	18.5% reduction in photosynthesis	30	Bogacka et al. 1990
Green alga, Scenedesmus quadricauda	-	-	8 days	68.1% reduction in photosynthesis	100	Bogacka et al. 1990
Green alga, Scenedesmus quadricauda	-	-	8 days	99.3% reduction in photosynthesis	337	Bogacka et al. 1990
Green alga, Scenedesmus quadricauda	Technical or analytical	-	22 hr	96% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Green alga, Scenedesmus quadricauda	92.2%	-	96 hr	EC50 (chlorophyll)	169	Fairchild et al. 1998
Green alga, Scenedesmus subspicatus	99.0%	-	4 days	EC50 (cell number)	110	Geyer et al. 1985
Green alga, Scenedesmus subspicatus	-	-	24 hr	24.8% inhibition of effective photosynthesis rate	12.3	Schafer et al. 1994
Green alga, Scenedesmus subspicatus	-	-	24 hr	57.4% inhibition of effective photosynthesis rate	37	Schafer et al. 1994
Green alga, Scenedesmus subspicatus	-	-	24 hr	93.4% inhibition of effective photosynthesis rate	111.1	Schafer et al. 1994
Green alga, Scenedesmus subspicatus	-	-	24 hr	100.0% inhibition of effective photosynthesis rate	333.3	Schafer et al. 1994

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	Effect	Concentration (µg/L)	Reference
			FRESHWA'	TER SPECIES		
Green alga, Scenedesmus subspicatus	98%	-	2 days	EC50 (cell numbers)	21	Kirby and Sheahan 1994
Green alga, Scenedesmus subspicatus	-	-	24 hr	50% reduction in dry mass	! 21.5	Reinold et al. 1994
Green alga, Scenedesmus subspicatus	-	-	24 hr	EC50 (net assimilation inhibition)	25	Zagorc-Koncan 1996
Green alga, Scenedesmus subspicatus	-	-	72 hr	EC50 (growth inhibition)	200	Zagorc-Koncan 1996
Green alga, Scenedesmus subspicatus	99%	-	60 days	NOEC (growth and photosynthetic oxygen evolution)	20	Behra et al. 1999
Green alga, <i>Scenedesmus</i> sp.	-	-	72-96 hr	60.2% growth inhibition <sup>g</sup>	50	Foy and Hiranpradit 1977
Green alga, <i>Scenedesmus</i> sp.	-	-	72-96 hr	72.4% growth inhibition <sup>g</sup>	100	Foy and Hiranpradit 1977
Green alga, <i>Scenedesmus</i> sp.	-	-	72-96 hr	81.6% growth inhibition <sup>g</sup>	200	Foy and Hiranpradit 1977
Green alga, <i>Scenedesmus</i> sp.	-	-	72-96 hr	84.7% growth inhibition <sup>g</sup>	400	Foy and Hiranpradit 1977
Green alga, <i>Scenedesmus</i> sp.	-	-	72-96 hr	83.7% growth inhibition <sup>g</sup>	800	Foy and Hiranpradit 1977
Green alga, <i>Scenedesmus</i> sp.	-	-	4 days	EC50 (biomass)	169	Fairchild et al. 1994a
Green alga, Selenastrum capricornutum	97.4%	-	5 days	Significantly reduced cell numbers	54	Parrish 1978
Green alga, Selenastrum capricornutum	97.4%	-	5 days	Minimum algistatic concentration	200	Parrish 1978
Green alga, Selenastrum capricornutum	97.4%	-	5 days	12% chlorophyll-a reduction	32	Parrish 1978
Green alga, Selenastrum capricornutum	97.4%	-	5 days	42% chlorophyll-a reduction	54	Parrish 1978
Green alga, Selenastrum	97.4%	-	5 days	76% chlorophyll-a reduction	90	Parrish 1978

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Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>			
Green alga, Selenastrum capricornutum	97.4%	-	5 days	92% chlorophyll-a reduction	150	Parrish 1978
Green alga, Selenastrum capricornutum	97.4%	-	5 days	96% chlorophyll-a reduction	200	Parrish 1978
Green alga, Selenastrum capricornutum	85.5%	47	7 days	13.8% increased biomass	100 <sup>i</sup>	Johnson 1986
Green alga, Selenastrum capricornutum	85.5%	47	7 days	36.2% decreased biomass	1,000 <sup>i</sup>	Johnson 1986
Green alga, Selenastrum capricornutum	85.5%	47	7 days	75.9% decreased biomass	1,000 <sup>i</sup>	Johnson 1986
Green alga, Selenastrum capricornutum	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	53ª	Larsen et al. 1986
Green alga, Selenastrum capricornutum	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	34 <sup>ª</sup>	Larsen et al. 1986
Green alga, Selenastrum capricornutum	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	42 <sup>b</sup>	Larsen et al. 1986
Green alga, Selenastrum capricornutum	80%	-	21 days	EC50 (biomass)	58.7ª	Turbak et al. 1986
Green alga, Selenastrum capricornutum	80%	-	21 days	EC50 (biomass)	410 <sup>b</sup>	Turbak et al. 1986
Green alga, Selenastrum capricornutum	80%	-	24 hr	EC50 $(0_2 \text{ evolution})$	69.7 <sup>k</sup>	Turbak et al. 1986
Green alga, Selenastrum capricornutum	80%	-	24 hr	EC50 $(0_2 \text{ evolution})$	854 <sup>1</sup>	Turbak et al. 1986
Green alga, Selenastrum capricornutum	-	-	5 days	EC50 (cell number)	100	Roberts et al. 1990
Green alga, Selenastrum capricornutum	-	-	5 days	EC50 (cell number)	95	Roberts et al. 1990

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	TER SPECIES		
Green alga, Selenastrum capricornutum	Reagent grade	171	30 min	EC50 (CO <sub>2</sub> fixation)	100	Versteeg 1990
Green alga, Selenastrum capricornutum	Reagent grade	171	30 min	EC50 (O <sub>2</sub> generation)	380	Versteeg 1990
Green alga, Selenastrum capricornutum	Reagent grade	171	4 days	EC50 (cell number)	50	Versteeg 1990
Green alga, Selenastrum capricornutum	99.9%	-	1 day	22.0% reduction in chlorophyll; 69.3% reduction in <sup>14</sup> C uptake	130	Abou-Waly et al. 1991a
Green alga, Selenastrum capricornutum	99.9%	-	3 days	53.2% reduction in chlorophyll; 42.4% reduction in <sup>14</sup> C uptake	130	Abou-Waly et al. 1991a
Green alga, Selenastrum capricornutum	99.9%	-	5 days	24.5% reduction in chlorophyll; 60.6% reduction in <sup>14</sup> C uptake	130	Abou-Waly et al. 1991a
Green alga, Selenastrum capricornutum	99.9%	-	7 days	11.6% reduction in chlorophyll; 31.5% reduction in <sup>14</sup> C uptake	130	Abou-Waly et al. 1991a
Green alga, Selenastrum capricornutum	99.9%	-	3 days	EC50 (chlorophyll-a)	283	Abou-Waly et al. 1991b
Green alga, Selenastrum capricornutum	99.9%	-	5 days	EC50 (chlorophyll-a)	218	Abou-Waly et al. 1991b
Green alga, Selenastrum capricornutum	99.9%	-	7 days	EC50 (chlorophyll-a)	214	Abou-Waly et al. 1991b
Green alga, Selenastrum capric- ornutum	92.2%	-	4 days	EC50 (chlorophyll)	117	Fairchild et al. 1994a, 1998
Green alga, Selenastrum capric- ornutum	-	-	72 hr	EC50 (growth)	200	Kallqvist and Romstad 1994
Green alga, Selenastrum capric- ornutum	-	-	72 hr	EC50 (growth)	110	Kallqvist and Romstad 1994
Green alga, Selenastrum capricornutum	Technical or analytical	-	22 hr	99% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	<u>Effect</u>	Concentration (µg/L)	Reference
			FRESHWA	ATER SPECIES		
Green alga, Selenastrum capricornutum	-	100	96 hr	EC50 (chlorophyll-a)	147	Gaggi et al. 1995
Green alga, Selenastrum capricornutum	-	-	72 hr	EC50	118.2	Radetski et al. 1995
Green alga, Selenastrum capricornutum	-	-	72 hr	EC50 (cell numbers)	359	Van der Heever and Grobbelaar 1996
Green alga, Selenastrum capricornutum	-	-	72 hr	EC50 (chlorophyll-a; spectrophotometric measurement)	902	Van der Heever and Grobbelaar 1996
Green alga, Selenastrum capricornutum	-	-	72 hr	EC50 (chlorophyll-a; fluorometric measurement)	960	Van der Heever and Grobbelaar 1996
Green alga, Selenastrum capricornutum	-	-	96 hr	EC50 (cell number; free culture)	200	Abdel-Hamid 1996
Green alga, Selenastrum capricornutum	-	-	96 hr	EC50 (cell number; immobilized culture)	220	Abdel-Hamid 1996
Green alga, Selenastrum capricornutum	-	-	96 hr	LC50	26	Caux et al. 1996
Green alga, Selenastrum capricornutum	-	-	96 hr	EC50 (cell numbers)	26	Caux et al. 1996
Green alga, Selenastrum capricornutum	-	-	30 min	EC50 (decrease in oxygen evolution)	222	Van der Heever and Grobbelaar 1997
Green alga, Selenastrum capricornutum	-	-	72 hr	EC50 (growth inhibition)	164.3	Benhra et al. 1997
Green alga, Selenastrum capricornutum	-	-	72 hr	EC50 (growth inhibition)	92.9 (Cryoalgotox)	Benhra et al. 1997
Green alga, Selenastrum	-	-	4 days	I50 (chlorophyll-a)	80	El Jay et al. 1997

