EFFECTS OF ATRAZINE ON EMBRYOS, LARVAE, AND ADULTS OF ANURAN AMPHIBIANS

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Abstract—We examined the effects of atrazine (0–20 mg/L) on embryos, larvae, and adult anuran amphibian species in the laboratory. Atrazine treatments did not affect hatchability of embryos or 96-h posthatch mortality of larvae of Rana pipiens, Rana sylvatica, or Bufo americanus. Furthermore, atrazine had no effect on swimming speed (measured for R. pipiens only). However, there was a dose-dependent increase in deformed larvae of all three species with increasing atrazine concentration. In adult R. pipiens, atrazine increased buccal and thoracic ventilation, indicating respiratory distress. However, because atrazine had no effect on hemoglobin, this respiratory distress was probably not indicative of reduced oxygen-carrying capacity of the blood. Frogs exposed to the highest atrazine concentration stopped eating immediately after treatment began and did not eat during the 14-d experiment. However, no decreases in mass were measured even for frogs that were not eating, probably because of compensatory fluid gain from edema. Atrazine concentrations found to be deleterious to amphibian embryos and adults are considerably higher than concentrations currently found in surface waters in North America. Therefore, direct toxicity of atrazine is probably not a significant factor in recent amphibian declines.

Keywords—Atrazine Amphibian decline Anuran Aquatic toxicity

INTRODUCTION

Populations of many amphibian species are dwindling across the globe. In the western United States alone, more than a dozen species of anurans have disappeared from much of their historic ranges [1]. In Canada, 17 of the 45 endemic amphibian species have suffered declines in population numbers [2]. The Wisconsin Frog and Toad survey has documented significant declines in spring peepers (Pseudacris crucifer), northern leopard frogs (Rana pipiens), Cope’s gray treefrogs (Hyla chrysoscelis), and further declines in populations of the already endangered Blanchard’s cricket frog (Acris crepitans blanchardi) [3]. Extensive and intensive agricultural practices in the midwestern United States implicate agricultural chemicals as a possible factor contributing to local amphibian declines [4]. In fact, there is often a lower species richness and abundance of amphibians at agricultural sites where pesticides are applied than at adjacent nonagricultural habitats [5,6]. However, because the presence of contaminants at agricultural sites is often confounded with less suitable habitat [5], it is difficult to prove causation from this association. Laboratory experiments on the effects of pesticides on amphibians can demonstrate causation by eliminating these confounding variables and thus act as an important complement to field studies.

Atrazine is one of the most heavily used pesticides in North America [7,8]. In 1993, an estimated 32,000 tons of active ingredient were applied in the United States alone, 69% of which was used in the 12 states comprising the Midwestern corn-growing region [7]. Atrazine concentrations in ponds, streams, and reservoirs commonly peak during May, June, and July following spring application [7,8]. It is during these three months that 16 of the 19 species of Wisconsin’s amphibians (WI, USA) are laying eggs and undergoing the brief period of embryo development [9]. Adult anurans may come into contact with contaminated terrestrial environments when migrating across fields where atrazine was applied in order to reach breeding ponds. Vernal pools, farm ponds, and other breeding sites adjacent to agricultural fields may be sinks for pesticides from runoff [10]. Calling males and breeding pairs in amplexus could be exposed to high concentrations of atrazine in these contaminated surface waters.

Though several studies have been conducted on the effects of atrazine on amphibian larvae [11–15], to our knowledge, no one has examined the effects of atrazine on adult frogs, and there has been only one study on amphibian embryos [16]. Harfenist et al. [17] stressed the importance of testing all three life stages (embryo, larvae, and adult) because of the wide range in sensitivity of different life stages to the same toxicant. Harfenist et al. also pointed out the need to test a number of species. Because so little is known about the relative sensitivity of different amphibian taxa, it is difficult to choose an indicator species. Therefore, we examined the effects of atrazine on hatchability, mortality, and deformity in embryos and larvae of three species of anurans native to Wisconsin, i.e., the leopard frog (Rana pipiens), the wood frog (Rana sylvatica), and American toad (Bufo americanus). We also examined swimming speed in 96-h posthatch R. pipiens larvae.

