

ACUTE TOXICITY OF ANTIMONY III
TO SEVERAL SPECIES OF FRESHWATER ORGANISMS

by

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Antimony (III) chloride is very soluble in water. The Merck Index (9th ed.) reports a solubility of 1 g SbCl_3 per 10.1 mL of water. This solution undergoes gradual hydrolysis to antimony oxychloride (SbOCl), which is further hydrolyzed to antimony trioxide (Sb_2O_3), both of which are only slightly soluble in water. All of the above compounds are more soluble in solutions of high chloride ion content.

Static, acute exposures were made with Sb (III) to seven species of freshwater organisms. These tests were necessary to complete the minimum data requirements for the generation of numerical National Water Quality Criteria. Exposures were made using SbCl_3 and a hydroid (Hydra sp.), an annelid (Lumbriculus variegatus), an amphipod (Gammarus pseudolimnaeus), a caddisfly (Pycnopsyche sp.) rainbow trout (Salmo gairdneri), fathead minnow (Pimephales promelas), and a duckweed (Lemna minor).

Antimony trioxide has very limited solubility in water. Many aquatic toxicity test exposures have been made in the past with this compound. As a result of its limited water solubility, much of the information reported in the toxicity literature may be misleading due to the very low concentration of Sb (III) in solution from this compound. We conducted a static acute exposure with the fathead minnow and Sb_2O_3 to compare its toxicity to that of SbCl_3 .

METHODS AND MATERIALS

Exposure Conditions

The water supply for organism acclimation and testing was the municipal water supply for the City of Superior, WI. The water was dechlorinated by charcoal filtration and sodium sulfite addition. Water temperature was controlled using either a constant temperature water bath (amphipod, annelid, caddisfly and fish tests) or controlled temperature rooms (duckweed and hydroid tests).

All acute toxicity tests were conducted with duplicate controls and exposure treatments except for the duckweed and hydroid tests which had quadruplicate

chambers. Tests consisted of at least two toxicant treatments with a dilution factor of 0.5 ^{2.}

Test chambers were of varying sizes. Rainbow trout, amphipods, caddisflies and annelids were tested together in 30 x 30 x 30 cm tanks containing 24 L of solution. The amphipods were placed into stainless steel mesh wire cylinders (8-10 cm length x 3 cm diameter), closed at either end and positioned horizontally on the bottoms of the aquaria. Each cylinder contained two leaf disks (Betula sp. and Populus sp.) 20 mm in diameter. The hydroid test was conducted in 100 mL beakers using 80 mL of solution. The duckweed test was conducted in 250 mL beakers using 200 mL of solution. Fathead minnow tests were conducted in 2 L beakers containing 2 L of solution. The number of organisms per test chamber consisted of ten for fish and invertebrates. Twenty fronds per test chamber were used in the duckweed test.

Test solutions were not aerated for any of the tests. Dissolved oxygen was measured in control, low, middle, and high exposures at the initiation of each test and every 48 h thereafter (Table 1). Total hardness (EDTA), total alkalinity and pH were taken at least once during each test on control, low, middle, and high exposures (Table 1) in accordance with Standard Methods (APHA 1980). Dissolved oxygen (mean percent saturation) ranged from 70.9 to 93.8 percent for all the tests; mean total (EDTA) hardness ranged from 46.9 to 60.5 mg·L⁻¹ as CaCO₃; mean total alkalinity ranged from 36.9 to 46.5 mg·L⁻¹ as CaCO₃; and pH means from 7.10 to 7.70.

Biomass loading requirements were not exceeded as stated by ASTM (1980). Tests were initiated by placing organisms in the exposure chambers within 30 min after the toxicant was added.

