

Chronic Toxicity of Atrazine to Sago Pondweed at a Range of Salinities: Implications for Criteria Development and Ecological Risk

L. W. Hall, Jr.,¹ R. D. Anderson,¹ M. S. Ailstock²

¹Agricultural Experiment Station, Wye Research and Education Center, College of Agriculture, University of Maryland at College Park, Box 169, Queenstown, Maryland 21658, USA

²Environmental Center, Anne Arundel Community College, 101 College Parkway, Arnold, Maryland 21012, USA

Received: 4 September 1996/Accepted: 9 March 1997

Abstract. The objective of this study was to conduct a series of 28-d partial life-cycle atrazine subchronic toxicity tests with sago pondweed, *Potamogeton pectinatus*, at salinities of 1, 6, and 12 ppt. These data will be used for development of a chronic estuarine criterion for atrazine in Maryland waters of Chesapeake Bay and to determine possible ecological risk for a sensitive nontarget species. The three endpoints used for this submerged aquatic macrophyte were final wet weight, final dry weight, and final number of rhizome tips at the termination of the 28-d test. Dry weight was determined to be the most sensitive endpoint. Chronic values from a one-way ANOVA using dry weight were 21.2, 21.2, and 10.6 $\mu\text{g/L}$ at salinities of 1, 6, and 12 ppt, respectively. Chronic values using wet weight were 21.2 $\mu\text{g/L}$ at all three salinities. A chronic value of 94.9 $\mu\text{g/L}$ was reported at all three salinities using rhizome tips as the endpoint. A two-way ANOVA was also used for analysis of data to increase the power of detecting differences among treatments and assess salinity interaction. The salinity effects were averaged in the two-way ANOVA. Both rhizome tips and dry weight were used in the two-way ANOVA; wet weight did not satisfy the equal variance assumption. The chronic value for rhizome tips was 94.9 $\mu\text{g/L}$ atrazine, which is the same value reported from the one-way ANOVA. There was no salinity effect and no interaction between salinity and atrazine concentration. The chronic value determined from the two-way ANOVA using dry weight was 5.3 $\mu\text{g/L}$; there was a salinity effect but no significant salinity/atrazine concentration interaction. Salinity was not reported to effect either dry weight or rhizome tips after 28-d exposures in the controls but wet weight was significantly lower at 12 ppt when compared with 1 and 6 ppt. Ecological risk to sago pondweed from atrazine exposure was judged to be low based on recent exposure data from the mainstem, tributaries, and streams in Chesapeake Bay.

vegetation. This herbicide has been identified by the Criteria and Standards Workgroup of the Chesapeake Bay's Toxic Subcommittee as a "Toxic of Concern" (US EPA, 1991). Presently, there are no human health or aquatic life surface water criteria for atrazine although this herbicide has been reported in the ground and surface waters of the Chesapeake Bay watershed (Hall and Anderson 1991; Hall *et al.* 1996a). The U.S. Environmental Protection Agency is working to establish both freshwater and marine criteria for atrazine but it is doubtful that these criteria will be completed in the near future (David Hansen, US EPA, personal communication). Due to limited resources, the US. Environmental Protection Agency does not establish estuarine criteria for atrazine or any other substance. Development of estuarine criteria is receiving high priority because we need to determine a "toxicity benchmark" for chemicals such as atrazine in the Chesapeake Bay watershed. Logical reasons for developing estuarine criteria are: (1) estuarine organisms may differ substantially in their sensitivity to some toxic substances due to their inherent differences from either freshwater or marine organisms, and (2) the bioavailability of some toxic substances may be different in estuarine water due to its unique chemistry, thus toxicity would be affected.

Toxicity data from eight different families, including an algal species or vascular plant, are required for the development of a chronic criterion for atrazine (unless an acute to chronic ratio approach is used). The chronic aquatic vascular plant data that is currently available for atrazine does not meet the various requirements for data acceptance recommended by the U.S. Environmental Protection Agency (Stephan *et al.* 1985). Since atrazine is a herbicide that is designed to be toxic to target plants (weeds), it is critical that scientifically valid plant data be used in the criterion development process.

The goals of this study were to: (1) conduct partial life cycle (subchronic) toxicity studies with atrazine at a range of salinities (1, 6, and 12 ppt) for a common submerged aquatic macrophyte found in Chesapeake Bay, the sago pondweed *Potamogeton pectinatus*; (2) use these data for the development of a chronic atrazine criterion for Maryland waters of Chesapeake Bay; and (3) assess possible ecological risk of atrazine to sago pondweed based on recent exposure data from the Chesapeake Bay mainstem, tributaries, and streams.