Atrazine can detrimentally affect the oxygen-carrying components of the blood. The number of red blood cells decreased in numerous taxa exposed to atrazine, including mice [18], dogs [19], and fish [20,21]. Furthermore, atrazine (1.1 mg/L) decreased oxygen consumption in fish (Tilapia mossambica) in a time-dependent manner (from a 12% decrease after 7 d to a 46% decrease after 90 d) [21]. Respiratory distress has been documented in adult ranids exposed to agricultural chemicals. In experiments with adult Rana temporaria exposed to...
ammonium nitrate fertilizer granules, Oldham et al. [22] found that the most useful indicator of a toxic effect was the ratio of the thoracic to buccal ventilation rates. They found that, when this ratio approached unity (i.e., frogs were taking as many thoracic breaths as buccal ventilations), continued exposure usually resulted in death [22]. We investigated the effects of cutaneous atrazine exposure on feeding behavior, mass, hemoglobin, and ventilation rate in adult *R. pipiens*.

**MATERIALS AND METHODS**

**Treatment solutions**

Treatment solutions were prepared by dissolving 20 mg of 99% pure atrazine (ChemService, West Chester, PA, USA) in charcoal-filtered (Barnstead/Thermolyne, Dubuque, IA, USA), dechlorinated water. A sample of this dilution water was analyzed by the Wisconsin State Lab of Hygiene and was found to have a pH of 7.67, conductivity of 538 µhos, alkalinity of 280 mg/L, hardness of 290 mg/L as CaCO₃, sodium at 3.3 mg/L, and chloride at 4.7 mg/L.

To select concentrations of treatment solutions for definitive toxicity tests, we first conducted range-finding tests [23,24] for both the embryo and adult experiments. Concentrations employed in the range-finding bioassays included environmentally relevant levels (0, 0.02, 0.2, 2, and 20 mg/L). We found reduced feeding and altered respiration rates in adult frogs exposed to 2 and 20 mg/L atrazine in the range-finding study. Likewise, we found an increase in malformations and erratic swimming in larvae in the 20 mg/L treatment, though not in the 2 mg/L treatment. We then conducted definitive toxicity tests in which the concentrations were a 60% dilution series bracketing the range in which we saw effects from the range-finding studies [23].

New stock solution was made up every 48 h, and a sample of each treatment solution was analyzed for atrazine concentration by enzyme-linked immunosorbent assay (Strategic Diagnostics, Newark, DE, USA). Measured atrazine concentrations did not differ from nominal concentrations. A regression of nominal versus measured atrazine concentrations yielded an R² of 0.9453 and showed the slope (1.012) not to be significantly different from one (Tₐ₈ = 0.333, p = 0.741) and the y-intercept (−0.141) not to be significantly different from zero (Tₐ₇ = −0.564, p = 0.576). The 95% confidence interval for atrazine concentrations measured by enzyme-linked immunosorbent assay enclosed each nominal concentration. We used nominal concentrations in all analyses.

**Exposure of adult *Rana pipiens***

Twenty-eight adult male northern leopard frogs (*R. pipiens*) measuring 7.5 to 9.0 cm in length were purchased from Kon’s Scientific (Germantown, WI, USA). The supplier ensured that the frogs were not collected from agricultural sites. Frogs were maintained and tested under a 14:10 h light:dark cycle (dark from 8 PM to 6 AM) and a room temperature of 22.0 ± 0.5°C. Relative humidity was 40 ± 2%, and the temperature of the treatment solutions in the aquaria ranged from 20.8 to 21.3°C. The frogs were placed individually in 2.5-gallon aquaria with screen covers. Treatments were randomly assigned to each tank at the beginning of the experiment. Four frogs were exposed to each treatment concentration of atrazine (0, 1.56, 2.59, 4.32, 7.2, 12, and 20 mg/L) for 14 d. Frogs were given a 10-d acclimation period prior to treatment. During this period, frogs were exposed to experimental conditions identical to treatment conditions except that water was used instead of treatment solution.