The criteria for death were lack of movement, absence of respiratory movement and lack of reaction to gentle prodding. Because death was not easily determined for Hydra sp., only an EC₅₀ was determined. The Hydra sp. EC₅₀ was

TABLE 1. Test Water Characteristics for Static Acute Toxicity Tests with Antimony (III). Values are Means \pm Standard Deviation, Range, and Number of Samples

Species	Dissolved Oxygen (%)	Total (EDTA) Hardness (mg·L ⁻¹ as CaCO ₃)	Total Alkalinity (mg·L ⁻¹ as CaCO ₃)	pH	Temperature °C
Hydra (<u>Hydra</u> sp.)	90.7 \pm 2.8 (85.7-94.2) n=12	46.9 \pm 2.07 (43.8-48.2) n=4	41.5 \pm 1 (40.0-42.0) n=4	7.70 \pm 0.07 (7.64-7.79) n=4	23.6 \pm 0.2 (23.3-24.0) n=60
Annelid (<u>Lumbriculus variegatus</u>)	70.9 \pm 13.6 (53.7-89.9) n=9	52.2 \pm 0.64 (51.7-52.9) n=3	36.9 \pm 4.4 (33.0-41.6) n=3	7.12 \pm 0.05 (7.03-7.20) n=9	15.5 \pm 0.51 (15.0-16.4) n=30
Amphipod (<u>Gammarus pseudolimnaeus</u>)	70.9 \pm 13.6 (53.7-89.9) n=9	52.2 \pm 0.64 (51.7-52.9) n=3	36.9 \pm 4.4 (33.0-41.6) n=3	7.12 \pm 0.05 (7.03-7.20) n=9	15.5 \pm 0.51 (15.0-16.4) n=30
Caddisfly (<u>Pycnopsyche</u> sp.)	70.9 \pm 13.6 (53.7-89.9) n=9	52.2 \pm 0.64 (51.7-52.9) n=3	36.9 \pm 4.4 (33.0-41.6) n=3	7.12 \pm 0.05 (7.03-7.20) n=9	15.5 \pm 0.51 (15.0-16.4) n=30
Rainbow trout (<u>Salmo gairdneri</u>)	70.9 \pm 13.6 (53.7-89.9) n=9	52.2 \pm 0.64 (51.7-52.9) n=3	36.9 \pm 4.4 (33.0-41.6) n=3	7.12 \pm 0.05 (7.03-7.20) n=9	15.5 \pm 0.51 (15.0-16.4) n=30
Fathead minnow (<u>Pimephales promelas</u>)	76.3 \pm 5.3 (65.1-82.9) n=12	48.5 \pm 1.78 (45.8-61.0) n=6	38.2 \pm 2.1 (35.0-40.0) n=6	7.10 \pm 0.06 (7.04-7.21) n=6	21.4 \pm 0.6 (20.8-23.5) n=60
Duckweed (<u>Lemna minor</u>)	93.8 \pm 4.0 (88.5-98.8) n=8	60.5 \pm 1.69 (58.1-61.7) n=4	46.5 \pm 5 (40.0-52.0) n=4	7.20 \pm 0.02 (7.18-7.24) n=4	24.0 \pm 2.93 (22.0-27.3) n=60

based on whether body column and tentacles were clubbed (i.e., shorter than controls). Exposures were checked every 24 h for death and/or effect.

Test Organisms

Test organisms were acquired from a variety of sources. Amphipods and caddisflies were collected from the Eau Claire River, Douglas County, WI; rainbow trout from the U.S. EPA Environmental Research Laboratory, Duluth, MN; Hydra sp., fathead minnows, duckweed and annelids from the University of Wisconsin-Superior culturing unit. Recommended procedures for care, handling and acclimation of test organisms were followed (ASTM 1980).

The organisms were acclimated to test water and exposure temperature for a minimum of seven days prior to test initiation.

Preparation of Exposure Solutions

Initial procedure - A concentrated stock solution of SbCl_3 was prepared without the aid of a carrier solvent or an increased chloride ion concentration by dissolving 1 g of SbCl_3 in 10 mL of water. In dilutions of this stock solution, or in preparations of a less concentrated stock, a fine white precipitate was noted. The precipitate, the result of hydrolysis, formed immediately and continued with time. The hydrolysis occurs in dilute Sb (III) solutions at a much greater rate than in the highly concentrated stock solution because of the reduced chloride ion concentration.

In the initial tests, SbCl_3 was weighed and transferred to a measured volume of water, mixed for <24 h and the pH adjusted. The solutions and precipitate that formed were evenly split between replicate tanks and the bioassay started. The amount of precipitate present in the tank combined with the low chloride ion concentration resulted in decreasing Sb concentrations with time. The percentage of Sb lost from solution during a 96-h test ranged from a low of 6% for the

lowest exposure concentration (nominally $25 \text{ mg}\cdot\text{L}^{-1}$ Sb) to 76% for the highest concentration (nominally $50 \text{ mg}\cdot\text{L}^{-1}$ Sb). The apparent trend was to lose a greater percentage of Sb from solution with increasing nominal Sb concentration. Variations in the percentage of Sb lost in a 96-h test between replicate exposures were as high as 25%.