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Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<u>Reference</u>
			FRESHWAT	<u>FER SPECIES</u>		
Green alga, Selenastrum capricornutum	Technical grade	-	96 hr	NOEC (biomass)	75	Fairchild et al. 1997
Green alga, Selenastrum capricornutum	Technical grade	-	96 hr	LOEC (biomass)	150	Fairchild et al. 1997
Green alga, Selenastrum capricornutum	Technical grade	-	96 hr	EC50 (biomass)	235	Fairchild et al. 1997
Green alga, Selenastrum capricornutum	99.7%	-	6 days	Lowest Complete Inhibition Concentration (growth)	2160	Schrader et al. 1997
Green alga, Selenastrum capricornutum	-	-	4 hr	EC50 (chlorophyll-a fluorescence)	232	Van der Heever and Grobbelaar 1998
Green alga, Selenastrum capricornutum	ACS	-	\$3 days	EC50 (growth)	164	Mayer et al. 1998
Green alga, Selenastrum capricornutum	99.7%	-	5 days	LOEC (growth)	220	Schrader et al 1998
Green alga, Stigeoclonium tenue	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	127ª	Larsen et al. 1986
Green alga, Stigeoclonium tenue	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	224 <sup>a</sup>	Larsen et al. 1986
Green alga, Ulothrix subconstricta	-	-	24 hr	EC50 ( <sup>14</sup> C uptake)	88 <sup>a</sup>	Larsen et al. 1986
Benthic diatom, Craticula cuspidata	98%	-	67 days chronic, 12 days acute	LOEC (chlorophyll-a)	83	Nelson et al. 1999
Diatom, Asterionella formosa	98%	-	96 hr	EC50	261	Seguin et al. 2001
Diatom, <i>Cyclotella meneghiniana</i> (Arizona race)	-	-	7 min	EC50 (photosynthesis)	99	Millie and Hersh 1987
Diatom, <i>Cyclotella meneghiniana</i> (Iowa race)	-	-	7 min	EC50 (photosynthesis)	105	Millie and Hersh 1987
Diatom, <i>Cyclotella meneghiniana</i> (Minnesota race)	-	-	7 min	EC50 (photosynthesis)	243	Millie and Hersh 1987

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	Effect	Concentration (µg/L)	Reference
			FRESHWAT	TER SPECIES		
Diatom, Cyclotella meneghiniana	Technical or analytical	-	22 hr	97% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Diatom, <i>Cyclotella</i> sp.	-	-	6 days	EC50 (growth)	430	Kallqvist and Romstad 1994
Diatom, Navicula accomuda	98%	-	96 hr	EC50	164	Seguin et al. 2001
Diatom, Navicula pelliculosa	97%	-	5 days	EC50 (cell number)	60	Hughes 1986; Hughes et al. 1986, 1988
Diatom, Navicula pelliculosa	97%	-	5 day exposure, 9 day recovery	NOEC	<100	Hughes 1986; Hughes et al. 1986, 1988
Diatom, Navicula pelliculosa	97%	-	5 day exposure, 9 day recovery	Algistatic concentration	1,710	Hughes 1986; Hughes et al. 1986, 1988
Diatom, Navicula pelliculosa	97%	-	5 day exposure, 9 day recovery	Algicidal concentration	>3,200	Hughes 1986; Hughes et al. 1986, 1988
Diatom, <i>Nitzschia</i> sp.	98%	-	96 hr	EC50	412	Seguin et al. 2001
Diatom, Nitzschia sp.	Technical or analytical	-	22 hr	99% inhibition of photosynthesis ( <sup>14</sup> C uptake)	2,667	Peterson et al. 1994
Mixed algal assemblage	98%	-	21 days	Shift in dominant algal abundance	30	Seguin et al. 2001
Algal assemblage	-	-	28 days	LOEC (biomass)	11	Girling et al. 2001
Cryptomonad, Cryptomonas pyrinoidifera	-	-	6 days	EC50 (growth)	500	Kallqvist and Romstad 1994
Duckweed, Lemna gibba	97%	-	5 days	EC50 (frond production)	170	Hughes 1986; Hughes et al. 1986, 1988
Duckweed, Lemna gibba	97%	-	5 day exposure 9 day recovery	NOEC (frond production)	<100	Hughes 1986; Hughes et al. 1986, 1988
Duckweed, Lemna gibba	97%	-	5 day exposure 9 day recovery	Phytostatic concentration	1,720	Hughes 1986; Hughes et al. 1986, 1988

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	TER SPECIES		
Duckweed, Lemna gibba	97%	-	5 day exposure 9 day recovery	Phytocidal concentration	>3,200	Hughes 1986; Hughes et al. 1986, 1988
Duckweed, Lemna minor	-	-	20 days	No effect upon growth; increased soluble protein content; increased photosynthesis and respiration	20	Beaumont et al. 1976a,b
Duckweed, Lemna minor	-	-	20 days	! 12% reduced growth; increased water and soluble protein content; increased photosynthesis and respiration	50	Beaumont et al. 1976a,b, 1978
Duckweed, Lemna minor	-	-	20 days	! 23% reduced growth; increased water and soluble protein content; increased photosynthesis and respiration	100	Beaumont et al. 1976a,b, 1978
Duckweed, Lemna minor	-	-	20 days	! 74% reduced growth; increased water, chlorophyll, and soluble protein content; increased photosynthesis and respiration	250	Beaumont et al. 1976a,b
Duckweed, Lemna minor	-	-	15 days	Increased total fatty acid and <sup>"</sup> -linolenic acid content; increased monogalatosyldia-cyl- glycerol percentage	100	Grenier et al. 1979
Duckweed, Lemna minor	-	-	15 days	Increased total fatty acid and "-linolenic acid content; decreased linoleic acid content; increased monoga- lactosyldiacyl-glycerol percentage	1,000	Grenier et al. 1979
Duckweed, Lemna minor	-	-	15 days	Increased amounts of polar lipids in chlorophyll-protein complexes of chloroplasts	248	Grenier et al. 1987
Duckweed, Lemna minor	-	-	10 days	Increased [ <sup>14</sup> C]- acetate incorporation into chloroplast lipids	248	Grenier et al. 1989

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	ATER SPECIES		
Duckweed, Lemna minor	-	-	2 days	Changes in chloroplast ultrastructure; increased chlorophyll content	248	Simard et al. 1990
Duckweed, Lemna minor	Technical or analytical	-	7 days	95% inhibition of growth	2,667	Peterson et al. 1994
Duckweed, Lemna minor	Technical	-	96 hr	NOEC (biomass)	75	Fairchild et al. 1997
Duckweed, Lemna minor	Technical	-	96 hr	LOEC (biomass)	150	Fairchild et al. 1997
Duckweed, Lemna minor	Technical	-	96 hr	EC50 (biomass)	153	Fairchild et al. 1997
Duckweed, Lemna minor	92.2%	-	4 days	EC50 (frond production)	92	Fairchild et al. 1998
Wild rice, Zizania aquatica	85%	-	83 days	Visibly senescent; 75% reduction in chlorophyll- a in leaves	50	Detenbeck et al. 1996
Wild celery, Vallisneria americana	-	-	42 days	EC50 (total leaf length)	163	Davis 1981; Forney and Davis 1981
Wild celery, Vallisneria americana	-	-	-	Reduced leaf growth and whole plant biomass	8	Cohn 1985
Wild celery, Vallisneria americana	-	-	-	Reduced tuber over- wintering success	4	Cohn 1985
Coontail, Ceratophyllum dermersum	85%	-	6-8 days	Reduced stem elongation	50	Detenbeck et al. 1996
Coontail, <i>Ceratophyllum</i> sp.	92.2%	-	14 days	EC50 (wet weight)	22	Fairchild et al. 1998
Cattail, Typha latifolia	85%	-	19 days	No effect upon growth	25	Detenbeck et al. 1996
Watermilfoil, Myriophyllum heterophyllum	92.2%	-	14 days	EC50 (wet weight)	132	Fairchild et al. 1998
Watermilfoil, Myriophyllum spicatum	-	-	28 days	EC50 (length)	1,104	Davis 1981; Forney and Davis 1981
Watermilfoil, Myriophyllum spicatum	-	-	24 hr	30% increase in net photosynthetic rate	10	Hoffmann and Winkler 1990
Watermilfoil, Myriophyllum spicatum	-	-	5 days	50% reduction in branch number	3,700	Bird 1993
Sago pondweed, Potamogeton pectinatus	-	-	28 days	Reduced biomass	100	Fleming et al. 1991

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWA	TER SPECIES		
Bushy pondweed, <i>Najas</i> sp.	92.2%	-	14 days	EC50 (wet weight)	24	Fairchild et al. 1998
Egeria, <i>Egeria</i> sp.	-	-	14 days	EC50 (biomass)	<38	Fairchild et al. 1994a
Elodea, Elodea canadensis	-	-	28 days	EC50 (length)	80	Davis 1981; Forney and Davis 1981
Elodea, Elodea canadensis	-	-	21 days	EC50 (length)	109	Davis 1981; Forney and Davis 1981
Elodea, Elodea canadensis	-	-	20 days	Dark respiration rate exceeded net photosynthesis rate	10	Hoffmann and Winkler 1990
Elodea, Elodea canadensis	85%	-	19 days	No effect upon growth	75	Detenbeck et al. 1996
Elodea, Elodea canadensis	92.2%	-	14 days	EC50 (wet weight)	21	Fairchild et al. 1998
Water moss, Fontinalis antipyretica	-	-	20 days	Dark respiration rate exceeded net photosynthesis rate	10	Hoffmann and Winkler 1990
Water moss, Fontinalis hypnoides	-	-	24 hr	90% reduction in net photosynthesis	2	Hoffmann and Winkler 1990
Water moss, Fontinalis squamosa	-	-	24 hr	20% reduction in net photosynthesis	10	Hoffmann and Winkler 1990
Mixed macrophytes, <i>Ceratophyllum</i> sp. and <i>Elodea</i> sp.	85.5%	47	30 days	18.3% increased biomass	10	Johnson 1986
Mixed macrophytes, <i>Ceratophyllum</i> sp. and <i>Elodea</i> sp.	85.5%	47	30 days	11.6% decreased biomass	100	Johnson 1986
Mixed macrophytes, <i>Ceratophyllum</i> sp. and <i>Elodea</i> sp.	85.5%	47	30 days	47.6% decreased biomass	1,000	Johnson 1986
Protozoa, Acanthamoeba castellanii	-	-	6 days	5% population decrease	100	Prescott et al. 1977
Protozoa, Acanthamoeba castellanii	-	-	6 days	14% population decrease	1,000	Prescott et al. 1977
Protozoa, Acanthamoeba castellanii	-	-	6 days	15% population decrease	4,000	Prescott et al. 1977
Protozoa, Acanthamoeba castellanii	-	-	6 days	40% population decrease	10,000	Prescott et al. 1977

