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Acute Toxicity of Antimony Chloride and its Effects on Oxygen Consumption of Common Carp (*Cyprinus carpio*)

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Abstract The purposes of this study were to investigate the acute toxicity and effects of sublethal antimony (sb) concertrations on respiratory activity changes in the common carp (Cyprinus carpio). Median lethal concentrations were determined in acute tests. The 96-h LC50 value was 14.05 (11.09~17.80) mg L^{-1}. Common carp were exposed to 4 different sublethal levels of antimony (1.0, 2.0, 4.0, and 8.0 mg L^{-1}) over a 28-day test period and a 14-day recovery period. On days 14 and 28, decreases in oxygen consumption were significant ($p < 0.05$) for the higher-exposure level groups (4.0 and 8.0 mg L^{-1}). An increase in oxygen consumption was observed in the recovery period (on day 42) compared to the respective groups at the same level on day 28 at the higher exposure levles.

Keywords Antimony · Common carp · Acute toxicity · Oxygen consumption

Antimony (Sb) compounds such as indium antimonide (InSb) and gallium antimonide (GaSb) are important materials for the manufacture of integrated circuits and optoelectronic devices in the semiconductor industry (Bustamante et al. 1997). Manufacturing processes devoted to the fabrication of GaSb-based semiconductor devices generate large volumes of wastes that contain the toxic metals antimony and gallium. In addition, both metals are listed as hazardous by the Environmental Protection Agency in the US (Takayanagi 2001). Previous reports indicated that mammalian exposure to trivalent forms of Sb can cause severe liver damage, hemolysis, hematuria, and circulatory disease. And antimony trichloride ($SbCl_3$) induced sister chromatid exchanges (SCEs) in V79 cells and apoptosis in human fibroblasts (HFs), a human bronchial epithelial cell line (BES-6), and a Chinese hamster ovary cell line (CHO-K1) (Venugopal and Luckey 1978; Huang et al. 1998).

Oxygen consumption is widely considered to be a critical factor for evaluating the physiological response and a useful variable for an early warning for monitoring aquatic organisms (Chinni et al. 2000). Like most fish, common carp (*Cyprinus carpio*) are oxygen regulators, i.e., they maintain their oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until a critical oxygen concentration is reached, and below which oxygen consumption begins to fall. Under conditions of stress, this critical oxygen concentration is likely to increase, reflecting the decreased capacity of the fish to cope with environmental perturbations. For common carp and trout, a shift in the critical oxygen concentration to a higher oxygen concentration has been observed when exposed to low pH or other stressors (Ultsch et al. 1980). Metabolic responses to changes in oxygen availability may vary, depending on the physiological state of the animal, level of activity, and surrounding temperature (Burggren and Roberts 1991). Because the common carp is an important cultured fish species in fishponds near semiconductor manufacturing districts in Taiwan, it is a suitable model species to study the toxicity of semiconductor-related metals. Therefore, the purposes of this study were to investigate the acute toxicity and effects of sublethal antimony concentrations on respiratory activity changes in the common carp.

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Materials and Methods

Common carp (*C. carpio*) were obtained from local commercial suppliers. Fish were transported to a glass aquarium in our laboratory, which is equipped with a water-cycling device; dechlorinated tap water (with a pH of 7.4–7.8, dissolved oxygen (DO) of 7.3–8.1 mg L^{-1}, and hardness of 38–45 mg $CaCO_3$ L^{-1}) was used during the entire experiment. The temperature was maintained at 25.0 ± 0.5°C, and the photoperiod was set at 12 h of light and 12 h of dark. Fish were acclimated for 2 weeks and fed an aquarium fish mixture twice a day. Fish (6 weeks old, 0.238 ± 0.046 g in body weight) were used for the acute toxicity tests and oxygen consumption tests in the initial experiments. Antimony trichloride (purity ≥ 99%) was purchased from Sigma (St. Louis, MO, USA). A stock solution was prepared in deionized water (1,000 mg L^{-1} Sb in 0.1% nitric acid).

