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# Acute and Chronic Toxicities of Arsenic(III) to Fathead Minnows, Flagfish, Daphnids, and an Amphipod

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**Abstract.** Acute and chronic toxicities of arsenic(III) (As) to four species of freshwater organisms were determined. All tests were flow-through exposures except the daphnid (*Daphnia magna*) tests which were static concentration renewal exposures. Acute exposures of fathead minnows (*Pimephales promelas*), flagfish (*Jordanella floridae*), and an amphipod (*Gammarus pseudolimnaeus*) to As resulted in 96-hr $LC_{50}$ or $EC_{50}$ estimates of 14,100, 14,400, and 874 μg/L, respectively. Daphnids were exposed to As with and without food resulting in 96-hr $EC_{50}$ estimates of 4,340 and 1,500 μg/L, respectively. Chronic exposures of 28 to 31 days duration were made for fathead minnows, flagfish, and daphnids. The chronic limit ranges (highest tested exposure concentration having no adverse effect and the lowest tested exposure concentration having an adverse effect) based upon the most sensitive measured parameters of body length and wet weight were 2,130 to 4,300 μg/L for fathead minnows and 2,130 to 4,120 μg/L for flagfish. Daphnids had chronic limits of 633 to 1,320 μg/L based upon survival and the measured parameters of reproduction and body length. Calculation of an acute test/chronic test ratio for fathead minnows, flagfish, and daphnids (fed and unfed) resulted in a range of values from 1.64 to 4.80.

Arsenic (As) is toxic to freshwater organisms at concentrations found in natural waters (Sanders and Cope 1966; Biesinger and Christensen 1972) and it also accumulates in some aquatic organisms (Spehar *et al.* 1980). Twenty percent of 722 samples of rivers and lakes in the United States contained concentrations greater than 100 μg/L As. Concentrations ranged from 0.1–1.6 μg/L in Lake Superior water to 243,000 μg/L in a lake near Searles, CA (National Academy of Sciences 1977).

Several U.S. Federal Agencies in conjunction with the National Pesticide Monitoring Program sampled freshwater fish from 106 sites within the U.S. monitoring various trace contaminants during 1976–77. Arsenic was one of the measured contaminants with whole body, wet weight concentrations ranging from 0.05 to 2.92 mg/kg (May and McKinney 1981).

Arsenic is used in metallurgy for the hardening of copper, lead and alloys and is used in the manufacture of certain types of glass (Windholz *et al.* 1976). Sodium arsenite is used as an insecticide and as a herbicide for aquatic vegetation (Kirk-Othmer Encyclopedia of Chemical Technology 1978).

Arsenic can be present in water in one of four valence states (−3, 0, +3, +5). It is rarely found in the metal form (0), and in the −3 state only at extremely low Eh values (Ferguson and Gavis 1972). Abdelghani *et al.* (1980), Spehar *et al.* (1980), and Ferguson and Gavis (1972) found arsenite (As(III)) to be more toxic to aquatic life than arsenate (As(V)).

The objectives of this study were to determine the acute toxicity of As(III) to fathead minnows (*Pimephales promelas*), flagfish (*Jordanella floridae*), a daphnid (*Daphnia magna*) and an amphipod

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(*Gammarus pseudolimnaeus*), and to study the sublethal effects of long-term As(III) exposure to fathead minnows, flagfish, and daphnids.

## Materials and Methods

### *Source and Culture of Test Organisms*

Flagfish and fathead minnow embryos and fry obtained from the culture unit at the Environmental Research Laboratory-Duluth (ERL-D), Duluth, MN were fed live brine shrimp (*Artemia* sp.) nauplii and reared in continuously flowing 25°C water.

Daphnids were also obtained from an ERL-D stock culture. They were reared in 25°C Lake Superior water and fed a mixture of ground trout pellets and yeast (ASTM 1980). The food mixture was prepared by homogenizing 60 g of fish food pellets with 1.5 g of bread-makers yeast in water. A stock solution was made at a concentration of 5,000 mg food/L of water and fed in quantity sufficient to give 30 mg/L of food in the exposure chamber.

