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Toxicity of Atrazine to Marine Invertebrates Under Flow-Through Conditions—Eastern Oyster (*Crassostrea virginica*) and Mysid Shrimp (*Americamysis bahia)*

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Abstract The triazine herbicide atrazine is routinely detected in freshwaters, and has also been detected in coastal waters in Europe and the US. Relatively few atrazine studies have been conducted with estuarine/marine invertebrates. This study sought to contribute additional invertebrate atrazine toxicity data using model species, Eastern oyster (*Crassostrea virginica*) and Mysid shrimp (*Americamysis bahia*). Specifically, we investigated (1) acute effects on survival and growth of Eastern oyster, and (2) acute and chronic effects on survival, reproduction, and growth of the Mysid shrimp. No oyster mortality was observed following 96-h exposure to up to 17 mg a.i./L, but shell growth was reduced by 0.55% to 16% in 1.0, 9.2, and 17 mg a.i./L treatments, resulting in a 96-h EC50 of > 17 mg a.i./L. In the 96-h Mysid test, mortality rates of 5 to 70% were observed in 1.7, 2.4, 3.6, and 6.4 mg a.i./L treatments, and the 96-h LC50 was 5.4 mg a.i./L. Sub-lethal effects were observed among surviving Mysids exposed to ≥ 2.4 mg a.i./L. After 28 days of exposure to up to 1.1 mg a.i./L, there were no effects on survival or reproduction. The LOEC was 0.50 mg a.i./L, based on reduced body size, and the NOEC was 0.26 mg a.i./L. Overall, the results indicate that atrazine is slightly toxic towards Eastern oyster and moderately toxic to the Mysid shrimp under acute exposure conditions. These data will help to fill a gap in the literature and inform risk assessment of potential effects of atrazine towards estuarine/marine communities.

Keywords Crustacean · Bivalve · Invertebrate toxicity · Pesticides

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1 Introduction

Atrazine is a triazine herbicide used primarily for selective control of broadleaf weeds in corn and sorghum crops (Moore et al., 2017). As a result of its wide adoption (USGS, 2020) and potential to enter waterways via runoff (Moore et al., 2017), it is routinely detected in North American surface waters (e.g., ECCC, 2011; Government of Canada, 2016; US EPA, 2019a). While much of its use occurs in the Midwestern US, major watersheds (e.g., Mississippi River) can accumulate atrazine and other herbicides and transport them to coastal waters (Clark et al., 1999; DeLorenzo et al., 2006). In addition, there are pockets of relatively intensive atrazine use (typically in corn) in several states (e.g., North Carolina, South Carolina, Texas; USGS, 2020) that could result in movement of atrazine more directly into estuarine and/or marine environments. For example, atrazine has been measured in estuarine samples collected from the mid-Texas coast at peak concentrations from 0.01 to 62.5 μg/L (Pennington et al., 2001) and in Chesapeake Bay from 0.006 to 300 μg/L (DeLorenzo et al., 2006). Detections of atrazine in other

coastal and estuarine waters in Europe (Nödler et al., 2013, 2014) and the US (Clark et al., 1999; DeLorenzo et al., 2006 and references therein) have also been reported, generally in the range of 0.001 to <10 μg/L.

Given that atrazine can reach marine environments under some use patterns, knowledge of potential effects in marine organisms is desirable to inform risk assessments. In the 2016 Refined Ecological Risk Assessment for Atrazine (US EPA, 2016), it was noted that although studies were available for marine invertebrates, typically only acute LC50/EC50 values were reported. Bouilly et al. (2004) also noted that relatively few atrazine toxicity studies have been conducted with marine invertebrates, considering the number of species within these taxa. Bivalve species have long been recognized as sentinel species for monitoring of anthropogenic chemicals (including pesticides) in estuarine/marine environments (Alvarez et al., 2014; Bouilly et al., 2004; Kim et al., 2008), and effects of atrazine have been studied previously in bivalve species such as Pacific oysters (*Crassostrea gigas*), Eastern oyster (*Crassostrea virginica*), and hard clam (*Mercenaria mercenaria*) (Britt et al., 2020; Gagnaire et al., 2003). However, toxicity data for tests using technical atrazine are relatively limited for this group of organisms.

Acute toxicity endpoints considered by the US EPA for risk assessment ranged from 0.048 to 13.3 mg/L, with the most sensitive organism being the 96-h LC50 for the juvenile stage of the opossum shrimp *Neomysis integer* (Noppe et al., 2007; US EPA, 2016). Based on this endpoint, US EPA deemed that atrazine posed a potential risk for estuarine or marine species under some use scenarios (US EPA, 2016, 2019b). Shrimp and other crustaceous species represent important components of estuarine/marine ecosystems, as well as being economically valuable fisheries species (Kruse, 2020), but the data relatively are limited. In addition to the opossum shrimp, acute studies with atrazine have been conducted on grass shrimp (*Palaemonetes pugio* 96-h survival; Key et al., 2007) and prawn (*Penaeus monodon* 24-h metamorphosis; Mercurio et al., 2018). Given the demonstrated relative sensitivity of the opossum shrimp to atrazine, additional information on other shrimp-like species will improve our understanding of potential effects and risks of atrazine in coastal environments.