Toxicity test methods for common carp were based on the *Standard guide for conducting acute tests with fishes* (EPA 1998). Ten fish of similar size were randomly sampled and placed in 20-L glass beakers. After 24 h of acclimatization, fish were exposed to different Sb concentrations (0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 20.0 mg L^{-1}) for 96 h or more. The control and each treated group were run in duplicate. During the experiment, dead fish were removed, and the mortality was recorded after 24, 48, 72, and 96 h. The LC_{50} of antimony and its 95% confidence limits for common carp were calculated using a Basic program from the probit analysis described by Finney (1971).

The oxygen consumption analysis was carried out using a method described by Chinni et al. (2000) with slight modification. Groups of 20 fish were randomly sampled and placed in 50-L glass beakers; triplicate fish were then respectively exposed to a test solution of 0.0, 1.0, 2.0, 4.0, or 8.0 mg L^{-1}. Sublethal levels of antimony were equivalent to approximately 7%, 14%, 28%, and 57% of the 96-h LC_{50} value (14.05 mg L^{-1}) according to acute toxicity tests. Twice a week, 50% of the water was renewed with standard water containing antimony to maintain constant environmental conditions throughout the entire experimental period. The exposure time was 4 weeks, followed by a 2-week recovery period in Sb-free water.

Both control and exposed samples were taken after 30 min (for acute exposure), and after 14, 28, and 42 days for estimation of oxygen consumption. Oxygen consumption tests are customarily carried out by sealing two fish in a 325-mL respiratory jar capacity with an oxygen electrode (Microprocessor Oximeter, WTW, Heidelberg, Germany). All respiratory jars contained up to 7 mg L^{-1} DO before the initial measurement. At each interval, two fish were put into a respiratory jar with an acclimatization time of 30 min as recorded earlier, and then the oxygen consumption was estimated. We allowed them to deplete the oxygen until death occurred, and the residual dissolved oxygen was measured using a multiple-range temperature and oxygen analyzer and recorder (Yokogawa, Tokyo, Japan). Oxygen consumption (QO_2, mg O_2 kg^{-1} h^{-1}) was calculated as follows:

$$QO_2 = \Delta\text{ppm} \times 1/\text{BW} \times V \times 1/t$$

where QO_2 is the amount of oxygen (Δppm) consumed in the interval t (h) and BW is the wet body weight (kg) at the start and at the end of the test period.

All values of oxygen consumption test were statistically analyzed by analysis of variance (ANOVA) using SAS (1988) statistical software. Duncan's multiple range test was used to evaluate the mean difference among individual groups at the 0.05 significance level.

Results and Discussion

Physicochemical factors (temperature, pH, and DO) were measured throughout each experiment (Table 1). All physicochemical parameters remained relatively constant throughout the experimental period.

Median lethal concentrations (LC_{50}) of antimony for common carp were obtained. Values for the 48-, 72-, and 96-h LC_{50} are presented in Table 2. It is clear that the

Table 2 Median lethal concentrations (LC_{50}) of antimony to common carp

LC_{50} (mg L^{-1}Sb)		
48 h	72 h	96 h
19.36 (15.45–24.26)	16.24 (12.62–20.91)	14.05 (11.09–17.80)

The 95% confidence limits are given in parentheses

Table 1 Physicochemical parameters monitored over the experimental period

Parameter	Control	1.0 mg L^{-1}	2.0 mg L^{-1}	4.0 mg L^{-1}	8.0 mg L^{-1}
Temperature (°C)	25.3 ± 0.3	25.2 ± 0.5	25.1 ± 0.3	24.8 ± 0.6	25.3 ± 0.5
pH	7.6 ± 0.39	7.5 ± 0.51	7.5 ± 0.55	7.5 ± 0.28	7.5 ± 0.58
DO (mg L^{-1})	7.4 ± 0.36	7.5 ± 0.63	7.6 ± 0.21	7.4 ± 0.39	7.5 ± 0.64