For 16 h each day (7 PM to 11 AM), aquaria were placed on a slant to provide the option of both an aequous and a dry environment, with 350 ml of charcoal-filtered, dechlorinated water covering 50% of the surface area of the aquarium bottom. The raised edge of the aquarium was 5.5 cm above the lower edge, providing a slope of 9° as a bank for the frogs and a maximum water depth of 3.3 cm. The aquaria were taken off the slant for 8 h each day (from 11 AM to 7 PM) so that the frogs experienced forced cutaneous exposure to the treatment solution. When aquaria were resting flat, the solution was deep enough (0.8 cm) to cover the undersurface of the frog without interfering with ventilation through the nares.

One hour after cutaneous exposure began each day (12 PM), the buccal and thoracic ventilation rate of each frog was measured by manually counting the number of ventilations for 1 min. The same individual took all measurements of ventilation rate and was blind to atrazine treatment.

Aquaria were cleaned and treatment solution was removed daily. While aquaria were empty and clean, a cricket (Nature’s Way, Ross, OH, USA) was presented to each frog. Each frog was allowed 20 min to feed, at which time any uneaten cricket was removed and feeding behavior was recorded (yes or no). Aquaria were then replenished with treatment solutions. Initial mass of each frog was recorded 2 d after the acclimation period began, and final mass was recorded at the end of the experiment.

On the third day of the acclimation period and at the end of the experiment, a blood sample was drawn via cardiac puncture from each frog under moderate anesthesia by immersion in 0.5-mg/L MS-222. Blood was immediately analyzed for hemoglobin concentration via the spectrophotometric cyanmethemoglobin method [25] using a Beckman DU-64 spectrophotometer and semimicro polystyrene cuvettes (Fisher 14-385-942).

**Exposure of embryos**

Egg masses of anurans were identified in the field by a dichotomous field guide [9]. Seven clutches of *R. pipiens* embryos were collected from a pond in Green Bay Shores State Wildlife Area, Sensiba Unit, near Suamico (WI, USA; Township 25N Range 20E, Section 25) on April 8, 1999, and were immediately transported to Russell Laboratory in Madison (WI, USA) and put under treatment at Gosner [26] stages 10 (early gastrula) to 12 (late gastrula). Three clutches of *R. sylvatica* embryos were collected from a pond near Watersmeet (MI, USA; Township 45N Range 38W, Section 26) on May 2, 1999, and were placed under treatment conditions at Gosner [26] stages 10 (early gastrula) to 12 (late gastrula). Four clutches of *B. americanus* embryos were collected from Graber Pond (Middleton, WI, USA; Township 7N Range 8E, Section 2) on May 18, 1999, and were placed under treatment conditions at Gosner [26] stages 8 (middlearge) to 12 (late gastrula). At the time egg masses were collected, a water sample was also collected from each location for determination of atrazine levels by enzyme-linked immunosorbent assay. Atrazine levels were negligible at each site compared with our test concentrations (Suamico = not detectable to 0.01 µg/L, Middleton = 0.06 µg/L, Watersmeet = 0.02 µg/L).

All tests were conducted according to American Society for Testing and Materials standard guidelines for acute toxicity tests for amphibians [23]. Fifty (*R. pipiens* and *B. americanus*)
or 30 embryos (R. sylvatica) from each clutch were exposed to six concentrations of atrazine (0, 2.59, 4.32, 7.2, 12, and 20 mg/L) in 100-mm × 20-mm glass petri dishes containing 75 ml of treatment solution. Exposure was static renewal; new treatment solutions were set up daily in separate petri dishes and allowed to equilibrate to temperature. Embryos were then transferred via plastic pipettes to new solutions. Embryos were maintained and tested under a 14:10 h light:dark cycle and a room temperature of 22.0 ± 0.5°C.

Hatchability (proportion of embryos successfully developing to free-swimming larvae) was assessed on day 6. Exposure was continued for 96 h posthatch to assess mortality, deformity [27], and swimming speed. Swimming speed was measured for 96-h posthatch larvae from the same mass of R. pipiens eggs according to methods previously described by Jung and Jagoe [28]. Deviations from the procedure of Jung and Jagoe were that we used a Sony CCD-TRV 36 video camera (Sony, Toyko, Japan) to film the swim trials and we used a Panasonic Hi-Fi video cassette recorder AG-1970P and a Panasonic editing controller AG-A96 (Panasonic, Osaka, Japan) with shuttle/jog dial for slow motion (frame-by-frame) analysis of maximum burst swimming speed.