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWA'	TER SPECIES		
Protozoa, Colpidium campylum	-	-	24 hr	EC50 (cell number)	>50,000	Roberts et al. 1990
Protozoa, Euglena gracilis	-	-	8 hr	! 11% inhibition of photosynthesis	10	Valentine and Bingham 1976
Protozoa, Euglena gracilis	-	-	8 hr	! 28% inhibition of photosynthesis	100	Valentine and Bingham 1976
Protozoa, Euglena gracilis	-	-	8 hr	! 83% inhibition of photosynthesis	1,000	Valentine and Bingham 1976
Protozoa, Tetrahymena pyriformis	-	-	24 hr	EC50	118,500	Huber et al. 1991
Protozoa, Tetrahymena pyriformis	-	-	48 hr	EC50 (cell number)	96,000	Schafer et al. 1994
Hydra, <i>Hydra viridis</i>	-	-	21 days	Reduced budding rate	5,000	Benson and Boush 1983
Rotifer, Brachionus calyciflorus	-	-	24 hr	LC50	7,840	Crisinel et al. 1994
Leech, Glossiphonia complanata	99.2%	-	27-28 days	LC50	6,300	Streit and Peter 1978
Leech, Helobdella stagnalis	99.2%	-	27-28 days	LC50	9,900	Streit and Peter 1978
Snail, Lymnaea palustris	97.8%	-	12 wk	No effect upon growth, fecundity or glycogen metabolism	125	Baturo et al. 1995
Snail, <i>Lymnaea palustris</i>	97.8%	-	12 wk	Inhibited B <i>a</i> PH and GST enzyme activities	5	Baturo and Lagadic 1996
Snail, Physa acuta	-	-	18 days	Increased grazing searching velocity and movement patterns	15	Roses et al. 1999
Mussel (glochidia larva), Anadonta imbecilis	97.3%	40-50	24 hr	LC50	>60,000	Johnson et al. 1993
Mussel (1-2 d old juvenile), Anadonta imbecilis	97.3%	40-50	48 hr	LC50	>60,000	Johnson et al. 1993
Mussel (7-10 d old juvenile), Anadonta imbecilis	97.3%	40-50	48 hr	LC50	>60,000	Johnson et al. 1993
Anostracan, Streptocephalus texanus	-	-	24 hr	LC50	>30,000	Crisinel et al. 1994
Cladoceran, Ceriodaphnia dubia	>99%	57.1	4 days	MATC	7,100	Oris et al. 1991

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWA	TER SPECIES		
Cladoceran, Ceriodaphnia dubia	>99%	57.1	4 days	MATC	14,100	Oris et al. 1991
Cladoceran, Daphnia magna	-	-	26 hr	LC50	3,600	Frear and Boyd 1967
Cladoceran, Daphnia magna	-	100	48 hr	BCF = 4.4	10	Ellgehausen et al. 1980
Cladoceran, Daphnia magna	-	100	48 hr	BCF = 2.2	10	Ellgehausen et al. 1980
Cladoceran, Daphnia magna	-	-	21 days	Reduced young production	2,000	Kaushik et al. 1985
Cladoceran, Daphnia magna	-	-	48 hr	10% mortality	22,000	Bogacka et al. 1990
Cladoceran, Daphnia magna	-	-	96 hr	30% mortality	16,900	Bogacka et al. 1990
Cladoceran, Daphnia magna	-	-	96 hr	60% mortality	48,300	Bogacka et al. 1990
Cladoceran, Daphnia magna	97.3%	40-50	48 hr	LC50	9,400 <sup>m</sup>	Johnson et al. 1993
Cladoceran, Daphnia magna	-	-	24 hr	EC50	>30,000	Crisinel et al. 1994
Cladoceran, Daphnia magna	-	-	48 hr	EC50	>30,000	Crisinel et al. 1994
Cladoceran, Daphnia magna	85%	-	48 hr	Significantly decreased survival	25	Detenbeck et al. 1996
Cladoceran, Daphnia magna	85%	-	48 hr	No effect upon survival	50	Detenbeck et al. 1996
Cladoceran, Daphnia pulex	-	-	3 hr	LC50	>40,000	Nishiuchi and Hashimoto 1967, 1969
Cladoceran, Daphnia pulex	99.2%	-	28 days	11.7% decreased survival and 28.2% decreased reproduction	1,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	28 days	4.2% decreased survival and 26.8% decreased reproduction	2,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	~70 days	41.7% decreased reproduction	2,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	28 days	20.2% decreased survival and 45.5% decreased reproduction	3,000	Schober and Lampert 1977
Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	Reference
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			FRESHWA	TER SPECIES		
Cladoceran, Daphnia pulex	99.2%	-	28 days	9.6% decreased survival and 48.3% decreased reproduction	4,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	28 days	42% decreased reproduction	5,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	70 days	48.2% decreased reproduction	5,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	28 days	14.9% decreased survival; 53.9% decreased reproduction	10,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	70 days	62.6% decreased reproduction	10,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	99.2%	-	28 days	96.5% decreased reproduction	20,000	Schober and Lampert 1977
Cladoceran, Daphnia pulex	-	-	10 min	10% reduction in food consumption	350	Pott 1980
Cladoceran, Daphnia pulex	-	-	10 min	50% reduction in food consumption	1,600	Pott 1980
Cladoceran, Daphnia pulex	98%	-	18 hr	LC50	! 700	Bowman et al 1981
Cladoceran (adult), Moina macrocopa	-	-	3 hr	LC50	>40,000	Nishiuchi and Hashimoto 1967, 1969
Cladoceran, Moina macrocopa	-	-	4-6 wk	40% mortality; 10% increase in potential production; reduced actual population growth	1,000	Shcherban 1972a,b
Amphipod (1 <sup>st</sup> instar), Gammarus fasciatus	94%	-	48 hr	LC50	5,700	Macek et al. 1976
Amphipod (approx 2 <sup>nd</sup> instar), <i>Hyalella azteca</i>	98%	-	18 hr	LC50	2,000	Bowman et al. 1981
White dotted mosquito, <i>Culex restuans</i>	98%	-	18 hr	LC50	! 60,000	Bowman et al. 1981
Midge (2 <sup>nd</sup> instar), Chironomus riparius	-	151	10 days	LC50	18,900	Taylor et al. 1991
Midge (~10 d), Chironomus tentans	97.1%	42-44	96 hr (fed)	LC50	>28,000	McNamara 1991b
Midge (4th instar), Chironomus tentans	99%	80-100	48 hr	LC50	>20,000	Pape-Lindstrom and Lydy 1997

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	FER SPECIES		
Midge (1st instar), Chironomus tentans	94%	-	48 hr	LC50	720	Macek et al. 1976
Midge (4th instar), Chironomus tentans	99%	-	48 hr	LC50	>1,000	Jin-Clark et al. 2002
Midge (3rd instar), Chironomus tentans	98.5%	40-52	48 hr	LC50 (fed)	>24,000	Springborn Smithers 2002
Midge (3rd instar), Chironomus tentans	98.5%	40-52	10 days	LC50	>24,000	Springborn Smithers 2002
Midge (3rd instar), Chironomus tentans	98.5%	40-52	10 days	EC50 (growth)	8,300	Springborn Smithers 2002
Midge (3rd instar), Chironomus tentans	98.5%	40-52	10 days	NOEC (survival)	16,000	Springborn Smithers 2002
Midge (3rd instar), Chironomus tentans	98.5%	40-52	10 days	NOEC (growth)	5,400	Springborn Smithers 2002
Rainbow trout (embryo), Oncorhynchus mykiss	80%	50	23 days (at hatching)	LC50	736	Birge et al. 1979
Rainbow trout (embryo), Oncorhynchus mykiss	80%	200	23 days (at hatching)	LC50	888	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	50	27 days (4 days post-hatch)	LC50	696	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	200	27 days (4 days post-hatch)	LC50	864	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	50	27 days (4 days post-hatch)	LC1	23.2	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	200	27 days (4 days post-hatch)	LC1	61.8	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	50	27 days (4 days post-hatch)	3% teratic larvae	43.2	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	50	27 days (4 days post-hatch)	6% teratic larvae	432	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchuls mykiss	80%	50	27 days (4 days post-hatch)	62% teratic larvae	4,020	Birge et al. 1979