All values are given as the mean ± SD; n = 3

Table 3 Effect of sublethal exposure to antimony on oxygen consumption (mg O_2 kg^{-1} h^{-1}) of common carp

	Control	1.0 mg L^{-1}	2.0 mg L^{-1}	4.0 mg L^{-1}	8.0 mg L^{-1}
Acute	0.553 ± 0.023[a]	0.630 ± 0.021[b]	0.664 ± 0.042[b]	0.778 ± 0.051[c]	0.871 ± 0.030[d]
Day 14	0.563 ± 0.019[a]	0.589 ± 0.043[a]	0.600 ± 0.015[a]	0.507 ± 0.005[b]	0.469 ± 0.003[c]
Day 28	0.548 ± 0.067[a]	0.574 ± 0.021[a]	0.568 ± 0.011[a]	0.445 ± 0.017[b]	0.404 ± 0.021[c]
Day 42	0.556 ± 0.046[a]	0.550 ± 0.040[a]	0.543 ± 0.038[a]	0.503 ± 0.016[b]	0.459 ± 0.013[c]

All values are given as the mean ± SD; n = 3. Values in the same row with different superscripts differ significantly at $p < 0.05$

higher the concentration, the shorter time in which the LC_{50} occurred in these animals. Comparing the toxicity of antimony with those of other metals studied such as mercury (96-h LC_{50}: 0.16 mg L^{-1}), copper (96-h LC_{50}: 0.64 mg L^{-1}), and cadmium (96-h LC_{50}: 2.15 mg L^{-1}) for the same species (Karan et al. 1998; Shyong 1999), it is clear that the toxicity of antimony is much less than those three metals. The 96-h LC_{50} value of antimony for 16-week-old zebrafish (*Brachydanio rerio*) (0.37 ± 0.11 g in body weight) was estimated to be 4.65 mg L^{-1} (Chen et al. 2006), indicating that the common carp is more tolerant to antimony exposure than the zebrafish. However, the 96-h LC_{50} value of antimony for tilapia larvae (*Oreochromis mossambicus*) is 18.9 mg L^{-1}. Common carp were found to be more susceptible than tilapia larvae to acute antimony toxicity (Lin and Hwang 1998).

The results of the oxygen consumption rates for the control and exposed common carp are presented in Table 3. After 30 min, there was an increase in the amount of oxygen consumed in exposed common carp; a maximum increase of 57.5% was observed at the highest exposure concentration (8.0 mg L^{-1}), and the increase was significant ($p < 0.05$) in exposed common carp in relation to antimony concentrations. On days 14, 28, and 42 (the last time point during the recovery period), decreases in oxygen consumption were significant ($p < 0.05$) for the higher-exposure level groups (4.0 and 8.0 mg L^{-1}). Moreover, the percent oxygen consumption decreased over their respective controls from acute exposure (after 30 min) to day 42 at the lower exposure levels (1.0 and 2.0 mg L^{-1}). However, an increase in oxygen consumption was observed in the recovery period (on day 42) compared to the respective same level groups on day 28 at the higher exposure levels (4.0 and 8.0 mg L^{-1}).

Our results suggest that measurements of oxygen consumption can be used to assess the effects of antimony on sublethal exposure levels of common carp. The determination of the critical oxygen concentrations for regulating oxygen consumption provides important information on the physiological condition of the organism (Chinni et al. 2000). On day 0, increased oxygen consumption in exposed common carp was observed in the exposed groups. Murty (1986) indicated that exposure to sublethal toxicant concentrations increases respiratory activity, resulting in increased ventilation, and increased uptake of the toxicant. Later, the decrease in oxygen consumption by common carp in the presence of antimony can possibly be attributed to injury to the gills. Karan et al. (1998) indicated that cytological damage occurs in common carp exposed to heavy metals, and it may manifest as a thickening of the branchial epithelium and increase the pollutant-blood diffusion distance, causing impaired gaseous exchange. This kind of change might have occurred in common carp upon exposure to antimony. However, some studies reported that during exposure to sublethal concentrations of copper and zinc, the above condition was reversed after 14 days in common carp (Karan 1998) and 30 days in *Cancer pagurus* (Spicer and Weber 1991) even in the continued presence of the toxicant. In the present investigation, common carp showed no recovery during the 28 days of exposure. A slight decrease in oxygen consumption was seen in common carp from days 0–28 of the exposure period for the lower-level groups (1.0 and 2.0 mg L^{-1}).