Amphipods were collected from the Eau Claire river near Gordon, WI and raised in continuously flowing 17 C Lake Superior water for one month prior to testing. They were fed maple (*Acer* sp.), birch (*Betula* sp.), and oak (*Quercus* sp.) leaves presoaked in Lake Superior water for one month.

### *Water Characteristics*

EDTA hardness, total alkalinity, acidity, and pH were measured in the control, low, middle, and high concentrations of all tests except the daphnid chronic exposure following methods described by the American Public Health Association (1980). Measurements were performed at least once (twice during the daphnid test) during each acute test and twice weekly in fish chronic tests. EDTA hardness, total alkalinity, and acidity measurements were performed once per week in control chambers of the daphnid life-cycle test, and pH was measured each time fresh solutions of arsenic/food mixtures were prepared.

Dissolved oxygen was measured with an oxygen meter (Yellow Springs Instruments Co., Inc. Model 54) every 48 hr in the control, low, middle, and high concentrations in all tests except the daphnid tests. Dissolved oxygen was measured in the daphnid tests at the beginning and end of the acute test and three times weekly in the chronic test immediately after the daphnids had been transferred to fresh As solutions.

Fish tests were conducted in University of Wisconsin-Superior laboratory water (City of Superior, WI municipal water) which comes from shallow wells beneath Lake Superior. The laboratory water was partially dechlorinated by activated carbon filtration and then treated with sodium sulfite for removal of residual chlorine (Seegert and Brooks 1978). Amphipod and daphnid tests were conducted in raw Lake Superior water.

Measured physical water parameters varied little between tests. Mean values ranged from 46.3 to 49.9 mg/L as $CaCO_3$ for total hardness, 37.2 to 45.5 mg/L as $CaCO_3$ for total alkalinity, 2.6 to 3.4 mg/L as $CaCO_3$ for acidity, 7.2 to 8.1 for pH (arithmetic mean), 79.6 to 99.3% of saturation for dissolved oxygen, and 81 to 131 μmhos/cm for conductivity. Mean water temperature for the four fish tests ranged from 23.0 to 25.8°C and was 18.5°C for the amphipod test. The two acute daphnid tests had mean water temperatures of 15.6°C and the chronic exposure was conducted at 20.8°C.

Water temperatures were measured daily. Temperatures were relatively stable in all tests except for the fathead minnow and flagfish chronic exposures which experienced temperature control failures for a portion of exposure days 19, 20 and 21 and 25, 26 and 27, respectively. Temperatures dropped 2.5, 6.4, and 9.4°C for the fathead minnows and 5.3, 9.2, and 12.2°C for flagfish on the three affected days. The changes occurred gradually over approximately 4–17 hr and were corrected during the next 8 hr. Mortalities did not increase and feeding appeared to continue as before the temperature changes.

### *Exposure Test Procedures*

All tests were conducted in proportional diluters (Mount and Brungs 1967) with the exception of the daphnid tests which were static exposures. The diluters were calibrated to provide 0.5 dilution factors for five toxicant concentrations in duplicate. Duplicate control chambers received dilution water only. Stock solutions of reagent grade sodium arsenite ($NaAsO_2$) were prepared in deionized water and delivered to the diluter mixing cell with a metering pump. Exposure water was from the same source as culture water. Fluorescent lights provided a daily photoperiod with 16 hr of light.

*Acute Tests:* Ten amphipods were placed in each duplicated exposure concentration. They were selected by size uniformity with no attempt to determine age. Exposure chambers with interior dimensions of 6.3 × 6.3 × 9.3 cm (l × w × h) were placed on the bottom of glass aquariums of 7 × 15 × 12.25 cm. The smaller chambers were constructed of glass on two sides and the bottom while two sides consisted of 202 Nitex® nylon mesh to allow exchange of exposure water from the larger chambers. Exposure water daily exchange rate was 12.9 chamber volumes. Effect was defined as lack of visual muscle response after gentle prodding. The average water temperature was 18.5 ± 0.9°C. Light intensity range was 215–334 lux 0.1 above the water surface. Mean exposure chamber concentrations (±s.d.) were 303 (±25.6), 583 (±65.7), 1,340 (±176), 2,400(±241), 5,250 (±163) μg As/L and <2 μg As/L in the controls.