Statistical analyses

SYSTAT® 7.0 [29] was used for all analyses. Normality and equal variance assumptions were verified by examination of residual plots for random distribution. For adults, buccal and thoracic ventilation rates were compared by analysis of variance (ANOVA). An average ventilation rate was calculated for each frog from the 10 d of data following treatment. Significant differences were further examined by Dunnett’s multiple comparison procedure to determine the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL). Hemoglobin and mass were measured twice for each frog during the course of the experiment, once 7 or 8 d, respectively, before treatment and once at the end of the experiment (14 d after treatment), so these parameters were compared before and after treatment by repeated measures ANOVA. Feeding behavior was recorded for 5 d prior to treatment and for 13 d during the 14-d treatment. All frogs ate during each of the 5 d prior to treatment. The number of days (out of the 13) that the frogs ate during exposure was compared among treatments by Kruskal–Wallis because of nonnormality. The NOAEL and LOAEL for inhibition of feeding were determined by Mann–Whitney. Because Mann–Whitney does not adjust the α = 0.05 for multiple comparisons, we divided the α = 0.05 by the number of comparisons made against the control, beginning with the highest concentration and descending until the NOAEL was reached.

For embryos, proportion data for hatchability, mortality, and deformity were adjusted with an arcsine square-root transformation to satisfy the homogenous variance and normality requirements [24]. Variation in these parameters was tested using all possible models conditional on four explanatory variables, i.e., atrazine concentration, species, atrazine-by-species interaction, and clutch. Clutch and species were considered categorical variables. Mallow’s Cp [30] was used as the basis for selecting the most appropriate model to explain the variation in the data. The effect of atrazine on swimming speed was analyzed by analysis of covariance with larval length (mm) as a covariate. In cases where atrazine was found to be a significant factor in explaining the variation in these variables, the NOAEL and the LOAEL were calculated by Dunnett’s multiple comparison procedure [24].

Fig. 1. (A) Buccal and (B) thoracic ventilation rates for adult Rana pipiens during cutaneous atrazine exposure. Four frogs were exposed in each atrazine concentration (n = 28 frogs) for 8 h/d for 14 d. An average was calculated for each frog for the 10 d of respiratory data following treatment, and these means were compared with analysis of variance (ANOVA). An * denotes ventilation rates significantly (p < 0.05) different from control by Dunnett’s multiple comparison. Values with the same lowercase letter are not significantly (p < 0.05) different by Fisher’s least significant difference.

RESULTS

Adults

Buccal ventilation rate differed significantly (F_{6,21} = 4.307, p = 0.006) among atrazine treatments and controls (Fig. 1A). There was a dose-dependent increase in buccal ventilation with
increasing atrazine concentration from 0 mg/L atrazine (control) to 7.2 mg/L atrazine. The first concentration with a significantly higher buccal ventilation rate than control (LOAEL) occurred at 4.32 mg/L ($p = 0.007$), and the NOAEL was 2.59 mg/L ($p = 0.239$). Mean buccal ventilation rate peaked in the 7.2 mg/L treatments and began falling as atrazine concentrations increased above 7.2 mg/L. Compared with frogs in the 7.2-mg/L treatment, frogs in the 20-mg/L treatment had a significantly slower mean buccal ventilation rate. However, the buccal cavity was being filled to a greater extent with each breath, with the throat having the appearance of an inflated vocal sac.

Thoracic ventilation rate also differed significantly ($F_{5,21} = 5.228, p = 0.002$) among atrazine treatments and controls (Fig. 1B). However, thoracic ventilation rates were not significantly different from controls in the first five atrazine concentrations but were significantly higher than controls in the highest two atrazine concentrations (12 and 20 mg/L). Thus, the NOAEL was 7.2 mg/L ($p = 0.441$) and the LOAEL was 12 mg/L ($p = 0.002$) by Dunnett's procedure.