Atrazine is a herbicide widely used for controlling broadleaf and grass weeds in corn, sorghum, sugarcane, and landscape

Materials and Methods

Culture Procedures

Culture procedures described in detail in other publications were used for sago pondweed (Fleming *et al.* 1988, 1991, 1993; Ailstock *et al.* 1991). Test specimens from a Chesapeake Bay axenic stock were provided by Anne Arundel Community College. Turions were sprouted in nutrient rich media (Murashige Shoot Multiplication Medium B, Carolina Biological Supply and 10-g/L sucrose in deionized water). Plants for the experiments were propagated from terminal sections of rhizomes comprised of a rhizome tip and two vegetative nodes. These plants were grown individually in 50-ml culture tubes containing 25 ml of the propagation media. After two to three weeks, plants were screened for uniformity of growth (approximate weight was 1.0 to 1.25 g) in order to initiate tests with similar size plants.

Test Procedures

Testing procedures described in detail in Fleming *et al.* (1988, 1991, 1993) and Hall *et al.* (1996b) were used (Table 1). All toxicity tests were conducted at 23 to 25°C in a temperature controlled room under full-spectrum florescent lighting providing about 70 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR (photosynthetically active radiation). Photoperiod was 16-h light: 8-h dark. All tests were static due to the minimal loss of atrazine that is expected based on 128-d degradation studies with atrazine (Hall *et al.* 1992).

Sago pondweed was exposed to five atrazine concentrations (300, 30, 15, 7.5, and 3.75 $\mu\text{g}/\text{L}$) and a control for 28 d at test salinities of 1, 6, and 12 ppt. Ten replicates (plants) were tested at each test condition in 1-L containers containing nutrient media and water (Table 2). Each atrazine test concentration was prepared by diluting a salinity adjusted stock solution (25 mg/L) of atrazine. Stock solutions were prepared 1 to 2 d prior to testing by dissolving 0.0515 g of atrazine (97.1% active ingredient supplied by Ciba-Geigy Corporation) in 2 L of filtered (0.4 μm) salinity adjusted diluent and continuously stirring with a magnetic stir bar to ensure all atrazine was in solution.

The endpoints used for these studies were related to biomass production and vegetative reproductive potential: dry weight, wet weight, and rhizome tips. All endpoints were determined on day 0 and day 28 (end of test). At the end of the test, endpoint measurements were compared among test conditions as well as comparisons of each test condition with the control value. The gain in biomass and rhizome tips during the 28-d exposure was used to determine atrazine effects. Standard water quality conditions of temperature, salinity, pH, and dissolved oxygen were measured in the controls on day 0 and at the various test conditions on day 28.

Atrazine Analysis

Aqueous samples for atrazine analysis were collected from selected conditions on day 0 and day 28. Atrazine was measured at the high (300 $\mu\text{g}/\text{L}$), medium (15 $\mu\text{g}/\text{L}$), and low (3.75 $\mu\text{g}/\text{L}$) concentrations and controls on day 0 and day 28 for each test salinity.

Atrazine analysis was conducted by New York State Agricultural Experiment Station of Cornell University using similar analytical procedures that have been used for our previous atrazine studies (Hall *et al.* 1992). Samples for atrazine analyses were collected in 200-ml polyethylene containers from the various test conditions at day 0 and day 28, frozen, and shipped to the contractor on dry ice by Federal Express courier service.

Water samples were frozen at -40°C until sample preparation for analysis was initiated. Samples were first warmed to room temperature.

Table 1. Test conditions for sago pondweed/atrazine toxicity experiments

1. Temperature	23—25°C
2. Lighting	70 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR
3. Photoperiod	16-h L:8-h D
4. Size of test vessel	1 L
5. Volume of test solution	750 ml
6. Age of test plants	4-5 weeks (\approx 1-1.5 g)
7. No. of plants per test vessel	1
8. No. of replicates per concentration	10
9. Feeding regime	Culture media
10. Aeration	Yes, with CO ₂ enrichment
11. Dilution water	Distilled water
12. Test duration	28d
13. Effect measured and	Wet weight, dry weight, and number of rhizome tips

Table 2. Culture media used for sago pondweed/atrazine toxicity tests

Murashige Shoot Multiplication Medium B		Bioassay Culture Media	
Components	mg/L	H ₂ O column	
NH ₄ NO ₃	1650.00	Synthetic freshwater solution	
KNO ₃	1900.00	Components	mg/L
CaCl ₂ (anhydrous)	333.00	NaHCO ₃	96.00
MgSO ₄ (anhydrous)	181.00	CaSO ₄ · 2H ₂ O	60.00
KH ₂ PO ₄	170.00	MgSO ₄	60.00
FeNaEDTA	36.700	KCl	4.00
H ₃ B ₃	6.20		
MnSO ₄ · H ₂ O	16.90	Substrate Murashiges Minimal	
ZnSO ₄ · 7 H ₂ O	8.60	Organic Medium	
KI	0.830	NH ₄ NO ₃	1650.00
Na ₂ MoO ₄ · 2 H ₂ O	0.250	KNO ₃	1900.00
CuSO ₄ · 5 H ₂ O	0.025	CaCl ₂ (anhydrous)	333.00
CoCl ₂ · 6 H ₂ O	0.025	MgSO ₄ (anhydrous)	181.00
NaH ₂ PO ₄ · H ₂ O	170.00	KH ₂ PO ₄	170.00
Adenine sulfate	80.00	FeNaEDTA	36.700
IAA*	2.00	H ₃ B ₃	6.20
inositol	100.00	MnSO ₄ · H ₂ O	16.90
Kinetin	2.00	ZnSO ₄ · 7 H ₂ O	8.60
Thiamine HCL	0.400	KI	0.830
Sucrose	10,000	Na ₂ MoO ₄ · 2 H ₂ O	0.250
pH	5.0	CuSO ₄ · 5 H ₂ O	0.025
		CoCl ₂ · 6 H ₂ O	0.025
		iInositol	100.00
		Thiamine HCL	0.400
		Agar	6000.00
		pH	5.6