Two acute toxicity tests with As and daphnids were completed at the UW-Superior laboratory. One test was with food added, and one was conducted without food. The exposure chambers were 250 mL beakers containing 200 mL of the toxicant solution. Six exposure concentrations and controls were prepared in duplicate for the unfed and fed tests. The mean exposure concentrations (±s.d.) were 1,040 (±109), 1,540 (±100), 2,210 (±32.1), 4,220 (±532), 7,850 (±214), 13,300 (±524) μg As/L and <2 μg As/L in controls for the fed test (30 mg/L of food) and 815 (±27.4), 1,080 (±23.1), 1,860 (±41.1), 2,440 (±35.5), 4,190 (±164), 6,460 (±917) μg As/L and <2 μg As/L in controls for the unfed test. The chambers were placed in a temperature controlled water bath and five daphnids, <24 hr old, were placed in each chamber.

Fish and amphipods were observed for deaths or effects at 3, 6, and 12 hr and twice daily thereafter. Daphnids were observed for effects (no movements or minor movements) once daily. Organisms were exposed to As for 96 hr and not fed 24 hr prior to and during the test except for the fed daphnid test which was fed during the test.

*Chronic Tests:* Fish embryos <24 hr old were collected from spawning substrates and observed under a microscope. Embryos with healthy appearance were selected for testing. Fish embryo-larval tests were conducted in the same tanks as the acute tests. Each duplicate tank contained two embryo incubation cups sus-

pended from rocker arms which oscillated once every 10 sec a distance of one half the cup depth. The cups were 4.4 cm I.D. by 9.0 cm deep glass cylinders with Nytex® mesh cemented to an open bottom. Fifty fathead embryos were randomly selected and transferred into each of 24 embryo incubation cups with a wide-mouth glass pipette. Thirty-four flagfish embryos were randomly selected and transferred into each of another set of 24 incubation cups. Flagfish embryos were treated in a 0.4 mg/L solution of malachite green for 10 min during days 1 and 2 of incubation. Eggs were observed daily and dead eggs were counted and removed. Fathead minnows and flagfish embryos hatched by days 6 and 7, respectively. On the day of hatching, fry were rinsed into petri plates and examined for survival and abnormalities. Ten healthy appearing fry from each embryo cup were returned to the chamber for a total of 20 fry/duplicate chamber of each species.

Chambers were cleaned and siphoned at least three times/week. The number of surviving fry were counted weekly. Fish were fed an excess of live brine shrimp three times daily and Tetra Min® baby fish food once daily.

Mean As concentrations (±s.d.) were 1,060 (±283), 2,130 (±386), 4,300 (±502), 7,400 (±490), 16,500 (±1,030) μg/L and <2 μg/L in controls for the fathead minnow test and 1,240 (±354), 2,130 (±375), 4,120 (±287), 7,600 (±612), 16,300 (±849) μg/L and <2 μg/L in controls for the flagfish test.

After As exposure of 29 days for fathead minnows and 31 days for flagfish (not inclusive of incubation time for either species), all fish were removed, measured, and weighed. Measurements were of standard length and wet weight after removal of excess water by allowing fish to lay on paper towels for a few seconds.

Daphnids selected for testing were <24 hr old. The exposures consisted of six concentrations and two sets of controls. For each exposure concentration and control, 10 stoppered 300 mL Erlenmeyer flasks were used (80 flasks for the entire test). At each exposure concentration and control, seven of the flasks contained one daphnid each and three contained five daphnids each.

A 2.0 L stock solution containing 6 mL of food (same food as used in the acute exposures) was prepared for each exposure concentration and approximately 200 mL were added to each flask. Daphnids were transferred to flasks with fresh solutions three times weekly using a fire-polished wide-mouthed glass pipette. Arsenic concentrations were measured for a randomly selected flask in each exposure group each time the solutions were replaced and before the daphnids were transferred. Flasks were randomly arranged in a water bath. Light intensity ranged from 215–646 lux. Mean As exposure concentrations (±s.d.) were 72.8 (±5.67), 132 (±4.52), 270 (±14.4), 633 (±34.0), 1,320 (±32.3), 2,670 (±59.5) μg/L and <2 μg/L in controls.