Feeding behavior differed among atrazine treatments ($\chi^2_{df} = 19.726, p = 0.0031$). $p$-Values of 0.025 were considered significant because of multiple comparisons ($\alpha = 0.05$ divided by two comparisons). Though all frogs were eating for the 5 d prior to treatment, the frogs in the 20-mg/L atrazine treatment did not eat during the 14-d experiment. In contrast, frogs in the other treatment groups ate between 84.6 (11 of 13 d) and 100% (13 of 13 d) of the time and did not significantly differ from control frogs. Thus, the NOAEL was 12 mg/L (Mann–Whitney U test statistic = 14.000, $p = 0.0455, \chi^2_{df}$ approximation = 4.000) and the LOAEL was 20 mg/L (Mann–Whitney U test statistic = 16.000, $p = 0.0082, \chi^2_{df}$ approximation = 7.000) (Fig. 2).

Mass of frogs (34.0 ± 0.634 g, $n = 28$) did not differ before and after atrazine exposure ($F_{1,21} = 0.301, p = 0.589$), nor did it differ by treatment level of atrazine ($F_{2,21} = 1.200, p = 0.345$); furthermore, there was no effect of exposure that depended on the level of atrazine ($F_{2,21} = 0.766, p = 0.605$). However, we did note that frogs in the 12-mg/L and the 20-mg/L atrazine treatments exhibited noticeable generalized edema compared with control frogs.

Hemoglobin (7.34 ± 0.230 g/dL, $n = 28$) did not differ before and after atrazine exposure ($F_{1,21} = 0.769, p = 0.391$), nor did it differ by treatment level of atrazine ($F_{2,21} = 1.090, p = 0.400$); furthermore, there was no effect of exposure that depended on the level of atrazine ($F_{2,21} = 0.665, p = 0.679$).

Embryos

Hatchability (0.930 ± 0.011, $n = 84$) did not differ among atrazine treatments and controls ($F_{5,78} = 0.470, p = 0.797$), though there were significant differences in hatchability by clutch ($F_{6,78} = 9.979, p < 0.001$) and species ($F_{2,81} = 7.171, p = 0.001$). Proportion of hatch for each species was for R. pipiens, 0.973 ± 0.006 ($n = 42$); for B. americanus, 0.910 ± 0.016 ($n = 24$); and for R. sylvatica, 0.857 ± 0.040 ($n = 18$).

Mortality (0.030 ± 0.006, $n = 84$) did not differ among atrazine treatments and controls ($F_{5,78} = 0.831, p = 0.531$), though there were significant differences by clutch ($F_{13,80} = 9.230, p < 0.001$) and by species ($F_{2,81} = 20.455, p < 0.001$). Proportion of mortality for each species was, for R. pipiens, 0.012 ± 0.003 ($n = 42$); for B. americanus, 0.021 ± 0.004 ($n = 24$); and for R. sylvatica, 0.087 ± 0.022 ($n = 18$).

Swimming speed of larvae (3.80 ± 0.208 cm/s, $n = 30$) did not differ among atrazine treatments and controls ($F_{5,78} = 2.097, p = 0.101$). Because larval length was not significant ($F_{1,23} = 2.274, p = 0.145$) in explaining swimming speed, length was excluded from the final model.

Deformity differed significantly among atrazine treatments and controls ($F_{5,78} = 16.363, p < 0.001$) (Fig. 3) and by clutch ($F_{1,3,20} = 1.867, p = 0.049$). The NOAEL for deformity was 2.59 mg/L atrazine ($p = 0.263$), and the LOAEL was 4.32 mg/L atrazine ($p = 0.012$). Of the 3,086 larvae that successfully hatched and did not die, 575 (18.6%) were deformed. Deformities were categorized according to standard Frog Embryo Teratogenesis Assay-Xenopus (FETAX) procedures [27]. The results were wavy tail, 53.6%; lateral tail flexure, 27.3%; facial edema, 12.0%; axial shortening, 3.5%; dorsal tail flexure, 3.3%; and blistering, 0.3%. There were no differences in deformity among species ($F_{2,81} = 0.890, p = 0.415$).

**DISCUSSION**

**Adults**

Ventilation rate can be a useful endpoint in toxicity studies with anuran amphibians. Because the volume of the buccal pump is relatively fixed in amphibians, the capacity for altering ventilation through changes in the depth of breathing is slight. Instead, a change in ventilation is primarily controlled by altering the number of breaths per unit time [31]. Under normal environmental conditions, the almost continuous buccal oscillations are infrequently interrupted by a thoracic breath (approximately 1/min in adult R. pipiens) [32]. Under hypoxic conditions, however, adult anurans increase the frequency of lung ventilation with decreasing $O_2$. Adult *Bufo paracanemis* doubled pulmonary ventilation when the partial pressure of $O_2$
cause ventilation rates of the control frog were high due to stress of handling and declined in the first hour; it is possible that the data depicted in the graphs for the first 30 min are confounded with increased ventilation from handling stress.