Aeration

Ambient air enriched to a final concentration of 3% CO₂ is delivered through a sponge stoppered lid via a 50- μl glass microcapillary pipette attached to microtubing. Enriched air is mixed using a Visablend Gas proportioner then delivered thru a flow meter at a rate of 2500 ml/h.

* Indole acetic acid

Fifty-milliliter aliquots from the original sampling containers were Solid-Phase Extracted (SPF) by employing a 12-station vacuum manifold, using Waters™ C₁₈ Sep-paks (Millipore Corp., Milford, MA). Sep-Pak cartridges were preconditioned by initial activation with HPLC MeOH and then washed with HPLC/nanopure H₂O. Samples were then loaded at a rate of 5-7 ml/min, and the cartridges were allowed to vacuum-dry for approximately 10 min. Loaded Sep-Paks were then frozen at -22°C, warmed to room temperature, and

Chronic Toxicity of Atrazine to Sago Pondweed

Table 3. Water quality data from the 28-d atrazine toxicity experiments with sago pondweed. The “D” used in this table is an abbreviation for day

Atrazine (µg/L)	Salinity (ppt)	Temperature (°C)		pH		DO (mg/L)		Salinity (ppt)	
		D=0	D=28	D=0	D=28	D=0	D=28	D=0	D=28
Control	1	24.2	23.4	9.24	6.49	6.4	8.1	1	1
Control	6	25.5	23.5	8.73	6.60	6.7	8.0	6	6
Control	12	25.9	22.5	8.65	6.65	6.4	7.8	12	12
3.75	1	–	23.3	–	6.54	–	8.2	–	1
3.75	6	–	23.5	–	6.63	–	8.0	–	6
3.75	12	–	23.1	–	6.62	–	7.6	–	12
7.50	1	–	23.0	–	6.55	–	8.1	–	1
7.50	6	–	23.9	–	6.56	–	7.8	–	6
7.50	12	–	22.5	–	6.78	–	8.2	–	12
15.0	1	–	23.3	–	6.50	–	8.1	–	1
15.0	6	–	23.8	–	6.58	–	7.8	–	6
15.0	12	–	23.0	–	6.76	–	7.5	–	12
30.0	1	–	22.9	–	6.50	–	7.9	–	1
30.0	6	–	23.6	–	6.59	–	7.9	–	6
30.0	12	–	23.2	–	6.64	–	7.7	–	12
300	1	–	23.4	–	6.55	–	7.5	–	1
300	6	–	23.4	–	6.54	–	7.6	–	6
300	12	–	23.5	–	6.59	–	7.4	–	12

Table 4. Nominal and measured concentrations of atrazine at various test conditions

Nominal Test Conditions (µg/L)	Salinity (ppt)	Atrazine Concentrations (µg/L)				% Change
		Day = 0	Recovery ^a	Day = 28	Recovery ^a	
Control	1	<2.0				
	6	<2.0				
	12	<2.0				
3.75	1	3.66	98%	2.88	77%	21
	6	4.14	110%	3.60	96%	13
	12	4.2	112%	3.36	90%	20
15	1	15.9	106%	13.2	88%	17
	6	14.1	94%	12.7	85%	10
	12	16.6	110%	13.0	86%	22
300	1	311	104%	295	98%	5
	6	282	94%	293	98%	4
	12	286	95%	302	101%	6

^aRecovery percentage based on atrazine concentration by GC analysis compared with that cited as the original or spike concentration

Table 5. Summary of NOEC, LOEC, and CV atrazine toxicity values (using one-way ANOVA) for sago pondweed at salinities of 1, 6, and 12 ppt

Endpoints	Salinity (ppt) and Toxicity Values (µg/L)								
	1			6			12		
	NOEC	LOEC	CV	NOEC	LOEC	CV	NOEC	LOEC	CV
Dry weight	15	30	21.2	15	30	21.2	7.5	15	10.6
Wet weight	15	30	21.2	15	30	21.2	15	30	21.2
Rhizome tips	30	300	94.9	30	300	94.9	30	300	94.9

aspirated to remove any residual water. Each Sep-Pak was eluted with 2 ml of HPLC grade acetonitrile into 13-ml glass test tubes, placed in a warm water bath, and blown down to dryness with high purity nitrogen. The dried eluates were redissolved in 1.0 ml redistilled HPLC benzene containing 0.10 µg of chlorothalonil (reference standard).