The specific objectives of this study were to determine (1) the acute effects of atrazine on survival and growth of Eastern oyster (*Crassostrea virginica*), and (2) the acute and chronic effects of atrazine on survival, reproduction, and growth of the Mysid shrimp *Americamysis bahia*. These species were selected based on their demonstrated sensitivities towards toxicants and substantial availability of historical data (i.e., standard test species). The Eastern oyster is a preferred species for the shell deposition test and the Mysid shrimp is a representative marine invertebrate with life-cycle characteristics suitable for performing continuous, chronic exposures to toxicants in laboratory exposure systems. Collectively, the results of these studies can contribute to our understanding of potential effects of atrazine on marine invertebrates.

2 Materials and Methods

A total of three individual studies were conducted to investigate potential effects of atrazine in marine invertebrates. A summary of the study methods is presented in the sections below, with additional details provided in the supplementary information. All studies were conducted at Smithers, located in Wareham, MA (formerly Springborn Smithers Laboratories). Procedures used in the Eastern oyster toxicity test were in concordance with the appropriate Standard Evaluation Procedure (US EPA, 1985) and the OPPTS (Draft) Guideline 850.1025 (US EPA, 1996a, b). The 96-h toxicity test was conducted from 11 to 15 March 2005. The Smithers in-house protocols for Mysid shrimp testing were consistent with standard procedures from US EPA (1989). The 96-h acute toxicity test was conducted from 21 to 25 July 1994 and the 28-d life-cycle study was conducted from 7 July to 4 August 2005.

2.1 Test Solutions

2.1.1 Test and Reference Substances

The test material (atrazine technical, 97.1%) for the acute Mysid test was received from CIBA-GEIGY Corporation (now Syngenta Crop Protection, Inc.), Greensboro, NC, on 11 March 1994. Dimethylformamide (DMF; CAS #68-12-2) was used in preparation of the stock solution and was included in the acute mysid test as a solvent control. The test material, atrazine technical (97.1%), and reference substance, atrazine (96.2%), used for the Eastern oyster acute test and mysid life-cycle test were received on 18 November 2004 from Syngenta Crop Protection, Inc., Greensboro, NC. Upon

receipt at the testing laboratory, the test and reference substances were stored at room temperature (approximately 20 °C) in a dark ventilated cabinet and were used to prepare test solutions and quality control samples, and calibration standards, respectively. Concentrations were adjusted for the purity of the test substance and are reported as milligrams of active ingredient per liter of solution (mg a.i./L).

2.1.2 Test Concentrations

Selection of nominal atrazine concentrations was based on preliminary tests conducted by the testing laboratory (results presented in the SI). The nominal concentrations used for the definitive 96-h test with Eastern oysters were 6.3, 13, 25, 50, and 100% dilutions of a water accommodated fraction (WAF) prepared from a 33 mg a.i./L test solution via serial dilution. For the 96-h Mysid test, nominal concentrations were 0.91, 1.5, 2.5, 4.2, and 7.0 mg a.i./L. For the 28-d life-cycle Mysid test, nominal concentrations of 0.063, 0.13, 0.25, 0.50, and 1.0 mg a.i./L were selected.

2.1.3 Test Dilution Water

Natural seawater from the Cape Cod Canal, Bourne, MA, was used as dilution and control water during both the Eastern oyster and acute mysid studies. The seawater had a salinity of 32 to 33‰ and a pH of 7.8 to 8.1. Artificial seawater, prepared by addition of hw-MARINEMIX® to freshwater, was used as dilution water for the life-cycle study. Newly prepared artificial seawater had alkalinity of 154 to 168 mg/L as $CaCO_3$, pH of 8.0 to 8.3, and salinity of 20 ± 3‰. Representative samples of natural and artificial dilution waters were analyzed for pesticides, PCBs, and metals (Tables S1, S2). None of these were detected at concentrations that are considered toxic (ASTM, 2002).

2.2 Test Organisms

2.2.1 Eastern Oyster

Eastern oysters (*Crassostrea virginica*) were obtained from Circle C Oysters, Ridge, MD. Upon arrival, the oysters were carefully examined to confirm reproductive immaturity, and general health. The oysters were of similar age and had a mean valve height of 34 ± 3.2 mm (n = 30). Oysters were held under flow-through seawater conditions for a 14-d acclimation period and were fed *Tetraselmus maculata* prepared in seawater. Mortality of < 1% was observed during the seven days before test initiation. Prior to testing, 3 to 5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge, and any oysters which appeared less than optimal were discarded. During the exposure, the oysters received supplemental feedings of algal suspension (*T. maculata,* ~10^7 cells/mL) three times daily.

2.2.2 Mysid Shrimp

Mysids (*Americamysis bahia*) were obtained from laboratory cultures maintained at Smithers. The brood stock was originally obtained from Aquatic BioSystems, Inc., Fort Collins, CO. Mysid shrimp cultures were fed live brine shrimp (*Artemia salina*) nauplii twice daily. Food samples were analyzed for pesticides, PCBs, and metals (Tables S3, S4), and none of these compounds were present at concentrations toxic to the test species (ASTM, 1985). For the acute test, the culture seawater had a salinity of 31 to 32 ‰, a pH of 7.5 to 7.7, temperature of 25 to 26°C, and dissolved oxygen (DO) concentration range of 87 to 103% of saturation. For the life-cycle test, mysids were cultured in several 76-L glass aquaria with a salinity of 20 to 22‰, and a pH range of 8.2 to 8.3. Test organisms were maintained under these respective conditions for a minimum of 14 days prior to testing. Mysids used in the toxicity tests were <24 h old and were collected using a variation of the method described by Reitsema and Neff (1980).