Survival and offspring production were recorded when the daphnids were transferred to fresh test solutions. At the termination of the test, length (apex of helmet to base of spine) of all adults was measured with an optical micrometer.

The chronic test endpoint was the chronic limit value. It is defined as the highest exposure concentration having no measured effect and the lowest exposure concentration having a measured effect upon the physiological parameters of weight and length for the fish species and reproduction and survival for daphnids.

### *Analytical Procedure for As*

Water samples were collected with volumetric pipets from mid-depth in the water column near the exposure chamber center. Samples were collected and analyzed for the fish and amphipod acute toxicity tests daily; twice weekly for the fish chronic toxicity tests, at 0, 48, and 96 hr for the daphnid acute toxicity tests, and three times weekly during the daphnid chronic toxicity test.

Samples were prepared according to Method 206.2 (US EPA 1979). Five mL samples of exposure water and 50 mL of control tank water were collected and diluted to near 100 mL with deionized water. Before final volume adjustment, samples were acidified (1% $HNO_3$, v/v) and fortified with 0.6% hydrogen peroxide ($H_2O_2$, v/v) and 0.1% nickel nitrate hexahydrate ($Ni(NO_3)_2{\cdot}6H_2O$, w/v). Sample digestion was omitted.

Analyses were completed with a Perkin Elmer Model 306 atomic absorption spectrophotometer equipped with deuterium arc background correction and a HGA 2100 graphite furnace. The detection limit with this procedure was 2 μg/L. The mean percentage agreement between duplicate samples and standard deviation was 97.4 ± 3.5, n = 50. The mean percentage recovery and standard deviation for this technique was 97.7 ± 4.8, n = 48. Filtered samples (0.45 μ) contained 104.2% ± 4.3 (n = 5) of the As concentration of non-filtered samples. The measured arsenic concentration of the US EPA Trace Metals–Water Pollution Quality Control Sample #1171-III, was 97.7% ± 3.0 (n = 20) of the true value.

### *Statistical Analysis*

$LC_{50}$ and $EC_{50}$ estimates were determined by the Trimmed Spearman-Karber method (Hamilton *et al.* 1977). Duplicate exposures resulted in no significant ($p \leq 0.05$) survival differences; therefore, they were combined for the calculated $LC_{50}$ estimates.

Chronic exposure effect and no-effect concentrations were determined by one-way analysis of variance (ANOVA) tests at $p \leq 0.05$ significance. Specific concentrations causing significant ($p \leq 0.05$ and $p \leq 0.01$) deleterious effects were identified using the Dunnett's one-tailed procedure (Steele and Torrie 1960). Duplicate exposures were combined within each fish species test. Multiple daphnid flasks were combined for survival as were the single daphnid flasks combined for survival and reproduction/adult. Multiple and single daphnid flasks were combined for total length measurements as no significant ($p \leq 0.05$) differences existed between the groups. Comparison of values measured as percentages were first converted by arcsine transformation (Steele and Torrie 1960) before applying statistical procedures.

## Results

### *Acute Tests*

Acute exposure of fathead minnows to As resulted in some deaths at the two highest exposure concentrations (52,100 and 99,700 μg/L) within two hr of initial exposure. No fish survived 96 hr exposures ≥25,000 μg/L, while all fish survived at exposures ≤5,060 μg/L. The 96 hr $LC_{50}$ and $EC_{50}$ (loss of equilibrium) estimates and 95% confidence limits (in parenthesis) were identical (Table 1).

Flagfish acutely exposed to As began dying within 2 hr after initial exposure to the highest exposure concentration (99,700 μg/L). Only one fish survived 96 hr exposure to concentrations ≥25,900