Recovery spikes were prepared from a 100-mg/L atrazine stock solution in HPLC acetone. Aliquots of 2.0, 6.0, and 12.0 ml were blown down to dryness, redissolved in 2-ml HPLC MeOH, and sonicated for several minutes to assure complete dissolution. After quantitatively

transferring to 50-ml graduated cylinders with ground glass tops, the 200-, 600-, and 1,200-mg/L spiking solutions were diluted to the 50-ml mark with 12, 6, and 1 ppt saline check samples, respectively. Method recovery spikes and water blanks (day-0 check waters) were then solid-phase extracted and prepared for analysis.

Atrazine analysis was conducted on a Hewlett-Packard 5890 Series II gas chromatograph (GC) equipped with a ⁶³Ni electron capture detector (ECD). The GC-ECD operating conditions were as follows: splitless injections of 1.0 µl at 280°C were conducted on a 30-m HP-5

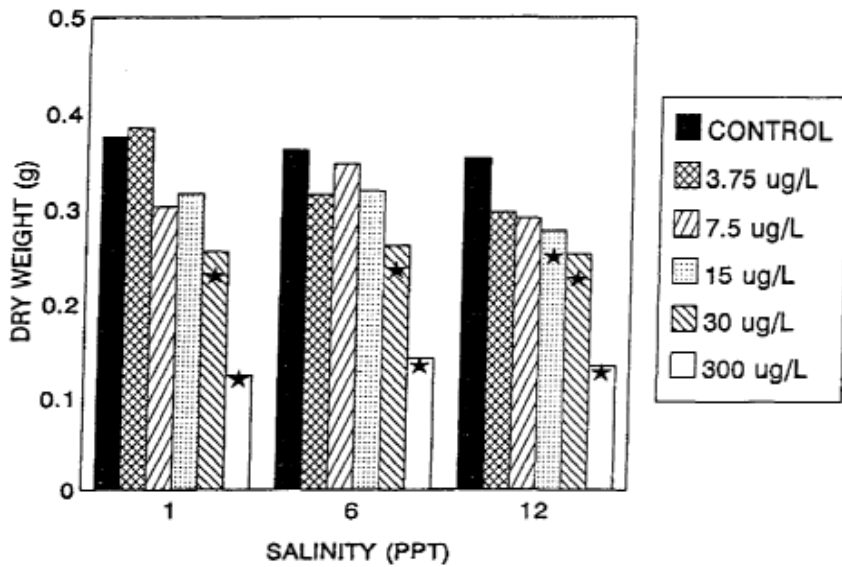


Fig. 1. Mean end dry weight data from one-way ANOVA at atrazine concentrations and salinities. An "*" indicates that the test condition was significantly different than the control.

* SIGNIFICANT DIFFERENCE FOUND (P=0.05)

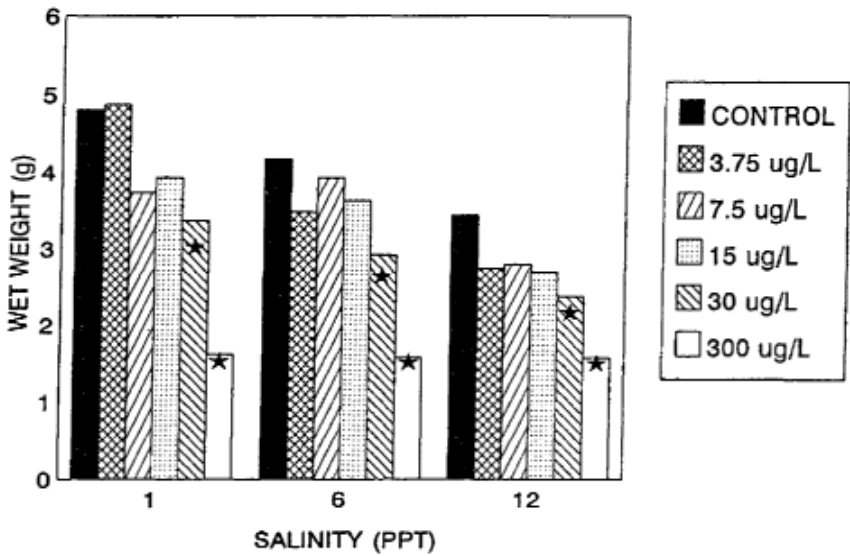
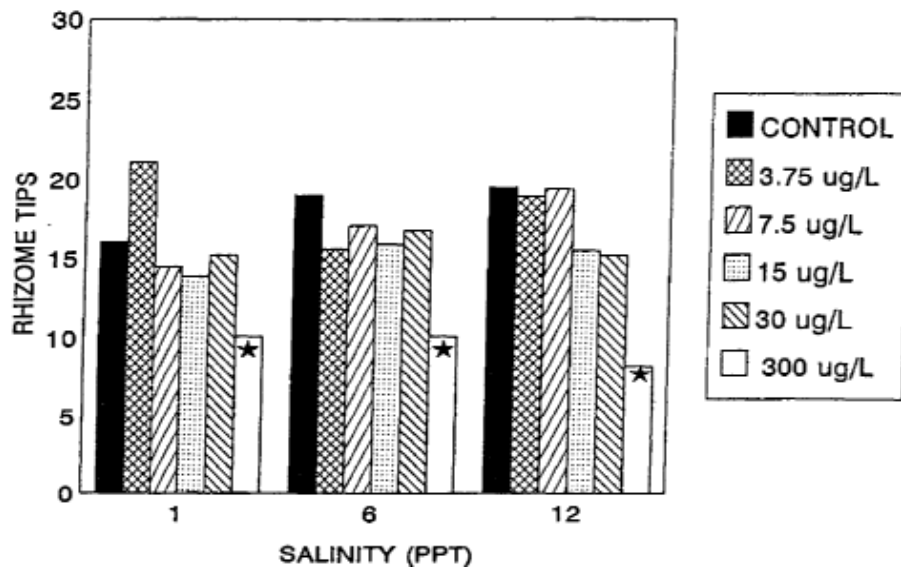