2.3 Test Conditions

2.3.1 Eastern Oyster Acute Test

The acute oyster exposure system consisted of a constant-flow serial diluter, a temperature-controlled water bath, and 12 glass exposure aquaria. The test system provided five concentrations of atrazine and a dilution water control, all in duplicate. Aquaria were placed in a temperature-controlled water bath designed to maintain a test solution temperature of 20 ± 2°C. The photoperiod was 16:8 h light:darkness throughout the test. The concentration of atrazine within the diluter was equivalent to the highest nominal concentration tested (100% WAF) and was subsequently diluted (50% dilution factor) to provide the remaining nominal test

concentrations: 50, 25, 13, and 6.3% WAF. The exposure was initiated by placing 20 oysters in each test aquarium (40 per treatment level and the control). Oysters were spaced equidistant from one another with their valve inflow openings facing toward the flow of water from the Teflon® circulator tube.

2.3.2 Mysid Shrimp Acute Test

The acute Mysid shrimp test was conducted in an exposure system consisting of a continuous-flow serial diluter, a temperature-controlled water bath, and 14 aquaria. The test system provided five concentrations of atrazine, a solvent control, and a dilution water control, all in duplicate. The photoperiod was 16:8 h, temperature was 25 ± 1°C, and light intensity was maintained at 270 to 910 lux. The test was initiated when ten mysids (20 organisms per treatment level and control) were distributed to each replicate aquarium. Mysids were fed live *A. salina* nauplii twice daily during the 96-h test.

2.3.3 Mysid Shrimp Life-Cycle Test

The life-cycle exposure system consisted of an intermittent-flow proportional diluter, temperature-controlled water bath, and 12 exposure aquaria. The exposure system provided five concentrations of the test substance and a dilution water control, maintained in duplicate. The test area was illuminated at an intensity of 340 to 700 lux, maintaining a photoperiod of 16:8 h. The water bath maintained the test solution temperatures at 26 ± 2 °C. Mysids were fed live brine shrimp nauplii twice daily. Following the pairing phase, nauplii were enriched with Selco® every other day.

Each exposure aquarium contained two retention chambers used to maintain non-paired mysids during the study, yielding 30 mysids per replicate vessel and 60 organisms for each treatment level and the control. Retention chambers were glass Petri® dishes, to which a Nitex® screen collar (350-μm) was attached. Pairing chambers, used to house sexually mature male and female organisms, were cylindrical glass jars (5.1 cm diameter, 10 cm high) with two 2-cm holes covered by 350-μm Nitex® screens.

The time to brood appearance (i.e., gravid females) was noted for all F0 mysids prior to pairing (beginning on Day 12). Upon sexual maturity (Day 14), male/female pairs within each exposure aquarium were transferred to ten glass pairing jars (one pair per jar). The remaining mysids were pooled and placed in one of the initial retention chambers for the duration of the test. Male mysids from this pool were used to replace dead males from the paired groups. Dead females in the pairing jars were not replaced. If development of brood pouches was delayed, all test organisms were maintained in the retention chambers until maturity was observed or until test termination.

2.4 Effects Monitoring

2.4.1 Eastern Oyster Acute Test

Biological observations (e.g., excessive mucous production or lack of fecal and pseudofecal production) were made at test initiation and every 24-h until termination. After 96 h of exposure, the oysters were removed from the test aquaria and the new shell growth was measured to the nearest 0.1 mm using a calibrated micrometer.

2.4.2 Mysid Shrimp Acute Test

Mortality, biological observations, and observations of the test solutions (e.g., precipitate, film on the solution's surface) were made at test initiation and at each subsequent 24-h interval until test termination. Effects were based on mortality, defined as the absence of movement and reaction to gentle prodding. Mortalities were recorded and removed from each aquarium every 24 h during the exposure period.

2.4.3 Mysid Shrimp Life-Cycle Test

Each retention chamber was gently lifted from the aquaria daily, placed in a dish on a light table, and dead organisms were counted. After pairing (Day 14), the number of dead males and females, the number of offspring, and any abnormal appearances or behaviors were recorded. Mortalities of F0 and F1 mysids were recorded and discarded when observed during the test. At test termination, all mysids were sacrificed by immersion in cold, deionized water. The mysids were carefully removed, blotted dry, and separated into male and female groups for each replicate exposure level. Individual body length was determined using a dissecting microscope (Olympus Optical). Following measurement, mysids were transferred to aluminum pans, dried at 60 ± 5 °C for 94 h, then placed in a desiccator. Individual total dry body weight was determined using

an analytical balance (American Scientific Products Model SP182). Reproductive success was calculated for each replicate aquarium as the ratio of the total number of offspring produced to the total number of females within each chamber per reproductive day. The number of female reproductive days was the number of days that an individual female was alive, counting from the day that offspring were first observed in any control pairing jar (i.e., Day 14 represented reproductive Day 1).

2.5 Water Quality and Analytical Measurements

During each of the three studies, pH, temperature, salinity, and DO concentration were measured daily in each replicate (details in SI). In addition, in each study, the dilution water control and the high, middle, and low test concentrations were sampled and analyzed for atrazine concentration prior to the start of the exposure. During the acute Eastern oyster study, samples were taken from one replicate solution of each treatment level and the control at each sampling interval. All samples were analyzed for atrazine by gas chromatography using nitrogen phosphorous detection (GC/NPD). For the acute Mysid test, each replicate for each treatment level and the controls were sampled at 0 and 96 h of exposure, and analyzed using GC. For the Mysid life-cycle test, samples were taken from alternate replicate test solutions of each treatment level and the control on test days 0, 7, 14, 21, and 28. All exposure solutions and quality control (QC) samples were analyzed by GC/NPD. The analytical limits of quantitation (LOQ) varied between studies, with a value of 0.179 μg/L for the acute mysid test and a value of 0.38 μg/L for both the Eastern oyster acute test and the mysid life-cycle test.