Chloride enrichment - A study with Sb (SbCl_3) demonstrated that a greater Sb concentration in solution could be maintained by use of a high chloride ion concentration. Sodium chloride was added to adjust the chloride ion concentration to $1000 \text{ mg}\cdot\text{L}^{-1}$. The initial measured Sb concentrations were 10.8, 26.6 and $51.6 \text{ mg}\cdot\text{L}^{-1}$ Sb. Ninety-six hours later the concentrations dropped to 10.3, 23.1 and $45.0 \text{ mg}\cdot\text{L}$ Sb, respectively.

Improved method - As a result of the problems encountered in preparing and maintaining exposure-concentrations in early tests, an improved method was developed for preparing stock solutions. Maximum concentrations of Sb were achieved by mixing the compound for between 0.5 h and 4 h, (adjust the pH during mixing), then filtering (Whatman 41) to remove the precipitate from the solution. Removal of the precipitate is extremely important in maintaining the Sb concentrations.

The maximum concentration maintained in a solution without organisms for 96 h was $35.0 \text{ mg}\cdot\text{L}^{-1}$ Sb. This solution, with a nominal Sb concentration of $250 \text{ mg}\cdot\text{L}^{-1}$, was determined to have an initial concentration of $36.4 \text{ mg}\cdot\text{L}^{-1}$ after having been filtered through a $0.45 \mu\text{m}$ membrane filter. In actual exposures using this method, concentrations decreased approximately 16% during a 96-h test.

Antimony trioxide exposure - An exposure of antimony trioxide (Sb_2O_3) with fathead minnows was conducted. The bioassay had replicate nominal concentrations of $50 \text{ mg}\cdot\text{L}^{-1}$ Sb and $100 \text{ mg}\cdot\text{L}^{-1}$ Sb; measured mean acid soluble concentrations were

3.4 and 5.0 mg·L⁻¹ Sb, respectively. No filtering of the stock solution was done, as previous experience had shown that filtering was ineffective. Solutions of Sb₂O₃ in lab water at nominal concentrations of 28, 58 and 110 mg·L⁻¹ Sb had measured dissolved (EPA 1979) Sb concentrations of 1.9, 2.6 and 3.3 mg·L⁻¹, respectively.

Statistical Treatment of Data

LC₅₀'s (EC₅₀'s) and their 95% confidence limits were calculated by the trimmed Spearman-Kärber method (Hamilton et al., 1977).

Analytical Technique

Water samples were collected and immediately acidified to 0.4% HCl (v/v, Baker Instra Analyzed) at 48 h intervals for the duration of the tests. A portion of the acidified sample was filtered using 25 mm 0.45 µm filters (Millipore, type HA) pre-cleaned in dilute hydrochloric acid (0.5 N). The filter-support assembly was conditioned with 30 mL of acidified sample and the successive 10 mL aliquot of sample was collected in a plastic bottle for acid soluble Sb measurements. A 10 mL aliquot of unfiltered sample was also saved for analysis. An agreement of 99+% between the acid soluble and unfiltered concentrations of the samples was noted. Therefore, the unfiltered sample concentrations have been excluded from this report. Unless otherwise stated, all concentrations or measurements reported are as acid soluble Sb.

An analytical stock solution (1000 mg·L⁻¹) of Sb (III) was prepared by dissolving antimony trichloride (SbCl₃, EM Chemicals, lot #5063, purity 99.2%), in a 50% (v/v) solution of HCl (Baker Instra-Analyzed). Analytical standards were prepared as required from the 1000 mg·L⁻¹ stock. Standards were 10% HCl (v/v) for flame analysis and 0.5% HCl for graphite furnace analysis.

Sample Analysis

An Instrumentation Laboratory Video 12 atomic absorption spectrophotometer was used to analyze samples of greater than $0.3 \text{ mg}\cdot\text{L}^{-1}$ Sb. The samples were adjusted to 10% HCl (v/v) prior to analysis. The light source was a Westinghouse antimony hollow cathode lamp. The spectral bandwidth was 0.3 nm, at a wavelength of 217.6 nm or 0.5 nm at a wavelength of 206.7 nm, a less sensitive wavelength. Conditions used included lean air-acetylene flame with a path length of 10 cm, auto integration, double beam mode, and deuterium arc background correction. The detection limit was $0.3 \text{ mg}\cdot\text{L}^{-1}$ Sb.