Fig. 2. Mean end wet weight data from one-way ANOVA at atrazine concentrations and salinities. An "*" indicates that the test condition was significantly different than the control.

* SIGNIFICANT DIFFERENCE FOUND (P=0.05)

ANOVA) and interactions between atrazine and salinity (two-way ANOVA). Normality and homogeneity of variance were determined with the Kolmogorov-Smirnov test and the Levene Median test, respectively. When data transformation was necessary to satisfy assumptions of ANOVA, the square root transformation was used.

The No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) were determined using Dunnett's procedures. The NOEC is the lowest concentration that is not statistically different than the control value. The LOEC is the lowest concentration that is statistically different ($p < 0.05$) from the control value. The chronic value (CV) was determined for each of the three endpoints (rhizome tips, wet weight, and dry weight) and salinities (1, 6, and 12 ppt) by calculating the geometric mean of the NOEC and LOEC.



* SIGNIFICANT DIFFERENCE FOUND (P=.05)

Fig. 3. Mean rhizome tips per plant from one-way ANOVA at atrazine concentrations and salinities. An "*" indicates that the test condition was significantly different than the control

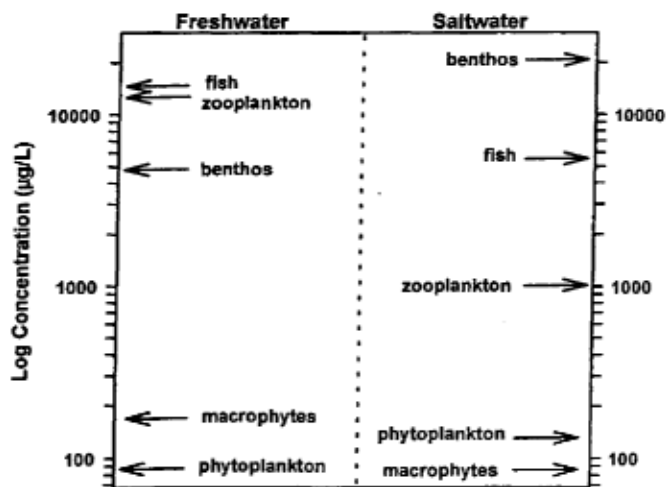


Fig. 4. Acute geometric means for atrazine toxicity data with freshwater and saltwater trophic groups (from Solomon *et al.* 1996)

Results

Water Quality and Atrazine Analysis

Water quality conditions measured during the study appeared adequate for survival of the test species (Table 3). There was a drop in pH and an increase in dissolved oxygen at various test conditions during the 28-d exposures. Nominal and measured concentrations of atrazine at day 0 and day 28 and recovery data for the selected test conditions are shown in Table 4. The mean loss of atrazine for all nine conditions was approximately 13% over the 28-d period. This is considered minimal loss in these types of experiments. Loss was generally less (<6%) at the higher atrazine concentration. The percent recovery for atrazine ranged from 77 to 112% for the selected test conditions.