**Table 1.** $LC_{50}$ Estimates (μg/L) for fathead minnow (*Pimephales promelas*), and flagfish (*Jordanella floridae*), and $EC_{50}$ estimates (μg/L) for an amphipod (*Gammarus pseudolimnaeus*) and a daphnid (*Daphnia magna*) exposed to arsenic(III) for various time intervals. Values in parentheses are the 95% confidence limits

| | 24 hr | 48 hr | 72 hr | 96 hr |
|---|---|---|---|---|
| Fathead minnow | 18,900 | 15,900 | 14,700 | 14,100 (12,500–15,900) |
| Flagfish | 18,300 | 16,200 | 15,900 | 14,400 (12,700–16,300) |
| Amphipod | [a] | 1,990[b] | 1,020[c] | 874 |
| Daphnid unfed | 2,160 | 1,500 (1,170–1,940) | 1,500 | 1,500 (1,170–1,940) |
| Daphnid fed | 7,250 | 4,630 (3,680–5,820) | 4,630 | 4,340 (3,520–5,360) |

[a] Insufficient deaths to calculate an $LC_{50}$
[b] 43 hr value
[c] 64 hr value

μg/L. No deaths occurred at exposure concentrations ≤5,060 μg/L. The 96 hr $EC_{50}$ (loss of equilibrium) was 200 μg/L less than the 96 hr $LC_{50}$ (Table 1).

Acute exposure of amphipods to As resulted in some immobilization at the two highest exposures (2,400 and 5,250 μg/L) within 19 hr of initial exposure. All the test organisms became affected at concentrations ≥1,340 μg/L after 96 hr of exposure and only one organism was immobilized at concentrations ≤583 μg/L. No control organisms were affected. Confidence limits were not reliable due to the lack of monotonically increasing mortality proportions (Table 1).

Unfed daphnids exposed to As responded quickly with most of the effects occurring within 24 hr of initial exposure. No organisms survived exposure concentrations ≥4,190 μg/L. The only change in the number of affected organisms between hr 48 and 96 of the test was in one control replicate where 3 of 5 organisms became distressed; however, this was not caused by exposure to As (Table 1).

Daphnids exposed to As and fed during the test were affected within 24 hr at concentrations ≥7,850 μg/L. After 48 hr of exposure to As, only a single organism (a control) was distressed at concentrations ≤2,220 μg/L; however, this was not caused by exposure to As. The test was continued to 96 hr with a small change in the $EC_{50}$ estimate (Table 1).

### *Chronic Tests*

Fathead minnow embryo survival at hatch was not significantly ($p \leq 0.05$) different from the control at any of the exposure concentrations. Percent hatch ranged from 86.1 to 93.1 (Table 2) for the various exposure concentrations and was 85.2 for the control fish. No significant differences ($p \leq 0.05$) were observed in the percent of fry that were abnormally developed at time of hatch.

Significant differences ($p \leq 0.01$) were found in survival, mean weight, and mean length of fry at test termination (Table 2). Only the highest exposure concentration of As (16,500 μg/L) reduced fry survival. Weight and lengths were significantly ($p \leq 0.01$) reduced at As exposure concentrations ≥4,300 μg/L. The chronic limits for fathead minnows exposed to As were 2,130 and 4,300 μg/L.

Exposure concentrations for flagfish embryos did not significantly ($p \leq 0.05$) affect percent hatch or the percent abnormally developed at hatch (Table 3). Survival of fry during the test was not adversely affected. Weight and standard length were significantly less ($p \leq 0.05$ or 0.01) at exposure concentrations of As ≥4,120 μg/L (Table 4). Hatching was delayed one day at the highest (16,300 μg/L) exposure concentration. The chronic limits for flagfish exposed to As were 2,130 and 4,120 μg/L.

All daphnids in the single organism flasks died at As exposure concentrations ≥1320 μg/L (Table 4). Survival was poor (6.6%) in the flasks with five organisms at 2680 μg/L As and significantly ($p \leq 0.01$) reduced at 1,320 μg/L. Deaths occurred principally during the first 14 days of exposure to As in single and multiple daphnid flasks.