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	Effect	Concentration (µg/L)	<u>Reference</u>
			FRESHWAT	FER SPECIES		
Rainbow trout (sac fry), Oncorhynchuls mykiss	80%	200	27 days (4 days post-hatch)	2% teratic larvae	13.6	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	200	27 days (4 days post-hatch)	3% teratic larvae	48.0	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	200	27 days (4 days post-hatch)	4% teratic larvae	416	Birge et al. 1979
Rainbow trout (sac fry), Oncorhynchus mykiss	80%	200	27 days (4 days post-hatch)	65% teratic larvae	4,020	Birge et al. 1979
Rainbow trout (juvenile), Oncorhynchus mykiss	99.3%	-	48 hr	LC50	5,660	Pluta 1989
Rainbow trout (juvenile), Oncorhynchus mykiss	-	-	28 days	Changes in renal corpuscle ultrastructure	5	Fischer-Scherl et al. 1991
Rainbow trout (juvenile), Oncorhynchus mykiss	-	-	28 days	Changes in renal corpuscle and tubule ultrastructure	10	Fischer-Scherl et al. 1991
Rainbow trout, Oncorhynchus mykiss	-	-	28 days	Slight ultrastructural changes in renal corpuscles	5	Schwaiger et al. 1991
Rainbow trout, Oncorhynchus mykiss	-	-	28 days	Slight histopathological changes in liver; increased ultrastructural changes in renal corpuscles	10	Schwaiger et al. 1991
Rainbow trout, Oncorhynchus mykiss	-	-	28 days	Ultrastructural changes in renal corpuscles and histopathological changes in liver	20	Schwaiger et al. 1991
Rainbow trout (juvenile), Oncorhynchus mykiss	93.7%	-	14 days	No effect upon survival, body weight, liver weight, or liver xenobiotic-metabolizing enzyme activities	10	Egaas et al. 1993
Rainbow trout (juvenile), Oncorhynchus mykiss	\$98%	-	10 days	Reduced plasma protein	3.0	Davies et al. 1994b
Rainbow trout (juvenile), Oncorhynchus mykiss	\$98%	-	10 days	Reduced plasma protein	50	Davies et al. 1994b
Rainbow trout (juvenile), Oncorhynchus mykiss	99%	380	5 wk	Ultrastructural alterations in kidney proximal tubules	12.4	Oulmi et al. 1995

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<u>Reference</u>
			FRESHWA'	FER SPECIES		
Rainbow trout (juvenile), Oncorhynchus mykiss	99%	380	5 wk	Ultrastructural alterations in kidney proximal and distal tubules	24.0	Oulmi et al. 1995
Atlantic salmon (parr), Salmo salar	-	-	30 min	Reduced olfactory response to female pheromone	2.0	Moore and Waring 1998
Goldfish, Carassius auratus	-	-	48 hr	LC50	>10,000	Nishiuchi and Hashimoto 1967, 1969
Goldfish (6-9 g), Carassius auratus	97.9%	-	24 hr (10 min flowing)	Burst swimming	0.5 (0.1 test dripping)	Saglio and Trijasse 1998
Common carp, Cyprinus carpio	-	-	48 hr	LC50	>10,000	Nishiuchi and Hashimoto 1967, 1969
Common carp (30-50 g), Cyprinus carpio	-	-	12 hr	! 125% increased serum cortisol	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	24 hr	! 300% increased serum cortisol	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	6 hr	! 40% increased serum cortisol	500	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	12 hr	! 60% increased serum cortisol	500	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	24 hr	! 250% increased serum cortisol	500	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	12 hr	! 60% increased serum glucose	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	24 hr	! 35% increased serum glucose	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	6 hr	! 15% increased serum glucose	500	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	12 hr	! 40% increased serum glucose	500	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	24 hr	! 70% increased serum glucose	500	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	72 hr	! 180% increased serum glucose; ! 40% decreased liver glycogen	1,000	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	4 hr	<ul> <li>25% increase in gill total ATPase activity;</li> <li>20% increase in gill Na-K dependent ATPase</li> </ul>	100	Hanke et al. 1983

Species	Chemical	Hardness (mg/L as CaCO <sub>2</sub> )	Duration	Effect	Concentration	Reference
Species	<u>entintun</u>	<u></u>	<u>FDFSHWA</u>	TED SDECIES		
			<u>FRESHWA</u>	<u>TER SPECIES</u>		
Common carp (30-50 g), Cyprinus carpio	-	-	6 hr	! 10% increase in gill total ATPase; ! 30% decrease in gill Na-K dependent ATPase	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	12 hr	<ul> <li>! 40% decrease in gill total ATPase;</li> <li>! 30% decrease in gill Na-K dependent ATPase</li> </ul>	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	24 hr	! 5% decrease in gill total ATPase; ! 25% decrease in gill Na-K dependent ATPase	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	4 hr	! 60% increase in serum AChE	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	6 hr	! 15% increase in serum AChE	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	12 hr	! 35% increase in serum AChE	100	Hanke et al. 1983
Common carp (30-50 g), Cyprinus carpio	-	-	24 hr	25% decrease in serum AChE	100	Hanke et al. 1983
Common carp, Cyprinus carpio	-	-	72 hr	Increased serum glucose and cortisol; decreased liver and muscle glycogen; decreased serum protein and cholesterol	100	Gluth and Hanke 1984, 1985
Common carp (juvenile), <i>Cyprinus carpio</i>	99.3%	-	48 hr	LC50	16,100	Pluta 1989
Common carp (juvenile), Cyprinus carpio	93.7%	141-223	96 hr (fed)	LC50	18,800	Neskovic et al. 1993
Common carp (juvenile), <i>Cyprinus carpio</i>	93.7%	141-223	14 days	Increased serum alkaline phosphatase; decreased alkaline phosphatase in heart, liver and kidneys; increased GPT in liver and kidneys; hyperplasia of some gill epithelial cells	1,500	Neskovic et al. 1993
Common carp (50-60 g), Cyprinus carpio	94%	-	14 days	NOEC (gill, liver, and kidney histopathology)	1,500	Poleksic et al. 1997
Fathead minnow (#24h), Pimephales promelas	97	60	7 days	NOEC (biomass)	\$4,900	Jop 1991b

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	FER SPECIES		
Fathead minnow (larvae), Pimephales promelas	85%	-	7	No effect upon survival	75	Detenbeck et al. 1996
Fathead minnow (juvenile), Pimephales promelas	85%	-	13 days	No effect upon survival or growth	75	Detenbeck et al. 1996
Channel catfish (embryo), Ictalurus punctatus	80%	50	4.5 days (at hatching)	LC50	272	Birge et al. 1979
Channel catfish (embryo), Ictalurus punctatus	80%	200	4.5 days (at hatching)	LC50	248	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	50	8.5 days (4 days post-hatch)	LC50	176	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	200	8.5 days (4 days post-hatch)	LC50	192	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	50	8.5 days (4 days post-hatch)	1% teratic larvae	22.4	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	50	8.5 days (4 days post-hatch)	4% teratic larvae	47.2	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	50	8.5 days (4 days post-hatch)	13% teratic larvae	344	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	50	8.5 days (4 days post-hatch)	69% teratic larvae	3,864	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	50	8.5 days (4 days post-hatch)	100% teratic larvae	37,360	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	200	8.5 days (4 days post-hatch)	1% teratic larvae	26.4	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	200	8.5 days (4 days post-hatch)	4% teratic larvae	43.2	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	200	8.5 days (4 days post-hatch)	16% teratic larvae	336	Birge et al. 1979

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	FER SPECIES		
Channel catfish (sac fry), Ictalurus punctatus	80%	200	8.5 days (4 days post-hatch)	47% teratic larvae	3,848	Birge et al. 1979
Channel catfish (sac fry), Ictalurus punctatus	80%	200	8.5 days (4 days post-hatch)	86% teratic larvae	37,360	Birge et al. 1979
Mosquitofish, Gambusia affinis	Technical	-	48 hr	No mortality	10,000	Darwazeh and Mulla 1974
Guppy, Poecilia reticulata	-	-	48 hr	LC50	38,200	Tscheu-Schluter 1976
Guppy, Poecilia reticulata	-	-	72 hr	LC50	31,600	Tscheu-Schluter 1976
Guppy, Poecilia reticulata	-	-	96 hr	40% mortality	28,600	Bogacka et al. 1990
Guppy, Poecilia reticulata	-	-	96 hr	53.2% mortality	37,200	Bogacka et al. 1990
Mozambique tilapia, <i>Tilapia mossambica</i>	-	-	90 days	Decreased red and white blood cell counts, hemoglobin, packed cell volume, mean corpuscular hemoglobin; decreased whole animal oxygen consumption; increased mean cell volume, blood volume and blood water content	1,100	Prasad et al. 1991a
Mozambique tilapia, Tilapia mossambica	-	-	30 days	Changed enzyme activity and levels of amino acids, proteins, ammonia, and urea in brain and liver	1,100	Prasad et al. 1991b
Mozambique tilapia, <i>Tilapia mossambica</i>	-	-	30 days	Increased lipase activity, free fatty acids, acetoacetate concentration, and total cholesterol in liver and muscle; decreased total lipids, glycerol and phospholipids in liver and muscle.	1,100	Srinivas et al. 1991
Mozambique tilapia, Tilapia mossambicus	-	-	90 days	Increased body weight, percent water, serum Na <sup>+</sup> and serum K <sup>+</sup> ; decreased serum Ca <sup>++</sup> , Mg <sup>++</sup> and HCO <sub>3</sub> <sup>-</sup>	1,100	Prasad and Reddy 1994