2.5.1 QA/QC

In each study, three QC samples were prepared at each sampling interval. A method validation study was conducted prior to the definitive exposures and established an average recovery of atrazine of 102 ± 8.0% from filtered seawater. Defined limits for acceptance of QC sample performance were set at 85.0 to 115%.

2.6 Statistical Analysis

Survival and growth data were assessed for homogeneity of variance using Bartlett's Test (Horning & Weber, 1985) and normality using Shapiro-Wilk's Test (Weber et al., 1989). When a solvent control was used, if significant differences were determined between negative control and solvent control results, treatment groups were compared to the solvent control. If controls were not significantly different, control data were pooled. Statistical analyses were performed using measured concentrations. All statistical tests used a 95% level of certainty except in the cases of Bartlett's Test and Shapiro-Wilk's Test, in which the 99% level of certainty was applied.

The median effect concentration (EC50) for Eastern oyster was the estimated concentration of test substance which reduced shell deposition (growth) of the exposed oysters by 50%, as compared to controls. If at least one test concentration caused a reduction in growth of greater than or equal to 50% of control, the LC50 and 95% confidence intervals (CIs) were calculated by probit analysis. The mean measured concentrations tested (0- and 96-h) were used to estimate the median lethal concentrations (LC50) and 95% CI at each 24-h interval of the exposure period. The LC50 values were calculated using one of: moving average angle analysis, probit analysis, or nonlinear interpolation, with 95% CIs calculated by binomial probability. If two or more statistical methods produced acceptable results, the method yielding the smallest 95% CI was selected. The No-Observed-Effect Concentration (NOEC) for the 96-h exposure period was determined using Williams' Test (Williams, 1971, 1972). The NOEC was defined as the highest concentration at, and below which, there was no statistically significant reduction in growth of oysters and no toxicant related mortalities or physical and behavioral abnormalities in mysids relative to control organisms. At the termination of the chronic mysid test, data from the paired (male/female) organisms were analyzed to establish treatment level effects on survival, growth (average dry body weight and average total body length), and reproduction. ToxStat® (Release 3.5, West, Inc. and Gulley, 1996) was used to estimate the theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty (i.e., the Maximum-Acceptable-Toxicant Concentration - MATC).

3 Results

In all three studies, concentrations of atrazine in the exposure solutions were generally consistent and the

delivery apparatus maintained the expected concentration-gradient (approximately 50% dilution factor) of the test substance. Throughout the exposure periods, no visible sign of undissolved test substance (e.g., precipitate) was observed in the mixing chamber, the chemical cells of the diluter system, and/or in any of the exposure solutions, as applicable. In each study, the water quality parameters measured were not statistically different among treatment or control groups, and remained within acceptable ranges for the survival/growth/reproduction of Eastern oyster or mysids, as applicable (Tables S5, S6).

3.1 Eastern Oyster Acute Test

3.1.1 Analytical Results

Results of this analysis established measured concentrations tested as 1.0, 2.0, 4.2, 9.2, and 17 mg a.i./L (Table 1). Analysis of five of the six QC samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 92.0 to 106% of the nominal fortified levels (1.00, 8.00, and 30.0 mg a.i./L).

3.1.2 Effects of Atrazine Exposure

New shell growth among control oysters at test termination averaged 3.61 mm (Fig. 1, Table S7). The Standard Evaluation Procedures (US EPA, 1985, Emended August 1990) for this study states that a minimum of 2.0 mm of new shell (based on the longest finger of new growth) must be deposited by the end of 96 h. The growth of the control group during this study was within the historical range (0.9 to 4.5 mm) compiled at Smithers (Table S8). Based on these data, the amount of shell deposition observed during this study is considered representative for this species and acceptable for establishing the relative toxicity of atrazine to Eastern oysters.

At test termination, no mortality or adverse effects were observed among oysters at any of the treatment levels tested. Fecal/pseudofecal material showing evidence of a white material that was believed to be test substance was observed in the highest treatment. The percent reduction in shell growth was 0.55%, 15%, and 16% for oysters exposed to the 1.0, 9.2, and 17 mg a.i./L treatment levels, respectively (Fig. 1). The remaining treatment levels (2.0 and 4.2 mg a.i./L) exhibited a positive response compared to the control. Since no concentration tested resulted in ≥ 50% reduction in shell deposition, the 96-h EC50 was empirically estimated to be > 17 mg a.i./L, the highest mean measured concentration tested. Williams' Test was used to compare the mean shell growth at the treatment levels to the control shell growth. No statistically significant difference was determined at any treatment level. Therefore, the No-Observed-Effect Concentration (NOEC) was determined to be 17 mg a.i./L. Further testing to determine an EC50 value was not performed since the concentration of the stock solution used to prepare the highest nominal test concentration approximated the functional water solubility of atrazine in natural seawater.