Toxicity Data

Atrazine toxicity data for sago pondweed at the three salinities are presented in Table 5 and Figures 1-3 (one-way ANOVA results). Dry weight was a more sensitive endpoint than either wet weight or rhizome tips. Chronic values using dry weight were 21.2, 21.2, and 10.6 $\mu\text{g/L}$ atrazine at 1, 6, and 12 ppt, salinity, respectively. The chronic value using wet weight as an endpoint was 21.2 $\mu\text{g/L}$ at all three salinities. A chronic value of 94.9 $\mu\text{g/L}$ was reported at all salinities using rhizome tips as an endpoint

Two-Way ANOVA was used to increase the power of detecting significant differences among treatments and identify salinity interactions. This statistical approach provided an average effect across the three salinities (marginal effect). Both rhizome tips and dry weight data were used in the two-way ANOVA; wet weight data did not satisfy equal variance assumptions. The chronic value for rhizome tips was 94.9 $\mu\text{g/L}$ atrazine, which is the same value reported from the one-way ANOVA. There was no salinity effect and no interaction between salinity and atrazine concentration. For dry weight, the chronic value was 5.3 $\mu\text{g/L}$ and there was a significant salinity effect but no significant salinity/atrazine concentration interaction. The chronic value for dry weight using the two-way ANOVA was approximately one-half of the lowest value reported for dry weight using the one-way ANOVA (10.6 $\mu\text{g/L}$ at 12 ppt).

Control data for the three endpoints were examined after 28 d at each salinity to determine possible salinity effects. For both dry weight and rhizome tips, there was no significance difference among the three salinities. However, wet weight values were significantly lower at 12 ppt when compared with 1 and 6 ppt conditions.

Discussion

The herbicide atrazine acts by inhibiting photosynthesis due to blockage of electron transport the Hill reaction of

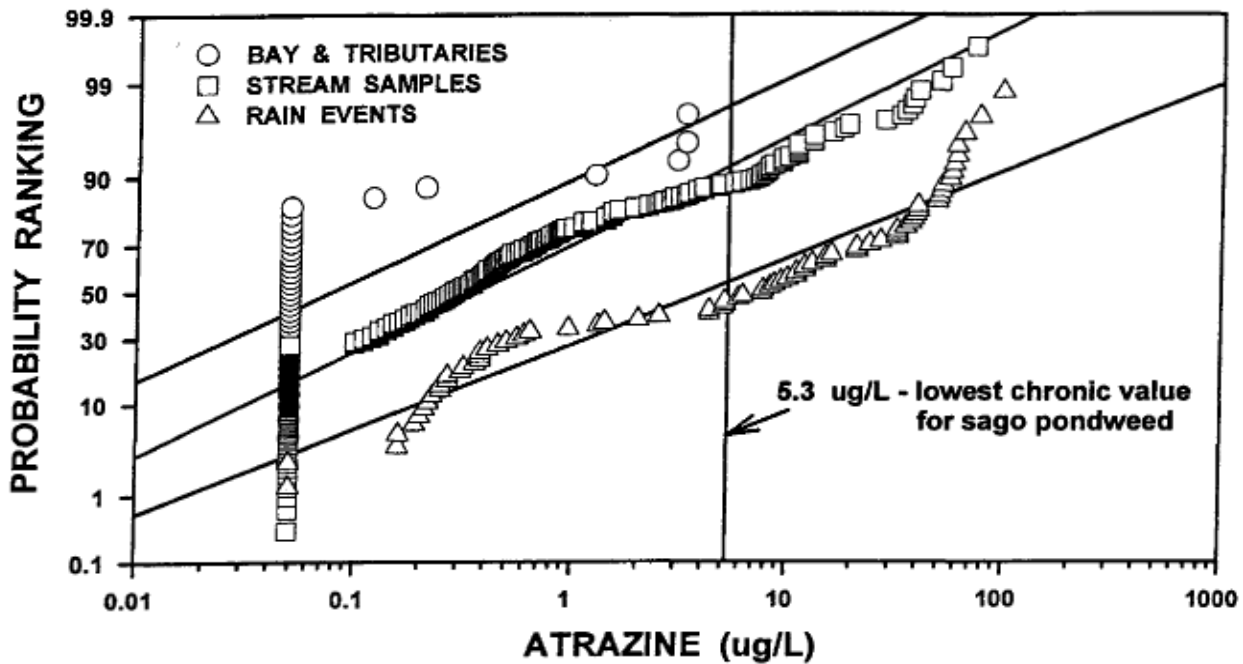


Fig. 5. Atrazine concentrations from 1995 sampling in the Chesapeake Bay mainstem/tributaries, streams and stream/rain events (see Hall *et al.* 1996a)

photosystem II. Since this pathway is primarily found in plants and not in animals, atrazine is predicted to be more toxic to plants. In a recent ecological risk assessment for atrazine in North American surface waters, laboratory toxicity data for 47 saltwater species in five trophic groups confirmed that plants were more sensitive to atrazine than animals (Solomon *et al.* 1996). Specifically, aquatic macrophytes were the most sensitive trophic group to atrazine (Figure 4). The lowest effect concentrations reported for saltwater aquatic macrophytes were as follows: 5 $\mu\text{g/L}$ caused reduced photosynthesis in *Potamogeton pectinatus* (Kemp *et al.* 1985) and 4 $\mu\text{g/L}$ reduced tuber development in *Vallisneria americana* (Cohn 1985). Both of these values are similar to our lowest chronic value of 5.3 $\mu\text{g/L}$ with sago pondweed using dry weight as an endpoint.