The presence of a minimal amount of matrix interference was indicated by standard additions. The mean agreement between the concentration from the calibration curve and standard additions was $89.3 \pm 0.05\%$ (n=3).

Approximately 10% of the samples were analyzed in duplicate and 10% were spiked with a known concentration of Sb (III). An overall mean agreement of duplicate analyses was $97.4 \pm 2.6\%$ (n=18), and the overall mean spike recovery was $103.4 \pm 9.0\%$ (n=20). An EPA reference sample, with a true value of $10.2 \text{ mg}\cdot\text{L}^{-1}$ Sb was analyzed. The mean value determined was $10.8 \pm 0.9 \text{ mg}\cdot\text{L}^{-1}$ Sb (n=33). Exposure concentrations were corrected for a mean spike recovery derived for each individual test.

Samples of less than $0.3 \text{ mg}\cdot\text{L}^{-1}$ were analyzed using graphite furnace atomic absorption spectrophotometry and standard additions. A Perkin-Elmer model 306 atomic absorption spectrophotometer was used employing a HGA 2100 graphite furnace, deuterium arc background correction, pyrolytically coated graphite, 2 sec analytical signal integration and manual injections of 20 μL . A Westinghouse antimony hollow cathode lamp supplied the 230.9 nm wavelength at a spectral bandwidth of 0.7 nm. The samples, which contained 0.4% HCl, were analyzed by standard additions due to matrix interferences.

RESULTS

Toxicity Tests

Adult Hydra sp. were exposed to five concentrations (0.3 ± 0.1 , 0.4 ± 0.2 , 0.7 ± 0.1 , 1.5 ± 0.1 and 3.3 ± 0.2 $\text{mg}\cdot\text{L}^{-1}$) of antimony, plus a control, in quadruplicate. Affected Hydra sp. formed clubbed tentacles and/or had a shortened body column and tentacles. The 96 h EC_{50} estimate and its confidence limits were 0.5 ($0.5 - 0.6$) $\text{mg}\cdot\text{L}^{-1}$ Sb (Table 2). Five percent of the control animals were affected.

Annelids, (mean length 23 ± 2 mm; mean weight, 0.006 ± 0.001 g) were exposed to two concentrations (11.4 ± 3.9 and 25.7 ± 2.2 $\text{mg}\cdot\text{L}^{-1}$) of antimony, plus a control in duplicate. Only 5% died in the highest exposure (25.7 $\text{mg}\cdot\text{L}^{-1}$) after 96 h. No LC_{50} or EC_{50} estimate could be made. No controls died.

Adult amphipods, (mean length, 7.2 ± 1.1 ; mean weight, 0.014 ± 0.005 g) were exposed to two concentrations (11.4 ± 3.9 and 25.7 ± 2.2 $\text{mg}\cdot\text{L}^{-1}$) of antimony, plus a control, in duplicate. Only 25% were dead in the highest exposure (25.7 $\text{mg}\cdot\text{L}^{-1}$) after 96 h; therefore, no LC_{50} or EC_{50} estimates could be made. No controls died.

Caddisflies, (mean length 18 ± 1 mm; mean weight, 0.113 ± 0.007 g) were exposed to two concentrations (11.4 ± 3.9 and 25.7 ± 2.2 $\text{mg}\cdot\text{L}^{-1}$) of antimony, plus a control, in duplicate. Only 5% in the highest exposure (25.7 $\text{mg}\cdot\text{L}^{-1}$) died after 96 h; therefore, an LC_{50} or EC_{50} estimate could not be made. No controls died.