Table 1 Concentrations of atrazine measured in exposure solutions during the 96-h exposure of Eastern oysters (*Crassostrea virginica)*

Nominal concentration (%)[a]	Measured concentration (mg a.i./L)		
	0 h	96 h	Mean[b]
Control	<0.16	<0.15	NA[c]
6.3	1.2	0.86	1.0
13	2.3	1.8	2.0
25	4.3	4.1	4.2
50	8.4	10	9.2
100	16	17	17

[a] Concentrations expressed as percent of a 33 mg a.i./L water accommodated fraction (WAF)

[b] Mean measured concentrations were calculated using the actual analytical (unrounded) results and not the rounded (two significant figures) presented in this table

[c] Not Applicable

3.2 Mysid shrimp acute test

3.2.1 Water Quality

Measured quality parameters (pH, DO, temperature, and salinity) evaluated during the acute mysid study are presented in Table 2 and Table S6. Daily and continuous (one control replicate) monitoring established a test solution temperature range of 25 to 26 °C.

3.2.2 Analytical Results

Mean measured concentrations of atrazine averaged 103% of the nominal fortified levels and defined the test

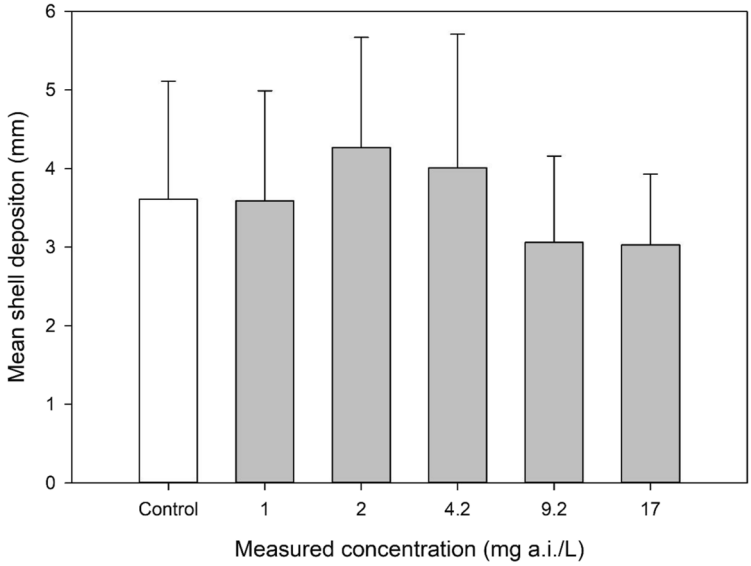

Fig. 1 Mean (±SD) shell deposition of Eastern oysters (*Crassostrea virginica)* after exposure to atrazine for 96 h (n=40 oysters per treatment). There were no significant differences among treatment groups relative to the control (p>0.05, Williams' Test)

concentrations as 1.2, 1.7, 2.4, 3.6, and 6.4 mg a.i./L (Table S9). Analyses of the QC samples resulted in measured concentrations which were generally consistent with the predetermined recovery range and averaged 91% of the nominal fortified levels (1.00 to 10.0 mg a.i./L).

3.2.3 Effects of Atrazine Exposure

Following test termination (96 h), 70% mortality was observed among organisms exposed to the highest mean measured concentration tested (6.4 mg a.i./L). Mortality of 5%, 5%, and 10% was observed among mysids exposed to 1.7, 2.4, and 3.6 mg a.i./L, respectively. Sub-lethal effects (e.g., darkened pigmentation, lethargy, erratic swimming behavior) were observed among surviving mysids exposed to 2.4, 3.6, and 6.4 mg a.i./L (Table S10). No mortality or sub-lethal effects were observed among mysids exposed to the lowest treatment level or the controls (Table 3).

The 24-, 48-, 72- and 96-h LC50 values and the NOEC established during this study are summarized in Table 3. The 96-h LC50 value for atrazine technical and mysids was calculated by probit analysis to be 5.4 mg a.i./L (95% confidence interval of 4.5 to 7.4 mg a.i./L). Since only 5% mortality and no adverse sub-lethal effects were observed among mysids exposed to 1.7 mg a.i./L atrazine technical, this concentration was established as the NOEC for this study. Performance of organisms in the 1.7 mg a.i./L treatment group was consistent with acceptable standards control organism performance (US EPA, 1996a, b). Based on the results of this study and on criteria established by US EPA (2017), atrazine technical would be classified as moderately toxic to *A bahia***.**

3.3 Mysid Shrimp Life-Cycle Test

3.3.1 Water Quality

During the 28-d exposure period, the concentration of DO was maintained between 85 to 106% of saturation and the pH of all solutions ranged from 8.2 to 8.3 (Table 2). Continuous and daily temperature monitoring established a temperature range of 23 to 28 °C during the exposure period. Salinity ranged from 19 to 21‰.

3.3.2 Analytical Results

Analyses of atrazine concentrations were performed on Days 0, 7, 14, 21, and 28 at all treatment levels. Based on measured concentrations, the treatment levels tested were defined as 0.068, 0.14, 0.26, 0.50, and 1.1 mg a.i./L (Table S11). Analysis of QC samples resulted in measured concentrations which were consistent with the predetermined recovery range (85.0% to 115%), and ranged from 88.9 to 103% (n = 15) of the nominal fortified levels (0.0400 to 2.00 mg a.i./L).