Chronic values for sago pondweed using the various endpoints, salinities, and types of statistical analysis ranged from 5.3 to 94.9 $\mu\text{g/L}$. All of these values are significantly lower than the 650 $\mu\text{g/L}$ reported by Correll and Wu (1982) to reduce oxygen consumption in sago pondweed in 21 to 42 d in saline conditions. In a freshwater sago pondweed atrazine toxicity study, Hemming *et al.* (1991) reported that 100 $\mu\text{g/L}$ reduced growth after 30 d of exposure. This value is similar to our highest chronic value of 94.9 $\mu\text{g/L}$ using rhizome tips as an endpoint.

Recent atrazine monitoring (exposure) data are available for the Chesapeake Bay watershed for comparison with the toxicity values reported for sago pondweed (Figure 5). In 1995, we measured atrazine in the following locations and frequencies in the Chesapeake Bay watershed: ten locations in the mainstem Chesapeake Bay and tributaries four times a year (March, May, June, and November), four stream sites in the Chester and Choptank River basins three times a week for a 26-week period (March-August), and the same four stream sites during one rain event at 4-h intervals for 72 h (Hall *et al.* 1996a). All mainstem

and tributary atrazine concentrations were less than 3.3 $\mu\text{g/L}$; therefore, even at the lowest chronic value of 5.3 $\mu\text{g/L}$ for sago pondweed, ecological risk is unlikely (Figure 5). For the four stream sites sampled for 26 weeks, the chronic value of 5.3 $\mu\text{g/L}$ was exceeded 9.8% of the time (Figure 5). Nearly all of these exceedences occurred during the last three weeks of May after the application of atrazine. Forty-five percent of the time atrazine exceeded the 5.3- $\mu\text{g/L}$ chronic value during the rain event sampling (Figure 5). As expected, atrazine concentrations are highest in the streams adjacent to corn fields when rainfall events occurred after atrazine application. These peak atrazine values were reported to decline significantly as the streams returned to base-flow conditions. Based on the limited temporal scale of these high values and the recovery potential of aquatic macrophytes, these atrazine exposures are judged to have low ecological risk.

Data generated in this study will be used to develop chronic estuarine water quality criterion for atrazine in Maryland waters of the Chesapeake Bay watershed. Since the estuarine environment includes a range of salinities, the interaction of salinity (1 to 12 ppt) on the toxicity of atrazine to resident species such as sago pondweed is a critical issue. Results from these experiments using the two-way ANOVA have demonstrated that salinity did not affect the toxicity of atrazine to sago pondweed when rhizome tips were used as endpoints, although there was a salinity effect when using dry weight as an endpoint. Growth of this macrophyte may have been somewhat impaired at the higher salinity, as suggested by the lower control weight value at 12 ppt when compared with the lower salinities.

There are three other animal studies available where the influence of salinity on the toxicity of atrazine was assessed (Hall *et al.* 1994, 1995). Maximum toxicity occurred in an acute study with the estuarine zooplankton, *Eurytemora affinis* at the lowest salinity when tested at 5, 15, and 25 ppt (Hall *et al.*

1994). Results from an 8-d chronic test with this zooplankton species showed that this species was more sensitive to atrazine exposure at either the high (25 ppt) or low (5 ppt) salinities when compared to the middle salinity of 15 ppt (Hall *et al.* 1995). In acute atrazine toxicity tests with sheepshead minnow, *Cyprinodon variegatus* larvae, atrazine was found to be more toxic at the highest salinity (25 ppt) when compared to 5 and 15 ppt (Hall *et al.* 1994). The above results for the three atrazine/salinity toxicity experiments show no consistent trend of salinity and atrazine toxicity interaction, but in all cases toxicity was affected by salinity.

Our results with sago pondweed showed that atrazine was more toxic at the higher salinity with one of three endpoints (dry weight). There are both chemical and biological reasons why salinity may influence the toxicity of atrazine: (1) atrazine bioavailability was different at different salinities; and (2) test species may be more physiologically effective in metabolizing and mitigating toxic effects of atrazine at different salinities. The bioavailability factor is a less convincing explanation since it is unlikely that the range of salinities used will greatly influence the bioavailable fraction since only a small arithmetic difference in interaction energy is produced by geometric changes in molar salt concentration (Kosower 1968). Results from studies with atrazine have demonstrated that salinity did not influence degradation and no significant loss of atrazine was reported after 128 day exposures in autoclaved saline water (Hall *et al.* 1992). The physiological reason is the more likely explanation for differences in toxicity observed at the three test salinities since growth of sago pondweed was somewhat reduced at the higher salinity.