Juvenile rainbow trout, (mean standard length 23 ± 1 mm; mean weight, 0.123 ± 0.013 g) were exposed to two concentrations (11.4 ± 3.9 and 25.7 ± 2.2 $\text{mg}\cdot\text{L}^{-1}$) of antimony, plus a control, in duplicate. Affected fish lost equilibrium. Deaths began occurring within 24 h after initial exposure with 45% dead in the

TABLE 2. LC₅₀ and EC₅₀ Estimates (95% Confidence Limits in Parentheses) for Static Exposures of SbCl₃ with Several Aquatic Organisms. [Units are mg·L⁻¹ as Antimony (III)]

Test Species	Average Weight ± s.d. (g)			LC ₅₀			EC ₅₀			
	24 hr	96 hr	48 hr	24 hr	96 hr	48 hr	24 hr	96 hr	48 hr	
Hydra (Hydra sp.)	-	-	-	-	-	-	2.0 (1.8-2.2)	0.5 (0.5-0.6)	1.0 (*)	-
Annelid (Lumbriculus variegatus)	-	>25.7	-	-	>25.7	-	-	>25.7	-	>25.7
Amphipod (Gammarus pseudo-limmaeus)	-	>25.7	-	-	>25.7	-	-	>25.7	-	>25.7
Caddisfly (Pycnopsyche sp.)	-	>25.7	-	-	>25.7	-	-	>25.7	-	>25.7
Rainbow trout (Salmo gairdneri)	-	>25.7	-	-	>25.7	-	-	>25.7	-	>25.7
Fathead minnow (Pimephales promelas)	20.8 (16.5-26.2)	14.4 (11.7-17.8)	17.4 (14.7-20.5)	20.8 (16.5-26.2)	14.4 (11.7-17.8)	17.4 (14.7-20.5)	20.8 (16.5-26.2)	14.4 (11.7-17.8)	17.4 (14.7-20.5)	14.4 (11.7-17.8)

* Confidence limits not reliable.

highest exposure ($25.7 \text{ mg}\cdot\text{L}^{-1}$) at 96 h. A 96 h LC_{50} or EC_{50} estimate could not be made due to insufficient mortalities. No control fish died.

Fathead minnows, 30-days old (mean standard length, 19 ± 1 mm; mean weight, 0.099 ± 0.025 g), were exposed to three concentrations (5.8 ± 0.2 , 11.7 ± 0.3 and $25.8 \pm 0.6 \text{ mg}\cdot\text{L}^{-1}$) of antimony from SbCl_3 and two concentrations (3.0 ± 0.3 and $4.5 \pm 0.2 \text{ mg}\cdot\text{L}^{-1}$) of antimony from Sb_2O_3 , plus respective controls, in duplicate. Affected fish lost equilibrium. Deaths began occurring in the SbCl_3 exposures within 24 h after initial exposure with 90% dead in the highest ($25.8 \text{ mg}\cdot\text{L}^{-1}$) exposure at 96 h. The 96 h LC_{50} estimate (95% confidence limits) was 14.4 ($11.7 - 17.8$) $\text{mg}\cdot\text{L}^{-1}$ Sb (Table 2). No control fish died. No fish were stressed or died in the Sb_2O_3 exposures.

Duckweed, was exposed to five concentrations (1.6 ± 0.2 , 3.3 ± 0.1 , 5.8 ± 0.6 , 12.5 ± 0.4 and $25.5 \pm 1.5 \text{ mg}\cdot\text{L}^{-1}$) of antimony, plus a control in quadruplicate. The mean numbers of fronds at the various antimony exposures are shown in Table 3. An EC_{50} could not be calculated due to insufficient inhibition of frond production in any exposure. However, a significant ($p < 0.05$) reduction in frond production occurred in the highest exposure concentration ($25.5 \text{ mg}\cdot\text{L}^{-1}$) as shown by least significant difference calculations (Dunnett's procedure).

TABLE 3. Frond Production by Duckweed (Lemna minor) Exposed for 96 Hr to $SbCl_3$. [Units are $mg \cdot L^{-1}$ Antimony (III)]

	Antimony (III) Concentrations					
	0	1.6	3.3	5.8	12.5	25.5
Mean number of fronds	45 ± 1	45 ± 3	47 ± 4	46 ± 1	42 ± 3	37 ± 6*
Percent effect ^{1/}	-	0.0	-8.0	-4.0	12.0	32.0

^{1/} Percent effect is the increase of fronds in the control

(C) minus the increase of fronds in the test concentration

(T) divided by the increase of fronds in the control multiplied by 100 (% effect = $\frac{C-T}{C} \times 100$).

* Significantly different from controls ($p \leq 0.05$).

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