Adult daphnid production and length of young at termination of exposure were significantly ($p \leq 0.01$) reduced at concentrations ≥1320 μg/L (Table 5). One exposure concentration (633 μg/L) resulted in a significant ($p \leq 0.05$) increase in total length when compared to controls and three exposure concentrations (72.8, 270, and 633 μg/L) resulted in significant ($p \leq 0.05$ or 0.01) increases above controls in the number of young produced per adult (Table 4). Based upon survival, production of young and

**Table 2.** Percent hatch, percent of hatch abnormally developed, percent survival from hatch to test termination, and mean weights and lengths for fathead minnows (*Pimephales promelas*) exposed to arsenic(III) for 29 days

| Concentration (μg/L) | % Hatch | % of Hatch abnormally developed | % Survival from hatch to test termination | Mean wet weight (g) | Mean standard length (mm) |
|---|---|---|---|---|---|
| Control | 85.2 | 2.6 | 95.0 | 0.058 | 17.0 |
| 1,060 | 92.6 | 6.4 | 70.0 | 0.056 | 17.0 |
| 2,130 | 90.9 | 12.0 | 90.0 | 0.050 | 16.3 |
| 4,300 | 93.1 | 6.0 | 77.5 | 0.041** | 15.6** |
| 7,400 | 91.1 | 11.9 | 97.5 | 0.026** | 13.3** |
| 16,500 | 86.1 | 9.2 | 22.5** | 0.012** | 11.2** |

** Statistically different from control; $p \leq 0.01$

**Table 3.** Percent hatch, percent of hatch abnormally developed, percent survival from hatch to test termination, and mean weights and lengths for flagfish (*Jordanella floridae*) exposed to arsenic(III) for 31 days

| Concentration (μg/L) | % Hatch | % of Hatch abnormally developed | % Survival from hatch to test termination | Mean wet weight (g) | Mean standard length (mm) |
|---|---|---|---|---|---|
| Control[a] | 57.0 | 3.5 | 77.5 | 0.057 | 13.23 |
| 1,240 | 66.7 | 3.3 | 85.0 | 0.051 | 12.65 |
| 2,130 | 65.0 | 2.2 | 80.0 | 0.052 | 12.72 |
| 4,120 | 87.4* | 1.7 | 95.0 | 0.044* | 12.11** |
| 7,600 | 78.5 | 0.9 | 82.5 | 0.034** | 10.85** |
| 16,300 | 76.7 | 1.0 | 75.0 | 0.012** | 7.87** |

[a] One of four replicates omitted due to fungus attack causing 100% embryo mortality
* Statistically different from control; $p \leq 0.05$
** Statistically different from control; $p \leq 0.01$

**Table 4.** Survival, production of young, and mean total length of adults for a daphnid (*Daphnia magna*) exposed to arsenic(III) for 28 days

| Concentration (μg/L) | Survival (%) | | Young per adult | Adult total length (mm) |
|---|---|---|---|---|
| | Single daphnid flasks | Multiple daphnid flasks | | |
| Control | 64.4 | 97.0 | 83.4 | 3.58 ± 0.22 |
| 72.8 | 57.0 | 87.0 | 126.2 | 3.67 ± 0.22 |
| 132 | 85.7 | 87.0 | 81.0 | 3.58 ± 0.18 |
| 270 | 57.0 | 93.0 | 115.2 | 3.72 ± 0.24 |
| 633 | 85.7 | 93.0 | 131.5 | 3.73 ± 0.28 |
| 1,320 | 0.0** | 53.0** | 0.0** | 3.19 ± 0.30** |
| 2,680 | 0.0** | 6.6** | 0.0** | 3.25[a] |

[a] Only one survivor
** Significantly different from controls; $p \leq 0.01$

length, the chronic limits for *Daphnia magna* exposed to As were 633 and 1,320 μg/L.

### *Acute/Chronic Ratios*

Acute/chronic (A/C) ratios were calculated by dividing the $LC_{50}$ or $EC_{50}$ values by the geometric mean of the chronic limits for each species. In the cases where both $LC_{50}$ and $EC_{50}$ values were available, the most sensitive of the two ($EC_{50}$) was used to compute the A/C ratio.

A/C ratios for As varied little for the fathead minnow, flagfish, and daphnid tests (Table 5). The ratios ranged from 1.64 to 4.80.

## Discussion

Amphipods were the most sensitive of the four species of organisms tested for acute toxicity. Spehar *et al.* (1980) found the same result in static tests involving five species (stonefly, *Pteronarcys dorsata;* snails, *Helisoma campanulata* and *Stagnicola emarginata;* rainbow trout, *Salmo gairdneri;* and an amphipod, *Gammarus pseudolimnaeus*). In their tests, amphipods showed 100% mortality after a 14-day exposure to 961 μg/L As(III). In this study, amphipods had a 96-hr $EC_{50}$ estimate of 874 μg/L of As. Surber and Meehean (1931) similarily found an amphipod, *Hyalella knickerbocker,* to be the most sensitive of 18 aquatic species tested with As(III).