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<u>Reference</u>
			<u>FRESHWA</u>	FER SPECIES		
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	8 days (4 days post-hatch)	LCI	7.4	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	8 days (4 days post-hatch)	LC10	44.9	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	8 days (4 days post-hatch)	LC50	410	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	4 days (to hatch)	1% teratic surviving larvae	51	Birge et al. 1980
Bullfrog (embryo and tadpole), <i>Rana catesbeiana</i>	80%	113	4 days (to hatch)	3% teratic surviving larvae	410	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	4 days (to hatch)	7% teratic surviving larvae	6,330	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	4 days (to hatch)	22% teratic surviving larvae	14,800	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	4 days (to hatch)	47% teratic surviving larvae	26,400	Birge et al. 1980
Bullfrog (embryo and tadpole), Rana catesbeiana	80%	113	4 days (to hatch)	100% teratic surviving larvae	45,800	Birge et al. 1980
Leopard frog (embryo and tadpole), <i>Rana pipiens</i>	80%	115	9 days (4 days post-hatch)	LC1	32.6	Birge et al. 1980
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	9 days (4 days post-hatch)	LC10	378.9	Birge et al. 1980
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	9 days (4 days post-hatch)	LC50	7,680	Birge et al. 1980
Leopard frog (embryo and tadpole), <i>Rana pipiens</i>	80%	115	5 days (to hatch)	2% teratic surviving larvae	110	Birge et al. 1980
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	5 days (to hatch)	2% teratic surviving larvae	210	Birge et al. 1980

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	<b>Reference</b>	
			FRESHWAT	<u>FER SPECIES</u>			
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	5 days (to hatch)	5% teratic surviving larvae	1,113	Birge et al. 1980	
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	5 days (to hatch)	9% teratic surviving larvae	6,540	Birge et al. 1980	
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	5 days (to hatch)	13% teratic surviving larvae	13,200	Birge et al. 1980	
Leopard frog (embryo and tadpole), Rana pipiens	80%	115	5 days (to hatch)	46% teratic surviving larvae	48,700	Birge et al. 1980	
Leopard frog (tadpole), Rana pipiens	85%	-	41 days	No effect upon growth or survival	25	Detenbeck et al. 1996	
Pickerel frog (embryo and tadpole), Rana palustris	80%	103	8 days (4 days post-hatch)	LC50	17,960	Birge et al. 1980	
Pickerel frog (embryo and tadpole), Rana palustris	80%	103	4 days (to hatch)	2% teratic surviving larvae	10,400	Birge et al. 1980	
Pickerel frog (embryo and tadpole), Rana palustris	80%	103	4 days (to hatch)	5% teratic surviving larvae	20,600	Birge et al. 1980	
Pickerel frog (embryo and tadpole), Rana palustris	80%	103	4 days (to hatch)	18% teratic surviving larvae	33,900	Birge et al. 1980	
American toad (embryo and tadpole), <i>Bufo americanus</i>	80%	-	7 days (4 days post-hatch)	LC50	>48,000	Birge et al. 1980	
American toad (embryo and tadpole), <i>Bufo americanus</i>	80%	-	3 days (to hatch)	2% teratic surviving larvae	490	Birge et al. 1980	
American toad (embryo and tadpole), <i>Bufo americanus</i>	80%	-	3 days (to hatch)	2% teratic surviving larvae	5,560	Birge et al. 1980	
American toad (embryo and tadpole), <i>Bufo americanus</i>	80%	-	3 days (to hatch)	3% teratic surviving larvae	10,800	Birge et al. 1980	
American toad (embryo and tadpole),	80%	-	3 days (to hatch)	6% teratic surviving larvae	24,800	Birge et al. 1980	

Bufo americanus

Species	Chemical	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			<u>FRESHWA</u>	TER SPECIES		
American toad (embryo and tadpole), <i>Bufo americanus</i>	80%	-	3 days (to hatch)	17% teratic surviving larvae	48,200	Birge et al. 1980
African clawed frog (embryo), <i>Xenopus laevis</i>	40.8%	-	96 hr	100% abnormal embryos	8,000	Morgan et al. 1996
African clawed frog (embryo), <i>Xenopus laevis</i>	40.8%	-	96 hr	LC50	126,000	Morgan et al. 1996
African clawed frog (embryo), <i>Xenopus laevis</i>	40.8%	-	96 hr	LOEC (teratogenesis)	1,100	Morgan et al. 1996
Tiger salamander, Ambystoma tigrinum	-	333	86 days	Stimulated plasma thyroxine; delayed development - retarded growth	82	Larson et al. 1998
American alligator, Alligator mississippiensis	99%	-	15 min	50% inhibition of ( <sup>3</sup> H) 17 <sup>°</sup> -estradiol binding	4,465	Vonier et al. 1996
Stream mixed algal species	80%	-	1 day to 3 wk	39-78% reduction in gross productivity	10	Kosinski et al. 1985; Kosinski and Merkle 1984
Stream mixed algal species	80%	-	3 days	Reduced net primary productivity	100	Moorhead and Kosinski 1986
Experimental stream periphyton community	80%	-	14 days	Severe population density reductions in several species; total destruction of <i>Cladophora glomerata</i>	1,000	Kosinski 1984
Stream mixed community	Technical	164-202	30 days	No effect upon macroinvertebrate community structure, periphyton production or biomass, and community P/R ratio	25	Lynch et al. 1985
Experimental laboratory stream community	96.5	-	2 wk	Decreased diurnal fluctuation and mean values for pH and dissolved oxygen; increased nitrate nitrogen; parameters rapidly returned to control levels when treatment ended	100	Malanchuk and Kollig 1985
Stream aufwuchs community	-	-	12 days	4% biomass reduction at 10°C	24	Krieger et al. 1988

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>
			FRESHWA	TER SPECIES		
Stream aufwuchs community	-	-	12 days	24% biomass reduction; 30% chlorophyll-a reduction at 25°C	24	Krieger et al. 1988
Stream aufwuchs community	-	-	12 days	47% biomass reduction; 40% chlorophyll-a reduction at 10°C	134	Krieger et al. 1988
Stream aufwuchs community	-	-	12 days	31% biomass reduction; 44% chlorophyll-a reduction at 25°C	134	Krieger et al. 1988
Natural stream periphyton community	98%	-	24 hr	No effect upon algal cell numbers or biomass	77.5	Jurgenson and Hoagland 1990
Natural stream plankton community	Commercial product	-	6 mo	Initial decrease in phytoplankton species (6 wks) followed by a recovery	! 0.5	Lakshminarayana et al. 1992
Stream algal and benthic invertebrate community	90%	-	14 days	No effect upon attached algal chlorophyll-a concentrations or benthic invertebrate populations	5	Gruessner and Watzin 1996
Artificial stream periphyton community	-	-	30 days	Community photosynthesis inhibited	100	Pearson and Crossland 1996
Pond microcosm, (static system)	98.2%	-	7 days	No effect upon diurnal oxygen production	5.0	Brockway et al. 1984
Pond microcosm, (static system)	98.2%	-	12 days	25-30% decreased oxygen production	50	Brockway et al. 1984
Pond microcosm, (static system)	98.2%	-	7 days	40-50% decreased diurnal oxygen production	100	Brockway et al. 1984
Pond microcosm, (static system)	98.2%	-	12 days	90% decreased diurnal oxygen production	500	Brockway et al. 1984
Pond microcosm, (static system)	98.2%	-	12 days	100% inhibition of diurnal oxygen production	5,000	Brockway et al. 1984
Pond microcosm, (static system)	Technical	-	40 days	NOEC (chlorophyll-a)	2,000	Diana et al. 2000
Pond microcosm, (static system)	Technical	-	40 days	NOEC (macrophyte biomass)	20	Diana et al. 2000
Pond microcosm, (static system)	Technical	-	40 days	NOEC (gray tree frog, Hyla versicolor growth)	20	Diana et al. 2000

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	Effect	Concentration (µg/L)	Reference
			FRESHWAT	FER SPECIES		
Lake microcosm plankton community	98%	-	10-21 days	Seasonal and species- dependent effects; growth generally stimulated for Chryptophytes and	10	Berard et al. 1999
				Chrysophytes, but inhibited in <i>Chlorella</i> vulgaris		
Freshwater microcosm	-	-	7 wk	No effects upon species composition of phytoplankton, zooplankton or benthic macroinvertebrates; slight decrease in photosynthetic activity	5.1	Van den Brink 1995
Periphyton-dominated microcosm	96.5%	-	1 day	77% decrease in daily net productivity	100	Hamala and Kollig 1985
Periphyton-dominated microcosm	96.5%	-	14 days	! 75% decrease in P/R ratio	100	Hamala and Kollig 1985
Phytoplankton, zooplankton and benthos microcosm	-	-	60 days	Reduced <sup>14</sup> C uptake/chlorophyll-a ratio	43.8	Stay et al. 1985
Phytoplankton, zooplankton and benthos microcosm	-	-	25 days	Reduced net primary productivity	! 50	Stay et al. 1985
Pond mesocosm community	-	-	70 days	Changed population densities of zooplankton (rotifers, crustaceans and insect larvae)	200	Peichl et al. 1984
Pond mesocosm community	-	-	121 days	Changed phytoplankton community composition; increased rotifer population	10	Peichl et al. 1985
Pond mesocosm community	41%	-	805 days	Reduced phytoplankton production and biomass, macrophyte, populations, and populations of benthic insect grazers, <i>Rana</i> <i>catesbiana</i> tadpoles, grass carp and bluegills	20	deNoyelles et al. 1982, 1989, 1994
Pond mesocosm community	41%	-	4 yr single annual application	Reduced photosynthesis in 24 hr bioassays, followed by recovery in 20-day bioassays and long-term pond studies	20-500	deNoyelles and Kettle 1985