Although heavy metals are often referred to as a common group of pollutants, each metal produces different problems in freshwater environments, and therefore metals have to be considered separately as well as in various combinations (Lloyd 1992). Because many wastewater discharges contain a mixture of pollutants, the combined effects of antimony with copper, cadmium, zinc, gallium, indium, and aluminum have to be evaluated. Frequent use of antimony compounds in semiconductor manufacturing has been accompanied by potentially increasing amounts of materials released as toxic wastes, which are harmful to health and the environment (Chelton et al. 1991). Inhibition of respiration is clearly evident from the present study. This finding suggests that antimony is a potential pollutant in aquatic environments, although limited knowledge on the adverse effects of antimony on aquatic animals has been reported to date.

References

Burggren W, Roberts J (1991) Respiration and metabolism. In: Prosser CL (ed) Environmental and metabolic animal physiology. Wiley-Liss, New York, pp 353–435

Bustamante J, Lennart D, Marie V, Bruce F, Sten O (1997) The semiconductor elements arsenic and indium induce apoptosis in rat thymocytes. Toxicology 118:129–136

Chelton CF, Glowatz M, Mosovsky JA (1991) Chemical hazards in the semiconductor industry. Ins Electr Electron Eng Trans Ed 34:269–288

Chen LH, Yang JL, Chen HC (2006) Effects of antimony chloride (III) on aquatic organisms: acute test, serum metabolic enzyme activities, and blood cell deformation. Environ Sci (accepted)

Chinni S, Khan RN, Yallapragada PR (2000) Oxygen consumption, ammonia-N excretion, and metal accumulation in *Penaeus indicus* postlarvae exposed to lead. Bull Environ Contam Toxicol 64:144–151

Environment Protection Agency (1998) Standard guide for conducting acute tests with fishes: static renewal test for common carp, NIEA B904.10B

Finney DJ (1971) Probit Analysis, 3rd edn. Cambridge University Press, London

Huang H, Shu SC, Shih JH, Kuo CJ, Chiu ID (1998) Antimony trichloride induces DNA damage and apoptosis in mammalian cells. Toxicology 129:113–123

Karan V, Vitorvic S, Tutundzic V, Poleksic V (1998) Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicol Environ Safety 40:49–55

Lin HC, Hwang PP (1998) Acute and chronic effects of antimony chloride ($SbCl_3$) on tilapia (*Oreochromis mossambicus*) larvae. Bull Environ Contam Toxicol 61:129–134

Lloyd R (1992) Pollution and Freshwater Fish. The Buckland Foundation, London

Murty AS (1986) Toxicity of Pesticides to Fish, vol ii. CRC Press, New York

Shyong WJ (1999) Studies on propagation, culture and environmental biology of Taiwan freshwater fish *Zacco barbata*. PhD dissertation, National Taiwan University, Taipei, Taiwan

Spicer JI, Weber RE (1991) Respiratory impairment in crustaceans and mollusks due to exposure to heavy metals. Comp Biochem Physiol C 100:339–342

Statistical Analysis System Institute (1988) SAS/STAT program. SAS Institute, Cary, NC

Takayanagi K (2001) Acute toxicity of waterborne Se(IV), Se(VI), Sb(III), and Sb(V) on red seabream (*Pargus major*). Bull Environ Contam Toxicol 66:808–813

Ultsch GR, Ott ME, Heisler N (1980) Standard metabolic rate, critical oxygen tension, and aerobic scope for spontaneous activity for trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*) in acidified water. Comp Biochem Physiol A 67:329–335

Venugopal B, Luckey TD (1978) Metal Toxicity in Mammals (II). Plenum Press, New York