Table 2 Ranges of water quality parameters measured in the exposure solutions during the 96-h and 28-d life-cycle exposures of mysids (*Americamysis bahia*) to atrazine

	Nominal concentration (mg a.i./L)	Salinitya (‰)	Dissolved oxygen (mg/L)b	Temperature (°C)[ac]	pH
96-h acute test	Control	32	5.8-6.6	25-26	7.8-8.0
	Solvent control	32	5.1-6.4	25-26	7.8-8.0
	0.91	32	4.8-6.1	25-26	7.8-7.9
	1.5	32	5.0-6.2	25-26	7.8-7.9
	2.5	32	5.1-6.1	25-26	7.8-7.9
	4.2	32	4.8-6.1	25-26	7.8-7.9
	7.0	32	4.6-5.7	25-26	7.7-7.9
28-d life-cycle test	Control	19-21	6.3-7.7	25-27	8.2-8.3
	0.063	19-21	6.5-7.5	25-27	8.2-8.3
	0.13	19-21	6.4-7.5	25-27	8.2-8.3
	0.25	19-21	6.6-7.5	25-27	8.2-8.3
	0.50	19-21	6.4-7.5	25-27	8.2-8.3
	1.0	19-21	6.5-7.4	25-27	8.2-8.3

[a] Values presented represent the daily temperature (Brooklyn alcohol thermometer) and salinity measured for all treatment levels and controls at the stated time interval

[b] Minimum dissolved oxygen concentration was 85% of saturation. Maximum dissolved oxygen concentration was 106% of saturation

[c] Continuous temperature monitoring of the control replicate A (Fisher Scientific minimum/maximum thermometer) established a test solution temperature range of 25 to 26°C during the 96-h test. Continuous temperature monitoring (control replicate A) established a temperature range of 23 to 28°C throughout the 28-d test

3.3.3 Effects of Atrazine Exposure

After 28 days of exposure, 72% survival was observed among control organisms, and mean survival rates in atrazine treatments ranged from 45 to 77% (Table S12). There were no statistically significant differences in survival among organisms exposed to any treatment level tested in comparison to the control using Williams' Test. Survival of mysids in the 0.068 mg a.i./L treatment level was lower than the survival observed in the four other treatment levels (i.e., 45%). The reduction in mysid survival in this treatment level was not dose-related and both growth and reproduction were comparable to control organism performance (Table 4).

Brood appearance (i.e., gravid females) was observed in the controls and all treatment levels by Day 14. Reproductive success for organisms exposed to the 0.068, 0.14, 0.26, 0.50, and 1.1 mg a.i./L treatment levels averaged 0.572, 0.738, 0.759, 0.697, and 0.431 offspring per female per reproductive day, respectively (Table 4). There was no significant reduction in reproductive success among organisms exposed to any treatment level tested when compared to the control (0.469 offspring per female per reproductive day; Williams' Test, $p>0.05$).

Measurements of growth, as total body length and dry body weight, for all surviving adult mysids at test termination are presented in Table S13. The average total body length of male mysids exposed to up to 1.1 mg a.i./L atrazine was 5.9 to 6.8 mm (Fig. 2). There was a significant reduction in average total body length among organisms exposed to the 0.50 and 1.1 mg a.i./L treatment levels (5.9 and 6.4 mm, respectively) compared to the control (7.1 mm). Average total body length among male mysids was unaffected by exposure to atrazine concentrations ≤ 0.26 mg a.i./L. For females, the average total body length ranged from 6.3 to 7.1 mm for mysids exposed to atrazine (Table S13). There was a significant reduction in average total body length at the 1.1 mg a.i./L treatment level (6.3 mm) compared to the control (7.3 mm), but total body length of female mysids was unaffected by exposure to atrazine concentrations ≤ 0.50 mg a.i./L (Fig. 2).

Table 3 Cumulative mortalities, LC50 (and corresponding 95% confidence interval), No Observed Effect Concentration (NOEC), and observations made during the 96-h flow-through toxicity test exposing mysid shrimp (*Americamysis bahia*) to atrazine

Mean measured concentration (mg a.i./L)	Cumulative mortality (%)											
	24 h			48 h			72 h			96 h		
	A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
Control	0	0	0	0	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0	0	0	0	0
1.2	0	0	0	0	0	0	0	0	0	0	0	0
1.7	0	0	0	10	0	5	10	0	5	10	0	5
2.4	0	0	0[a]	0	10	5[a]	0	10	5[a]	0	10	5[a]
3.6	0	0	0[a]	10	10	10[a]	10	10	10[a]	10	10	10[a]
6.4	10	20	15[a]	50	40	45[a]	70	60	65[a]	80	60	70[a]
LC50 (mg a.i./L)[b]	>6.4[c]			7.5 (5.4–1.7)[q]			5.7 (4.6–8.2)[q]			5.4 (4.5–7.4)[q]		
96-h NOEC (mg a.i./L)[b]										1.7		

[a] Sub-lethal effects (i.e., darkened pigmentation, lethargy, abnormal swimming behavior) was observed in one or more of the surviving mysids (details provided in Table S9)

[b] Based on mean measured concentrations of atrazine (as active ingredient)

[c] LC50 value was empirically estimated to be greater than the highest mean measured concentration tested (i.e., 6.4 mg a.i./L); therefore, the corresponding 95% confidence interval could not be calculated

[d] LC50 value and 95% confidence interval (in parentheses) was calculated by probit analysis

Average dry body weight of male mysids exposed to atrazine ranged from 0.78 to 0.89 mg (Fig. 3, Table S13). There was a significant reduction in average dry body weight among organisms exposed to the 0.50 and 1.1 mg a.i./L treatment levels (0.80 and 0.78 mg, respectively) compared to the control (0.90 mg). Male dry weight was unaffected by exposure to atrazine concentrations ≤ 0.26 mg a.i./L. Likewise, there was a statistically significant reduction in female dry body weight as a result of exposure to atrazine at 0.50 and 1.1 mg a.i./L levels, with mean weights of 1.18 and 1.08 mg, respectively, compared to 1.29 mg in the control (Fig. 3). There was no significant effect at concentrations ≤ 0.26 mg a.i./L, with body weights ranging from 1.13 to 1.27 mg.