**Table 5.** Acute $LC_{50}$ and $EC_{50}$ estimates, chronic limits, and acute/chronic ratios for three species of aquatic organisms exposed to arsenic(III)

| Species | $LC_{50}$ or $EC_{50}$ concentration (μg/L) | Chronic limits (μg/L) | $\frac{\text{Acute}}{\text{chronic}}$ ratio |
|---|---|---|---|
| Fathead minnow (*Pimephales promelas*) | 14,100[a] | 2,130–4,300 | 4.66 |
| Flagfish (*Jordanella floridae*) | 14,200[b] | 2,130–4,120 | 4.80 |
| Daphnid (*Daphnia magna*) unfed | 1,500[b] | — | 1.64[c] |
| Daphnid (*Daphnia magna*) fed | 4,340[b] | 633–1,320 | 4.75 |

[a] $LC_{50}$ and $EC_{50}$ estimate for 96 hr exposure
[b] $EC_{50}$ estimate for 96 hr exposure
[c] Calculated, using the chronic limits for the fed chronic exposure

Fathead minnow and flagfish juveniles had similar 96-hr $LC_{50}$ estimates of 14,100 and 14,400 μg/L As, respectively. Cardwell *et al.* (1976) conducted flow-through tests with measured As(III) concentrations (μg/L) and found the following 96 hr $LC_{50}$ estimates: fathead minnow juveniles, 15,660; flagfish fry, 28,130; goldfish juveniles (*Carassius auratus*), 26,042; brook trout adults (*Salvelinus fontinalis*), 14,964; channel catfish juveniles (*Ictalurus punctatus*), 18,096; and bluegill juveniles (*Lepomis macrochirus*), 41,760. Our fathead minnow tests agreed well with the results of Cardwell *et al.* (1976) but differed by a factor of approximately two for flagfish. Brook trout and channel catfish had 96-hr $LC_{50}$ estimates similar to our fathead minnow and flagfish results.

Acute static exposures of As to young daphnids resulted in 48-hr $EC_{50}$ estimates of 1,500 and 4,630 μg/L for the unfed and fed tests, respectively. It is possible that the increased tolerance of daphnids fed during exposure to As may be due to their improved condition or the binding of As with food particles making them biologically less available. Anderson (1946) found the *D. magna* 48-hr $EC_{50}$ estimate to be 5,278 μg/L in a static unmeasured As(III) test, and Biesinger and Christensen (1972) obtained a 48-hr $EC_{50}$ estimate of 7,400 μg/L As(V) in a static unmeasured sodium arsenate (As(V)) test.

In the chronic effects tests, daphnids were the most sensitive to As of the three species tested. Reproductive impairment occurred between 630 and 1,320 μg/L As for a 28-day static measured test. Biesinger and Christensen (1972) found 16% reproductive impairment at 520 μg/L As(V) and 50% impairment at 1400 μg/L As(V) in a 21-day static unmeasured test.

Comparisons of acute test $LC_{50}$ estimates with chronic exposure chronic limits were made for the fathead minnow, flagfish, and daphnid tests. Test conditions were similar for fish acute and chronic tests but differed in the daphnid tests. Water temperature during the daphnid tests averaged 15.6 and 20.8°C for acute and chronic tests, respectively. The effect of this temperature difference is unknown. Kersting (1978) showed the *D. magna* had the best growth efficiency at 10°C declining to zero efficiency slightly above 20°C. Adema (1978) demonstrated that $LC_{50}$ estimates of two test compounds in 21 day tests were only slightly changed by test temperatures of 15, 20, and 24°C for the same daphnid species.

Based upon our test data, it appears that the chronic toxicity of As(III) can be reliably estimated with acute toxicity values. A/C ratios for the three tested species varied from 1.64 to 4.80. If the unfed daphnid test is omitted much better agreement is achieved.

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