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>				
	FRESHWATER SPECIES									
Pond mesocosm community	97%	-	9-112 days	Significant reductions of herbivorous benthic insect species richness, abundance, and total insect emergence (89%), shift to earlier emergence for some herbivorous species; destabilization of ecosystem	20ª	Dewey 1986; Dewey and deNoyelles 1994				
Pond mesocosm community	97%	-	9-112 days	Significant reductions of herbivorous benthic insect species richness, abundance, and total insect emergence (95%), shift to earlier emergence for some herbivorous species; reduced species evenness; destabilization of ecosystem	100 <sup>n</sup>	Dewey 1986; Dewey and deNoyelles 1994				
Pond mesocosm community	97%	-	9-112 days	Significant reductions of herbivorous benthic insect species richness, abundance, and total insect emergence (85%), shift to earlier emergence for some herbivourous species; reduced species evenness; destabilization of ecosystem	500 <sup>n</sup>	Dewey 1986; Dewey and de Noyelles 1994				
Pond mesocosm community	40.8%	-	8 wk	Altered macrophyte community species composition; no effects upon primary productivity, total plant biomass, zooplankton or fish	50	Fairchild et al. 1994a				
Pond mesocosm plankton community	-	-	2 mo	No effect	5	Juttner et al. 1995				
Pond mesocosm plankton community	-	-	2 mo	Decreased O <sub>2</sub> , pH and conductivity	10	Juttner et al. 1995				
Pond mesocosm plankton community	-	-	2 mo	Decreased phytoplankton populations	182	Juttner et al. 1995				

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>	
			FRESHWATER SPECIES				
Pond mesocosm plankton community	-	-	2 mo	Reduced peak egg ratios in <i>Daphnia longispina</i> and elimination of <i>Polyarthra</i> sp. rotifers	318	Juttner et al. 1995	
Pond microbial microcosm community	98.6%	! 70	21 days	NOEC for concentrations of Mg, Ca and dissolved oxygen	10	Pratt et al. 1988	
Pond microbial microcosm community	98.6%	! 70	21 days	MATC for concentrations of Mg, Ca and dissolved oxygen	17.9	Pratt et al. 1988	
Pond microbial microcosm community	98.6%	! 70	21 days	LOEC for concentrations of Mg, Ca and dissolved oxygen	32.0	Pratt et al. 1988	
Pond microbial microcosm community	98.6%	! 70	21 days	NOEC for protozoan colonization, biomass protein, chlorophyll-a, and potassium concentration	110	Pratt et al. 1988	
Pond microbial microcosm community	98.6%	! 70	21 days	MATC for protozoan colonization, biomass protein, chlorophyll-a, and potassium concentration	193	Pratt et al. 1988	
Pond microbial microcosm community	98.6%	! 70	21 days	LOEC for protozoan colonization, biomass protein, chlorophyll-a and potassium concentration	337	Pratt et al. 1988	
Phyto- and zooplankton microcosm community	-	-	42 days	No or little effect upon net primary productivity, P/R ratio, and pH	! 15	Stay et al. 1989	
Phyto- and zooplankton microcosm community	-	-	42 days	Reduced net primary productivity, P/R ratio, and pH	! 84	Stay et al. 1989	
Experimental pond community	-	-	39 days after treatment	EC50 ( <sup>14</sup> C uptake)	96	Larsen et al. 1986	
Experimental pond community	-	-	43 days after treatment	EC50 ( <sup>14</sup> C uptake)	131	Larsen et al. 1986	

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>
			<u>FRESHWA</u>	TER SPECIES		
Experimental pond community	-	-	101 days after treatment	EC50 ( <sup>14</sup> C uptake)	109	Larsen et al. 1986
Experimental pond community	-	-	177 days after treatment	EC50 ( <sup>14</sup> C uptake)	24	Larsen et al. 1986
Experimental pond community	-	-	249 days after treatment	EC50 ( <sup>14</sup> C uptake)	27	Larsen et al. 1986
Experimental pond community	-	-	259 days after treatment	EC50 ( <sup>14</sup> C uptake)	37	Larsen et al. 1986
Experimental pond community	-	-	373 days after treatment	EC50 ( <sup>14</sup> C uptake)	100	Larsen et al. 1986
Mixed pond community	99.2%	-	4 mo	Elimination of <i>Lemna minor</i> population	60-120	Gunkel 1983
Mixed pond community	99.2%	-	4 mo	Rapid succession of algal species; reduced reproduction rate in Daphnia pulicaria	60-120	Gunkel 1983
Pond mesocosm community	99%	-	2 yr	Decreased green algal species, cell numbers and cladoceran populations; increased cryptomonad cell numbers	20	Neugebauer et al. 1990
Pond mesocosm community	99%	-	2 yr	Decreased green algal species, cell numbers and cladoceran populations; increased cryptomonad cell numbers	100	Neugebauer et al. 1990
Pond mesocosm community	99%	-	2 yr	Decreased green algal species, cell numbers and cladoceran populations; increased cryptomonad cell numbers	300	Neugebauer et al. 1990

Species	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<u>Duration</u>	Effect	Concentration (µg/L)	<u>Reference</u>
			<u>FRESHWA</u>	<u>FER SPECIES</u>		
Pond mesocosm community	Reagent grade	-	2 уг	Atrazine applied in May and June each year: decreased abundance of <i>Endochironomus</i> <i>nigricans</i> in June and of total macroinverte- brates in both May and June, followed by recovery in July; epiphytes decreased in abundance in June, followed by recovery in July; detritovore abundance decreased in May, followed by recovery in June; generalists decreased in May and June, followed by recovery in July	20	Huggins et al. 1994
Pond mesocosm community	Reagent grade	-	2 yr	Results similar to those at 20 $\mu$ g/L in May and June; <i>Caenis</i> sp. significantly increased in July; also increased abundance of <i>Caenis</i> sp., total macroinverte- brates, detritovores and generalists in late July	100	Huggins et al. 1994
Pond mesocosm community	Reagent grade	-	2 yr	Results similar to those at 20 and 100 : g/L in May and June: <i>Caenis</i> sp. were significantly reduced in abundance in early July but not in late July; the abundance of epiphytes decreased, while the abundance of total macroinverte- brates and generalists increased in late July	500	Huggins et al. 1994
Mixed algae from pond	-	-	>3 hr	Increased fluorescence rate for photosystem II	10	Ruth 1996
Lake limnocorral community	80%	-	34 days	Reduced periphyton ash- free dry weight	80-140	Herman et al. 1986
Lake limnocorral community	80%	-	9 wk (2 applications 6 weeks apart)	36-67% reduction in chlorophyll-a, organic matter, and total peri- phyton algal biomass	80-140 (first application); ! 110-190 (second application)	Herman et al. 1986

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	Duration	Effect	Concentration (µg/L)	Reference
			FRESHWAT	FER SPECIES		
Lake limnocorral periphyton community	80%	-	50 days	! 50% reduction in ash- free dry weight	80	Hamilton et al. 1987
Lake limnocorral periphyton community	80%	-	230 days	Reductions of ! 60% in biomass, ! 22% in cell numbers, and ! 32% in number of species	80	Hamilton et al. 1987
Lake limnocorral periphyton community	80%	-	56 days	Reductions of ! 50% in chlorophyll-a, ! 32% in biomass, ! 14% in cell numbers, and " 33% in number of species	140	Hamilton et al. 1987
Lake limnocorral periphyton community	80%	-	56 days	Reductions of ! 55% in chlorophyll-a, ! 68% in biomass, ! 19% in cell numbers, and ! 48% in number of species	1,560	Hamilton et al. 1987
Lake limnocorral community	80%	-	Two exposures 35 days apart	Different phytoplankton species assemblages for up to 114 days after second application; increased Secchi disc readings and decreased levels of dissolved oxygen, chlorophyll, and organic carbon; phyto- plankton communities were similar by day 323.	100 (1 <sup>st</sup> applic.) 155 (2 <sup>nd</sup> applic.)	Hamilton et al. 1988, 1989
Lake mesocosm plankton community	-	-	18 days	Decreased chlorophyl-a, dissolved oxygen, nauplii, <i>Daphnia</i> , <i>Cyclops</i> ; increased particulate organic carbon	1	Lampert et al. 1989
Lake mesocosm plankton community	-	-	10 days°	Decreased algal photosynthetic production, dissolved oxygen and <i>Daphnia</i> population; apparent recoveries after about 25 days	0.1	Lampert et al. 1989
Lake bacterial and algal species in microcosm study	-	-	-	Decreased algal population density and decreased "scope for change in ascendance" of community	250	Genoni 1992

<u>Species</u>	<u>Chemical</u>	Hardness (mg/L as <u>CaCO<sub>3</sub>)</u>	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>
			<u>FRESHWA</u>	TER SPECIES		
Lake mesocosm community	-	-	20 days	No effect upon tolerance to atrazine by phytoplankton and periphyton communities or upon length of <i>Cladocera</i> ; minor changes in species composition, POC/PON ratio and chlorophyll concentration	20	Gustavson and Wangberg 1995
Lake mesocosm phytoplankton community	-	-	20 days	EC50	58	Gustavson and Wangberg 1995
Lake mesocosm phytoplankton community	-	-	20 days	EC50	52	Gustavson and Wangberg 1995
Lake mesocosm periphyton community	-	-	20 days	EC50	52	Gustavson and Wangberg 1995
Lake mesocosm periphyton community	-	-	20 days	EC50	54	Gustavson and Wangberg 1995
Lake phytoplankton	-	-	3 hr	EC50 (carbon assimilation)	100	Brown and Lean 1995
Lake phytoplankton	-	-	3 hr	EC50 (phosphate assimilation)	14,000	Brown and Lean 1995
Lake phytoplankton	-	-	3 hr	EC50 (ammonium assimilation)	>33,000	Brown and Lean 1995
Stream periphyton community	85.5%	-	<4 hr	LOEC (chlorophyll-a)	109	Day 1993
Stream phytoplankton community	-	-	Spring season	Reduction in populations of green algae	40.4 maximum	Caux and Kent 1995
Wetland mesocosm community	85%	-	9-27 days	Decreased periphyton gross productivity; increased dissolved nutrients	15	Detenbeck et al. 1996