Male mysid body length and male and female dry body weight were determined to be the most sensitive performance criteria, so the Lowest-Observed-Effect Concentration (LOEC) was 0.50 mg a.i./L and the NOEC was 0.26 mg a.i./L. Therefore, the geometric mean MATC was calculated to be 0.36 mg a.i./L.

4 Discussion

As previously mentioned, atrazine toxicity data are relatively limited for marine invertebrates and the intention of this study was to help fill a data gaps in this regard and facilitate public access to the corresponding data. In the current study, Eastern oyster was found to not be sensitive to atrazine at the tested concentrations, with a NOEC of 17 mg a.i./L. In the acute mysid test, reduced survival and sub-lethal effects were observed in *A. bahia* at concentrations ≥ 2.4 mg a.i./L, and the 96-h LC50 was 5.61 mg/L. Under chronic exposure conditions, concentrations ≥0.50 mg a.i./L caused significant changes to mysid morphometrics but no effects on reproduction. As such, the resultant MATC value was 0.36 mg a.i./L. These results indicate that *A. bahia* is more sensitive to atrazine than Eastern oyster, and that the calculated MATC value for mysids would be protective of both species.

Other studies in oysters have been conducted at lower concentrations. In a previous study with juvenile Eastern oysters, Britt et al. (2020) observed a trend towards

Table 4 Summary of the first generation (F0) survival and reproductive success (offspring/female/reproductive day) at termination of the 28-d life-cycle exposure of mysids (*Americamysis bahia)* to atrazine. There were no significant differences among treatment groups relative to the control (p>0.05, Williams' Test)

Mean measured concentration (mg a.i./L)		Percent survival[ab]	Number of females producing young	Cumulative number of offspring	Percent of females producing young	Reproductive success (average number of offspring/female/reproductive day
Control	A	73	10	64	100	0.485
	B	70	9	56	90	0.452
	Mean	72		60	95	0.469
0.068	A	43	9	62	90	0.512
	B	47	8	65	100	0.644
	Mean	45		64	95	0.572
0.14	A	47	9	83	90	0.762
	B	73	10	89	100	0.718
	Mean	60		86	95	0.738
0.26	A	47	10	101	100	0.886
	B	73	7	63	88	0.618
	Mean	60		82	94	0.759
0.50	A	67	9	98	90	0.824
	B	70	9	72	90	0.576
	Mean	68		85	90	0.697
1.1	A	73	7	53	88	0.680
	B	80	9	28	90	0.255
	Mean	77		41	89	0.431

[a] Values presented have been rounded to two significant figures

[b] Daily survival data are presented in Table S10

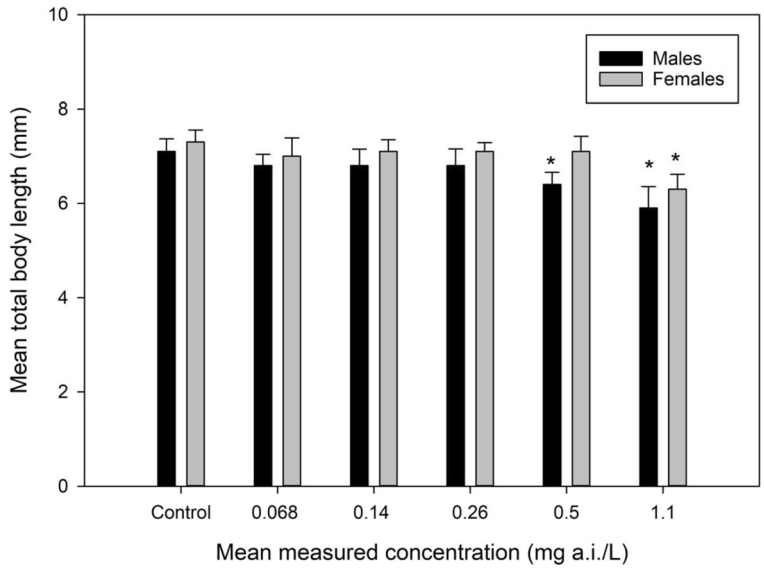

Fig. 2 Mean (±SD) total body length of first generation (F0) male (n=10–18) and female (n=12–19) mysids (*Americamysis bahia*) measured at the termination of the 28-d life-cycle exposure to atrazine. Significant differences relative to the respective control are indicated with an asterisk (p>0.05, Williams' Test)

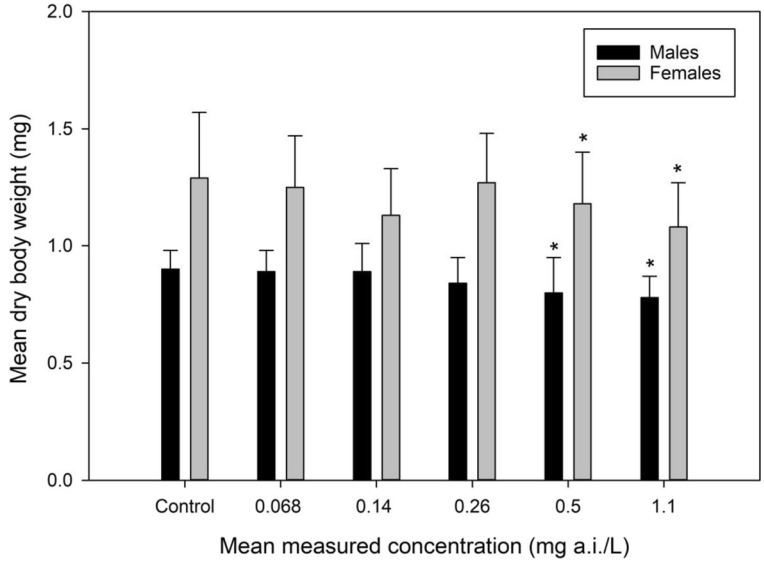

Fig. 3 Mean (±SD) dry body weight of first generation (F0) male (n=10–18) and female (n=12–19) mysids (*Americamysis bahia*) measured at the termination of the 28-d life-cycle exposure to atrazine. Significant differences relative to the respective control are indicated with an asterisk (p>0.05, Williams' Test)