In the current study, frogs responded to increasing atrazine concentrations by increasing buccal ventilation rate up to a point. Beyond this point, thoracic ventilation increased and buccal ventilation began to decline again. At the highest concentration of atrazine, buccal rate was significantly lower than its peak at 7.2 mg/L, though it still remained elevated over controls. As in Oldham et al.’s study [22], we found a significant linear relationship ($F_{1,36} = 16.264$, $p = 0.0004$, $R^2 = 0.385$) when $R$ value was regressed against atrazine concentration. However, even though $R$ values increased with increasing concentration of atrazine, they did not approach unity and rarely exceeded 0.30, even in the highest atrazine treatments. While Oldham et al. presented a decrease in buccal ventilation with increasing exposure to fertilizer [22], atrazine increased both buccal and thoracic ventilation rates in our study.

The number of circulating erythrocytes determines the hemoglobin concentration and therefore the blood $O_2$-carrying capacity [31]. Atrazine can impact detrimentally the oxygen-carrying capacity of the blood by reducing the number of pluripotent stem cells and reticulocytes destined to become mature erythrocytes [18] and by directly decreasing the number of erythrocytes and the hemoglobin levels [19–21] in mice [18], dogs [19], and fish [20,21]. However, we have no evidence to support the hypothesis that atrazine can decrease the oxygen-carrying capacity of the blood in frogs since there were no significant differences in hemoglobin levels before and after the 14-d exposure to atrazine. Thus, it is unlikely that the increases in ventilation rates that we observed were due to a compromised ability of the blood to carry and transport sufficient oxygen.

Buccal and thoracic ventilation rates may have increased in response to a lower availability of oxygen in waters containing atrazine. Atrazine has been reported to decrease the oxygen tension in water, which can result in anoxia and mortality in fish [20,35]. In static acute toxicity tests with shrimp (Penaeus duorarum) exposed to atrazine in aerated seawater, dissolved oxygen concentrations declined 45 to 73% in atrazine treatments (1–15 mg/L) compared with 15 to 30% in controls after 96 h [36]. Another plausible explanation for the increase in the ventilation rate of R. papiens in our study is that the faster ventilation was merely a reflection of an increase in metabolism required for detoxification and excretion of atrazine. It is also possible that the increased ventilation rate had nothing to do with either a lower availability of or an increased need for oxygen but perhaps was merely indicative of atrazine exerting a toxic effect on the physiological systems that control ventilation.

A dramatic inhibition in feeding behavior occurred in frogs exposed to the highest concentration of atrazine (Fig. 2). The complete halt in feeding of the frogs exposed to 20 mg/L atrazine could be considered an endpoint of imminent mortality if the exposure continued to the point of starvation. The fact that we saw no decrease in the mass of frogs in the 20-mg/L treatment after 13 d without food indicates that the expected weight loss was compensated by edema. These findings support other studies in which atrazine decreased food consumption. Bluegill (Lepomis machiiocichrus) exposed to 500 μg/L in flow-through tests for 28 d were lethargic, ate poorly, and decreased from 10 to 4 kPa [33]. Rice et al. [34] proposed that amphibians exposed to Pb might respond as if in hypoxic conditions because Pb exposure can damage erythrocytes, decrease hemoglobin levels, and impair respiratory surfaces. They found that Rana catesbeiana larvae exposed to both Pb and low $O_2$ displayed higher buccal ventilation rates than larvae exposed to either treatment separately. Even under high $O_2$ conditions, Pb-exposed larvae made more trips to the surface to gulp air than unexposed larvae.