Acknowledgements. We are grateful to the U.S. Environmental Protection Agency Chesapeake Bay Program (Contract Number U00P6002880) and the Maryland Department of the Environment for funding this study. Special consideration is extended to Mr. Richard Batiuk, Dr. Deirdre Murphy, and Mrs. Mary Jo Garreis for comments on the study design. Terry Spittler and Linn Lavin of Cornell University are acknowledged for conducting atrazine analyses. Dr. Elgin Perry is acknowledged for his advice on the statistical analysis. Mary Hancock is acknowledged for typing. This study was partially supported by Maryland Agricultural Experiment Station (MAES) Project Number MD-X-2 and is MAES Scientific Article Number A7914, MAES Contribution Number 9249.

References

- Ailstock MS, Fleming WJ, Cooke, TD (1991) The characteristics of axenic culture systems suitable for plant propagation and experimental studies of the submersed aquatic angiosperm, *Potamogeton pectinatus* (sago pondweed). *Estuaries* 14:57-64
- Cohn, SL (1985) An evaluation of the toxicity and sublethal effects of atrazine on the physiology and growth of the aquatic macrophyte, *Vallisneria spiralis* L. Ph.D. Dissertation. American University, Washington, DC
- Correll DL, Wu TL (1982) Atrazine toxicity to submersed vascular plants in simulated estuarine microcosms. *Aquat Bot* 14:15 1-158
- Fleming WJ, Ailstock MS, Monet JJ (1993) Net photosynthesis and respiration of sago pondweed (*Potamogeton pectinatus*) exposed to herbicides. In: Hughes J, Biddinger G, Mones E (eds) Third Symposium on Environmental Toxicology and Risk Assessment: Aquatic, Plant and Terrestrial, ASTM STP. American Society for Testing and Materials, Philadelphia, PA, pp 1-14
- Fleming WJ, Ailstock MS, Momot JJ, Norman CM (1991) Responses of sago pondweed, a submerged aquatic macrophyte, to herbicides in three laboratory culture systems. In: Gorsuch WR, Lower WR, Wang W, Lewis MA (eds) Plants for Toxicity Assessment: Second Volume ASTM STP 1115. American Society for Testing and Materials, Philadelphia, PA, pp 267-275
- Fleming WJ, Momot JJ, Ailstock MS (1988) Bioassay for phytotoxicity of toxicants to sago pondweed. In: Advances in Chesapeake Bay Research, Proceedings of a Conference, Chesapeake Research Consortium, Solomons, MD, pp 43 1-440
- Hall LW Jr, Ailstock MS, Anderson RD (1996b) Standard operating procedures for conducting sub-chronic aquatic toxicity tests with sago pondweed *Potamogeton pectinatus*: A submersed aquatic angiosperm. Report, USEPA Chesapeake Bay Program Office, Annapolis, MD
- Hall LW Jr, Anderson RD (1991) A review of estuarine aquatic toxicity data for the development of aquatic life criteria for atrazine in Chesapeake Bay. Report, Maryland Department of Environment, Baltimore, MD
- Hall LW Jr, Anderson RD, Kilian JV (1996a) Monitoring of atrazine and metolachlor in the mainstem, major tributaries and small streams of the Chesapeake Bay watershed. Draft Report, Ciba Geigy Corporation, Greensboro, NC
- Hall LW Jr, Ziegenfuss MC, Anderson RD, Spinier TD, Leichtweis HC (1992) The effects of salinity on the degradation of atrazine. Report, Ciba-Geigy Corporation, Greensboro, NC
- (1994) Influence of salinity on atrazine toxicity to a Chesapeake Bay copepod (*Eurytemora affinis*) and fish (*Cyprinodon variegatus*). *Estuaries* 17:181-186
- Hall LW Jr, Ziegenfuss MC, Anderson RD, Tierney DP (1995) The influence of salinity on the chronic toxicity of atrazine to an estuarine copepod: Implications for development of an estuarine criterion. *Arch Environ Contam Tox* 28:344-348
- Kemp WM, Boynton, WR, Cunningham II, Stevenson JC, Jones TW, Means JC (1985) Effects of atrazine and linuron on photosynthesis and growth of the macrophytes, *Potamogeton perfoliatus* and *Myriophyllum spicatum* L. in an estuarine environment. *Mar Environ Res* 16:255-280
- Kosower EM (1968) An Introduction to Physical Organic Chemistry. John Wiley and Sons, New York
- Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, LaPoint TW, Kendall RJ, Giddings, JM, Giesy JP, Hall LW Jr, Williams WM (1996) Ecological risk assessment of atrazine in North American surface waters. *Environ Tox Chem* 15:31-76
- Stephan CE, Mount DI, Hansen DI, Gentile JH, Chapman GA, Brungs WA (1995) Guidelines for deriving numerical water quality criteria for the protection of aquatic organisms and their uses. Report, USEPA, Office of Research and Development, Washington, DC
- USEPA (United States Environmental Protection Agency) (1991) Chesapeake Bay toxics of concern list information sheets. Report. USEPA. Chesapeake Bay Program, Annapolis, MD