reduced growth following pulsed exposure to atrazine at 0.003 to 0.030 mg/L; however, the result was not dose-dependent and statistical significance from the controls was not indicated. In another study, a related species, Pacific oyster (*Crassostrea gigas*), was exposed to atrazine at concentrations of 0.01 and 0.1 mg/L for 3 weeks. Exposure to atrazine did not result in significant effects on haemocyte viability or cellular processes (i.e., peroxidase activity, esterase activity, cellular division; Gagnaire et al., 2003). Based on the available data for oyster species, this taxonomic group appear to be relatively insensitive to atrazine at environmentally relevant concentrations (i.e., 0.1 mg/L or less; Van Der Kraak et al., 2014; Clark et al., 1999; DeLorenzo et al., 2006 and references therein).

Studies have also been conducted with another bivalve species, the hard clam (*Mercenaria mercenaria*). Juveniles were used by Lawton et al. (2006) in both acute and chronic aqueous bioassays with atrazine. The reported 96-h LC50 was 5.61 mg/L for acute aqueous exposure, and the estimated MATC was 0.63 mg/L (based on survival). Significant reductions in dry weight were observed at concentrations of 10 mg/L and 20 mg/L. For the chronic assay, the 10-d NOEC was 0.5 mg/L, the LOEC was 1.0 mg/L, and the MATC was 0.71 mg/L (based on survival and reduced length; Lawton et al., 2006). These results suggest slightly greater sensitivity in the clam species than the Eastern oyster, but a relatively consistent result across marine bivalves that effects on survival and growth would not be expected under conditions previously measured in fresh (USEPA, 2019a, b; ECCC, 2011; Government of Canada, 2016; Van Der Kraak et al., 2014) or marine (Clark et al., 1999; DeLorenzo et al., 2006 and references therein) waters. The hard clam endpoints were also consistent with those observed in mysids in the current study (i.e., LC50 value of 5.4 mg/L).

Previous studies in shrimp and shrimp-like species suggest that *A. bahia* had intermediate sensitivity to atrazine relative to other related species. For example, in a previous study, Noppe et al. (2007) reported that exposure to atrazine at up to 0.001 mg/L for 3 weeks did not affect moulting of mysid shrimp (*N. integer)*, but the 96-h LC50 was 0.048 mg/L (versus 5.4 mg/L for *A. bahia* in the current study). This greater sensitivity of *N. integer* than other invertebrate species was also noted by Noppe et al. (2007). In another study, metamorphosis of larval prawn (*Penaeus monodon*) was inhibited following 24 h exposure to atrazine at a concentration of 0.9 mg/L (24-h LOEC; Mercurio et al., 2018), indicating that this endpoint is more sensitive than the survival or sub-lethal effects in the current study for which the 24-h LC50 was >6.4 mg a.i./L and 96-h NOEC was 1.7 mg a.i./L. Another species, the grass shrimp (*Palaemonetes pugio*), larvae and adults were quite tolerant of exposure to atrazine, with reported 96-h LC50 values of >10 mg/L and 9 mg/L, respectively (Key et al., 2007). With concentrations of atrazine being unlikely to exceed 0.1 mg/L in freshwater or marine environments (USEPA, 2019a; ECCC, 2011;

Government of Canada, 2016; Van Der Kraak et al., 2014; Clark et al., 1999; DeLorenzo et al., 2006), *A. bahia* and *P. pugio* would not be expected to experience deleterious effects as a result of atrazine exposure. However, the more sensitive *N. integer* species could be affected under relevant conditions, and its use as a representative species for risk assessment would be protective of the bivalve and shrimp-like species tested to date.

Overall, the toxicity responses observed in the current study were consistent with results from previous investigations, and indicate that atrazine is slightly toxic towards Eastern oyster and moderately toxic to *A. bahia* under acute exposure conditions (based on toxicity categories from US EPA, 2017). The peak concentration measured historically in Chesapeake Bay was 0.3 mg/L, which approaches the most sensitive threshold determined by this study (i.e., MATC of 0.36 mg a.i./L for Mysid shrimp). However, the results from the current study and coastal monitoring data suggest atrazine is unlikely to pose risks for Eastern oyster or Mysid shrimp under typical conditions (i.e., measured mean/median concentrations < 0.01 mg/L; DeLorenzo et al., 2006). However, continued monitoring of estuarine/marine waters would be advised. Collectively, these data will help to fill a gap in the literature and inform risk assessment of potential effects of atrazine towards marine communities.

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J. Anderson—writing—original draft, writing—review and editing, visualization

M. Hanson—writing—review and editing.

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Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication All authors consent to the submission and publication in the journal.

Conflict of Interest R. Brain is an employee of Syngenta Crop Protection, LLC. J. Anderson and M. Hanson are independent contractors for Syngenta Crop Protection, LLC.

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