In the study of Oldham et al. [22], adult R. temporaria were cutaneously exposed to ammonium nitrate fertilizer on moistened chromatography paper or soil. They conducted statistical analyses on the ratio of lung to buccal inflations ($R$ value) and only presented graphs of the separate ventilation rates for three individual frogs. Ventilation of the control frog stabilized within an hour after being placed in the aquarium and remained steady, with a buccal ventilation of approximately 80 breaths/min and a thoracic ventilation of approximately 5 breaths/min. The graphs of ventilation rates of frogs exposed to ammonium nitrate show an initial thoracic rate higher than the control (40–80 breaths/min) and an initial buccal rate higher than control (130–140 breaths/min). Thoracic ventilation remained high and buccal ventilation steadily declined in both of the exposed frogs for 30 min when the experiment was terminated as the ratio of ventilation rates approached unity. The mean $R$ value, measured at 5-min intervals during the first hour of exposure, differed significantly ($F_{4,30} = 7.91$, $p < 0.05$) among treatments with high $R$ values associated with high concentrations of fertilizer. It is unclear whether Oldham et al. allowed ventilation rates to stabilize before initiating exposure and recording measurements.
swarm erratically [37]. Weanling male Wistar rats (n = 6) fed 900 mg/kg atrazine in an ad libitum diet for three weeks had lower (p < 0.01) terminal body weights (117 g) than controls (144 g) [38]. Daily food intake in the third week was significantly lower (p < 0.001) in the rats offered the 900 mg/kg diet (10.5 g/d) than in controls (13.9 g/d) [38]. In contrast, leeches (Glossiphonia complanata) exposed to atrazine (1–16 mg/L) for 28 d actually ingested up to four times as many limpets (Ancyclus fluviatilis) as control leeches [39]. However, this increase in ingestion may have been in response to a reduction in the carbon content of the algae that the limpets were eating and not a direct toxic response to atrazine [39].

Embryos

We found no differences in hatchability of embryos or mortality of 96-h posthatch larvae among atrazine treatments and controls. The highest concentration that we tested (20 mg/L) was lower than 96-h LC50 values reported for Xenopus larvae (100 mg/L) [16], early-stage B. americanus larvae (26.5 mg/L [12] to >48 mg/L [14]), and early-stage R. petersi (47.6 mg/L) [12]. However, Howe et al. reported late-stage larvae to be more sensitive to atrazine exposure than early-stage larvae with 96-h LC50s within the range we tested, i.e., 14.5 mg/L LC50 for R. petersi and 10.7 mg/L LC50 for B. americanus larvae [12]. They proposed that the greater sensitivity of late-stage larvae to atrazine may be due to the added physiological stress brought on by the onset of metamorphosis. In comparing our results with those of Howe et al., it is tempting to attribute the low mortality of larvae in our study to age differences. However, the disparity between our findings and those of Howe et al. may also be due to differences in formulation of atrazine. They used field-grade formulations of atrazine whereas we exposed animals to pure compound. Field-grade formulations contain other chemicals such as solvents and surfactants that may be toxic to amphibians. Birge et al. reported an LC50 of 0.41 mg/L for bullfrog (R. catesbeiana) larvae [14]. It appears that the species that we tested were more resistant to atrazine than R. catesbeiana larvae.

In FETAX experiments with Xenopus laevis, Morgan et al. [16] reported an EC50 for deformity of 33 mg/L and a LOAEL of 11 mg/L. We found no significant differences among species with respect to the effects of atrazine on deformity (Fig. 3). The LOAEL for deformity was 4.32 mg/L, compared with 11 mg/L that Morgan et al. found for Xenopus. The suite of deformities that we saw (blistering and edema, tail and axial malformations) were similar to the types of deformities reported by Morgan et al. (edema, gut, and tail malformations).

Though levels as high as 740 μg/L [8] have been detected in runoff waters from treated cornfields, atrazine concentrations in rivers and streams rarely exceed 20 μg/L in North America [7]. In other parts of the world, however, atrazine levels are within the range in which we found effects. For instance, atrazine concentrations in streams in agricultural regions of Czechoslovakia reached 13.5 mg/L in 1990 [40]. The concentrations of atrazine that negatively impacted the anuran amphibian embryos, larvae, and adults in our laboratory experiments are considerably higher than levels typically encountered in surface waters in North America. With respect to the parameters that we examined, it is not likely that atrazine is a significant factor in the recent declines of many species of amphibians in North America.

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