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	<u>Effect</u>	Concentration <u>(µg/L)</u>	<u>Reference</u>				
	SALTWATER SPECIES									
Green alga, <i>Chlamydomonas</i> sp.	-	30	90 min	EC50 (oxygen evolution)	60	Hollister and Walsh 1973				
Green alga, <i>Chlorella</i> sp.	-	30	90 min	EC50 (oxygen evolution	143	Hollister and Walsh 1973				
Green alga, Chlorococcum sp.	Technical	30	90 min	EC50 (oxygen evolution)	100	Walsh 1972				
Green alga, Chlorococcum sp.	80.0%	30	90 min	EC50 (oxygen evolution)	400	Walsh 1972				
Green alga, Chlorococcum sp.	Technical	30	90 min	EC100 (oxygen evolution)	400	Walsh 1972				
Green alga, Chlorococcum sp.	80.0%	30	90 min	EC100 (oxygen evolution)	800	Walsh 1972				
Green alga, Chlorococcum sp.	Technical	30	10 days	EC50 (growth)	100	Walsh 1972				
Green alga, Chlorococcum sp.	80.0	30	10 days	EC50 (growth)	100	Walsh 1972				
Green alga, Chlorococcum sp.	Technical	30	10 days	EC100 (growth)	500	Walsh 1972				
Green alga, Chlorococcum sp.	80.0%	30	10 days	EC100 (growth)	500	Walsh 1972				
Green alga, Chlorococcum sp.	-	30	90 min	EC50 (oxygen evolution)	80	Hollister and Walsh 1973				
Green alga, Dunaliella tertiolecta	Technical	30	90 min	EC50 (oxygen evolution)	300	Walsh 1972				
Green alga, Dunaliella tertiolecta	80.0%	30	90 min	EC50 (oxygen evolution)	600	Walsh 1972				
Green alga, Dunaliella tertiolecta	Technical	30	90 min	EC100 (oxygen evolution)	700	Walsh 1972				
Green alga, Dunaliella tertiolecta	80.0%	30	90 min	EC100 (oxygen evolution)	1,000	Walsh 1972				
Green alga, Dunaliella tertiolecta	Technical	30	10 days	EC50 (growth)	300	Walsh 1972				
Green alga, Dunaliella tertiolecta	80.0%	30	10 days	EC50 (growth)	400	Walsh 1972				
Green alga, Dunaliella tertiolecta	Technical	30	10 days	EC100 (growth)	1,200	Walsh 1972				
Green alga, Dunaliella tertiolecta	80.0%	30	10 days	EC100 (growth)	1,500	Walsh 1972				
Green alga, Dunaliella tertiolecta	-	30	90 min	EC50 (oxygen evolution)	159	Hollister and Walsh 1973				

		Salinity			Concentration				
<u>Species</u>	<u>Chemical</u>	<u>(g/kg)</u>	<u>Duration</u>	<u>Effect</u>	(µg/L)	<u>Reference</u>			
SALTWATER SPECIES									
Green alga, Dunaliella tertiolecta	97%	-	5 days	EC50 (cell number)	170	Hughes 1986; Hughes et al. 1986, 1988			
Green alga, Dunaliella tertiolecta	97%	-	5 day exposure, 9 day recovery	NOEC (cell numbers)	< 100	Hughes 1986; Hughes et al. 1986, 1988			
Green alga, Dunaliella tertiolecta	97%	-	5 day exposure, 9 day recovery	Algistatic concentration	1,450	Hughes 1986; Hughes et al. 1986, 1988			
Green alga, Dunaliella tertiolecta	97%	-	5 day exposure, 9 day recovery	Algicidal concentration	>3,200	Hughes 1986; Hughes et al. 1986, 1988			
Green alga, Dunaliella tertiolecta	-	-	15 min	EC50 (oxygen evolution)	270	Samson and Popovic 1988			
Green alga, Dunaliella tertiolecta	-	-	15 min	EC50 (complementary area)	37	Samson and Popovic 1988			
Green alga, Dunaliella tertiolecta	-	-	96 hr	EC50 (cell number)	132	Gaggi et al. 1995			
Green alga, Dunaliella bioculata	Technical	-	48 hr	35% reduction in growth	216	Felix et al. 1988			
Green alga, Dunaliella bioculata	Technical	-	48 hr	85% reduction in growth	3,240	Felix et al. 1988			
Green alga, Dunaliella bioculata	Technical	-	48 hr	100% growth inhibition	21,570	Felix et al. 1988			
Green alga, Nannochloris oculata	-	15	7 days	21% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	11% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	12% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	34% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	35% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	33% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	42% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	35% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>			
SALTWATER SPECIES									
Green alga, Nannochloris oculata	-	15	7 days	28% change in doubling time	50	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	46% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	35% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	21% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	59% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	52% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	47% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	57% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	56% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	54% change in doubling time	100	Karlander et al. 1983; Mayasich et al. 1986			
Green alga, Nannochloris oculata	-	15	7 days	change in doubling time	15	Mayasich et al. 1987			
Green alga, <i>Neochloris</i> sp.	-	30	90 min	EC50 (oxygen evolution)	82	Hollister and Walsh 1973			
Green alga, Platymonas sp.	-	30	90 min	EC50 (oxygen evolution)	102	Hollister and Walsh 1973			
Diatom, Achnanthes brevipes	-	30	90 min	EC50 (oxygen evolution)	93	Hollister and Walsh 1973			
Diatom, Amphora exigua	-	30	90 min	EC50 (oxygen evolution)	300	Hollister and Walsh 1973			
Diatom, Cyclotella nanna	-	30	90 min	EC50 (oxygen evolution)	84	Hollister and Walsh 1973			
Diatom, Isochrysis galbana	Technical	30	90 min	EC50 (oxygen evolution)	100	Walsh 1972			
Diatom, Isochrysis galbana	80.0%	30	90 min	EC50 (oxygen evolution)	200	Walsh 1972			
Diatom, Isochrysis galbana	Technical	30	90 min	EC100 (oxygen evolution)	200	Walsh 1972			
Diatom, Isochrysis galbana	80.0%	30	90 min	EC100 (oxygen evolution)	500	Walsh 1972			

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	Effect	Concentration (µg/L)	<b>Reference</b>
			<u>SALTWA</u>	TER SPECIES		
Diatom, Isochrysis galbana	Technical	30	10 days	EC50 (growth)	100	Walsh 1972
Diatom, Isochrysis galbana	80.0%	30	10 days	EC50 (growth)	100	Walsh 1972
Diatom, Isochrysis galbana	Technical	30	10 days	EC100 (growth)	200	Walsh 1972
Diatom, Isochrysis galbana	80.0%	30	10 days	EC100 (growth)	200	Walsh 1972
Diatom, Isochrysis galbana	-	30	90 min	EC50 (oxygen evolution)	100	Hollister and Walsh 1973
Diatom, Minutocellus polymorphus	-	-	72 hr	EC50 (cell numbers)	50	Walsh et al. 1988
Diatom, Monochrysis lutheri	-	30	90 min	EC50 (oxygen evolution)	77	Hollister and Walsh 1973
Diatom, Navicula inserta	-	30	90 min	EC50 (oxygen evolution)	460	Hollister and Walsh 1973
Diatom, Nitzschia closterium	-	30	90 min	EC50 (oxygen evolution)	287	Hollister and Walsh 1973
Diatom, Nitzschia (Ind. 684)	-	30	90 min	EC50 (oxygen evolution)	434	Hollister and Walsh 1973
Diatom, Nitzschia sigma	-	20	7 days	Reduced photosynthesis	220	Plumley and Davis 1980
Diatom, Nitzschia sigma	-	20	7 days	Reduced chlorophyll and cell number	2,200	Plumley and Davis 1980
Diatom, Phaeodactylum tricornutum	Technical	30	90 min	EC50 (oxygen evolution)	100	Walsh 1972
Diatom, Phaeodactylum tricornutum	80.0%	30	90 min	EC50 (oxygen evolution)	200	Walsh 1972
Diatom, Phaeodactylum tricornutum	Technical	30	90 min	EC100 (oxygen evolution)	200	Walsh 1972
Diatom, Phaeodactylum tricornutum	80.0%	30	90 min	EC100 (oxygen evolution)	600	Walsh 1972
Diatom, Phaeodactylum tricornutum	Technical	30	10 days	EC50 (growth)	200	Walsh 1972
Diatom, Phaeodactylum tricornutum	80.0%	30	10 days	EC50 (growth)	200	Walsh 1972

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>				
	SALTWATER SPECIES									
Diatom, Phaeodactylum tricornutum	Technical	30	10 days	EC100 (growth)	500	Walsh 1972				
Diatom, Phaeodactylum tricornutum	80.0%	30	10 days	EC100 (growth)	500	Walsh 1972				
Diatom, Phaeodactylum tricornutum	-	30	90 min	EC50 (oxygen evolution)	100	Hollister and Walsh 1973				
Diatom, Phaeodactylum tricornutum	-	-	7 days	Limited effect on doubling time	50	Mayasich et al. 1987				
Diatom, Skeletonema costatum	-	-	48 hr	EC50 (cell numbers)	20	Walsh et al. 1988				
Diatom, Stauroneis amphoroides	-	30	90 min	EC50 (oxygen evolution)	348	Hollister and Walsh 1973				
Diatom, Thalassiosira fluviatilis	-	20	7 days	Reduced chlorophyll	220	Plumley and Davis 1980				
Diatom, Thalassiosira fluviatilis	-	20	7 days	Reduced cell number and photosynthesis	2,200	Plumley and Davis 1980				
Diatom, Thalassiosira fluviatilis	-	30	90 min	EC50 (oxygen evolution)	110	Hollister and Walsh 1973				
Red alga, Porphyridium cruentum	-	30	90 min	EC50 (oxygen evolution)	79	Hollister and Walsh 1973				
Kelp, Laminaria hyperborea	-	-	28 days	LOEC (growth of new sporophytes)	10	Hopkins and Kain 1971				
Kelp, Laminaria hyperborea	-	-	24 hr	LOEC (respiration)	>1,000	Hopkins and Kain 1971				
Kelp, Laminaria saccharina	70%	30	2 days	No effect on sexual reproduction	33.2	Thursby and Tagliabue 1990				
Kelp, Laminaria saccharina	70%	30	2 days	66% reduction in fertilization	72.2	Thursby and Tagliabue 1990				
Redheadgrass pondweed, Potamogeton perfoliatus	-	8-12	2 hr	IC50 (photosynthesis)	77	Jones and Winchell 1984				
Redheadgrass pondweed, Potamogeton perfoliatus	99.7%	10	4 hr	IC50 (photosynthesis)	80	Jones et al. 1986				
Euraisian watermilfoil, Myriophyllum spicatum	-	8-12	2 hr	IC50 (photosynthesis)	104	Jones and Winchell 1984				
Aquatic vascular plant, Zannichellia palustris	-	8-12	2 hr	IC50 (photosynthesis)	91	Jones and Winchell 1984				

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	Effect	Concentration (µg/L)	<u>Reference</u>
			<u>SALTWA</u>	TER SPECIES		
Widgeon grass, Ruppia maritima	-	8-12	2 hr	IC50 (photosynthesis)	120	Jones and Winchell 1984
Vallisneria, Vallisneria americana	-	3	42 days	47% decrease in growth as length, and 48% decrease as dry weight	100	Forney 1980; Forney and Davis 1981
Vallisneria, Vallisneria americana	-	6	42 days	27% decrease in growth as length, and 30% decrease as dry weight	100	Forney 1980; Forney and Davis 1981
Vallisneria, Vallisneria americana	-	3	42 days	27% decreased in growth as length, and 41% decrease as dry weight	320	Forney 1980; Forney and Davis 1981
Vallisneria, Vallisneria americana	-	6	42 days	32% decrease in growth as length, and 29% decrease as dry weight	320	Forney 1980; Forney and Davis 1981
Vallisneria, Vallisneria americana	-	5	47 days	67% reduction in leaf production & 76% reduction in leaf area	12	Correll and Wu 1982
Eelgrass, Zostera marina	-	-	24 hr	Reduced net oxygen evolution	100	Kemp et al. 1982a
Eelgrass, Zostera marina	-	-	24 hr	No net oxygen evolution	1,000	Kemp et al. 1982a
Eelgrass, Zostera marina	97.2%	14	10 days	100% growth inhibition	1,900	Schwarzchild et al. 1994
Turtlegrass, Thalassia testudinum	Technical 99.7%	30	40 hr	EC50 (photosynthesis)	320	Walsh et al. 1982
Salt-marsh grass, Spartina alterniflora	97.1%	-	35 days	Increased peroxidase activity	30	Lytle and Lytle 1998
Salt-march grass, Spartina alterniflora	97.1%	-	35 days	No effect upon shoot growth, lipid peroxidation products or chlorophyll production; enhanced peroxidase activity	3,100	Lytle and Lytle 1988
Salt-marsh rush, Juncus roemerianus	97.1%	-	35 days	Reduced chlorophyl-a; Increased peroxidase activity and lipid peroxidation products	30	Lytle and Lytle 1998
Salt-marsh rush, Juncus roemerianus	97.1%	-	35 days	Reduced shoot growth, chlorophyll-a, chlorophyll-a; increased lipid peroxidation products	3,800	Lytle and Lytle 1998

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	<u>Effect</u>	Concentration (µg/L)	<u>Reference</u>	
SALTWATER SPECIES							
Eastern oyster (juvenile), Crassostrea virginica	Technical 99.7%	28	96 hr	EC50 (shell growth)	>1,000	Butler 1964; Mayer 1987	
Copepod, Acartia tonsa	97.1%	30-34	72 hr	LC50	6,100	McNamara 1991b	
Copepod, Acartia tonsa	97.1%	30-34	48 hr	LC50	8,400	McNamara 1991b	
Copepod, Acartia tonsa	97.1%	30-34	24 hr	LC50	15,000	McNamara 1991b	
Brown shrimp (juvenile), Penaeus aztecus	Technical 99.7%	30	48 hr	EC50	1,000	Mayer 1987	
Brown shrimp, Penaeus aztecus	-	-	24 hr	20% mortality	1,000	Butler 1964	
Brown shrimp, Penaeus aztecus	-	-	48 hr	30% mortality	1,000	Butler 1964	
Mud crab (field), <i>Panopeus</i> sp.	80%	-	70 days	No effect on number per m <sup>2</sup> after a single application	10,000,000	Plumley et al. 1980	
Drift line crab (field), Sesarma cinereum	80%	-	70 days	No effect on number per m <sup>2</sup> after a single application	10,000,000	Plumley et al. 1980	
Fiddler crab (field), Uca pugnax	80%	-	70 days	No effect on number per m <sup>2</sup> after a single application	1,000,000	Plumley et al. 1980	
Fiddler crab (field), Uca pugnax	80%	-	70 days	94% reduction in number per m <sup>2</sup> relative to control after a single application	10,000,000	Plumley et al. 1980	
Fiddler crab, <i>Uca pugnax</i> (animals collected in August)	80%	20	8 days	25% mortality of large males; 100% mortality of large females; 100% mortality of small males; 75% mortality of small females	100,000	Plumley et al. 1980	
Fiddler crab, <i>Uca pugnax</i> (animals collected in August 1977)	80%	20	8 days	50% mortality of large males; 100% mortality of large females; 75% mortality of small males, 50% mortality of small females	1,000,000	Plumley et al. 1980	

Species	<u>Chemical</u>	Salinity (g/kg)	<b>Duration</b>	<u>Effect</u>	Concentration (µg/L)	<u>Reference</u>
			SALTWA	TER SPECIES		
Fiddler crab, <i>Uca pugnax</i> (animals collected in November)	80%	20	30 days	No effect on survival of small males	1,000,000	Plumley et al. 1980
Fiddler crab, <i>Uca pugnax</i> (animals collected in March)	80%	20	9 days	No effect on survival of small males	1,000,000	Plumley et al. 1980
Fiddler crab, Uca pugnax (animals collected in August 1978)	80%	20	9 days	60% mortality	100,000	Plumley et al. 1980
Fiddler crab, Uca pugnax (animals collected in August 1978)	80%	20	9 days	90% mortality	180,000	Plumley et al. 1980
Fiddler crab, Uca pugnax (animals collected in August 1978)	80%	20	9 days	80% mortality	320,000	Plumley et al. 1980
Fiddler crab, <i>Uca pugnax</i> (animals collected in August 1978)	80%	20	9 days	90% mortality	560,000	Plumley et al. 1980
Fiddler crab, <i>Uca pugnax</i> (animals collected in August 1978)	80%	20	9 days	90% mortality	1,000,000	Plumley et al. 1980
Fiddler crab, Uca pugnax (animals collected in August 1978)	80%	20	9 days	100% mortality	10,000,000	Plumley et al. 1980
Spot (juvenile), Leiostomas santhurus	Technical 99.7%	29	48 hr	LC50	>1,000	Butler 1964; Mayer 1987
Estuarine microbial community	-	7-25	9 days	Effects on phototrophic component: chlorophyll- a, carbon assimilation, biovolume, and changes	40	DeLorenzo et al. 1999a

in species composition

	Salinity			Concentration		
Species	<b>Chemical</b>	(g/kg)	<b>Duration</b>	<u>Effect</u>	(µg/L)	<u>Reference</u>
			SALTWA	TER SPECIES		
Estuarine microbial community	97%	-	24 hr	Effects on phototrophic component: chlorophyll-a, carbon assimilation, and biovolume	47	DeLorenzo et al. 1999b
Mesocosm, Mixed marine phytoplankton	Residue grade	-	15 days	Reduced pH, particulate carbohydrates, chlorophyll, photosynthesis, primary production; increased dissolved organic phosphorus, dissolved organic nitrogen, and dissolved amino acids	0.12	Bester et al. 1995
Mesocosm, Mixed marine phytoplankton	Residue grade	-	15 days	Reduced pH, particulate carbohydrates, chlorophyll, photosynthesis, primary production; increased dissolved organic phosphorus, dissolved organic nitrogen, and dissolved amino acids	0.56	Bester et al. 1995
Mesocosm, Mixed marine phytoplankton	Residue grade	-	15 days	Reduced pH, particulate carbohydrates, chlorophyll, photosynthesis, primary production; increased dissolved organic phosphorus, dissolved organic nitrogen, and dissolved amino acids	5.80	Bester et al. 1995

<sup>a</sup> Test was run using a Taub and Dollar (1964) medium.

<sup>b</sup> Test was run using an algal assay medium (U.S. EPA 1971).

<sup>c</sup> Algae were pre-conditioned for 4 days with 531 : g/L of atrazine.

<sup>d</sup> Test performed with an atrazine-sensitive strain.

<sup>e</sup> Test performed with an atrazine-resistant strain

<sup>g</sup> Colorimetric determination.

<sup>h</sup> Only 2.3 to 4.7 percent of this concentration remained on day 7.

<sup>i</sup> Test performed with water from microcosm 30 days after atrazine had been introduced.

<sup>j</sup> Test performed directly with atrazine in water without a microcosm exposure.

<sup>k</sup> EC50 obtained using an algal assay medium.

<sup>1</sup> EC50 obtained using creek water as the test medium.

<sup>m</sup> Animals were fed at 24 hr.

<sup>n</sup> Two single annual applications at nominal concentration indicated.

<sup>o</sup> Atrazine concentrations were below detection after 10 days; however, the study continued for 42 days.

<sup>&</sup>lt;sup>f</sup> Nephelometric determination.

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