January 2007 *Volume 2 Issue 1*

Trade Science Inc.

Environmental Science

An Indian Journal

Current Research Paper

ESAIJ, 2(1), 2007 [53-58]

Effects Of $SbCl_3$ On Aquatic Organism: Acute Test, Serum Metabolic Enzyme Activities, And Blood Cell Deformation

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Received: 16th November, 2006
Accepted: 30th November, 2006

Web Publication Date: 27th December, 2006

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ABSTRACT

The purposes of this study were to investigate the effects of antimony (Sb) on acute toxicity, on serum metabolic enzyme activities as well as on erythrocyte morphological changes in the blood stream of zebrafish (Brachydanio rerio). Median lethal concentrations were determined in acute tests. The 96-h LC_{50} value was 4.65 (3.37-6.42) mg/L. During 2 weeks testing period, zebrafish were exposed to 3 different sublethal levels of antimony (0.5, 1.5, and 3.0 mg/L) in laboratory toxicity tests. Serum metabolic enzyme activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) was analyzed. An increase of enzyme activity in serum was observed, particularly at the highest exposure concentrations. Moreover, erythrocyte morphological deformation in the bloodstream proposes acting as a promising indicator of water pollution levels.

KEYWORDS

Antimony;
Zebrafish;
Acute toxicity;
Metabolic enzyme;
Erythrocyte.

INTRODUCTION

Antimony (Sb) is used extensively in traditional manufacturing such as battery, rubber, cement, glass, paints, medicine, and steel industries[15]. Recently, antimony compounds like as indium antimonide (InSb) and gallium antimonide (GaSb), important intermetallic compounds in the semiconductor de-

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vice manufacture[12]. The metal is listed as hazardous in EPA of USA[20]. Soluble antimony salts are more toxic than similar lead (Pb) or arsenic (As) compounds, and trivalent Sb salts are ten times more toxic than pentavalent salts[8]. Previous reports on mammals exposure to trivalent forms of Sb could cause severe liver damage, hemolysis, hematurea, and circulatory disease. And antimony chloride ($SbCl_3$) induced sister chromatid exchanges (SCE) in V79 cells and apoptosis in human fibroblasts (HF), human bronchial epithelial cell line (BES-6), and chinese hamster ovary cell line (CHO-K1)[18,10].

Accidental industrial spills might lead to high concentrations of toxic materials in the aquatic environment as well as effects on freshwater ecosystems with acute and chronic toxicity. As to aquatic animals, acute toxicity of antimony to juvenile sheepshead minnows (Cyrinodan variegatus) and larval tilapia (Oreochromis mossambicus) were carried out[9,22]. However, there is limited knowledge of antimony on freshwater fish, and further effects of the metal need to be better understood.

Pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes[26]. The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In some situations, poisonous materials may accumulate in the liver to toxic levels and cause pathological alterations[28]. Considerable articles of liver ultrastructural alterations induced by heavy metals in aquatic animals[2,13]. The use of metabolic serum enzyme evaluation has been advocated to provide a warning of potentially damaging changes in stressed specific organisms. Cell injury of certain organs leads to the release of tissue-specific enzymes into the bloodstream[6]. Elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were presumably due to damage to the liver. However, other organs may also have been damaged, like the kidney and gills[3]. Alkaline phosphatase (ALP) is composed of several isoenzymes that are present in practically all tissues of the body, especially in cell membranes. These enzymes catalyze the hydrolysis of monophosphate esters and have wide substrate specificity[17]. A large amount of studies revealed that elevated metabolic enzyme activities induced by heavy metals in aquatic vertebrates, such as copper, cadmium, mercury and gallium[7,14,29].

Erythrocytes are the most abundant cell type found in the peripheral blood and function in respiration by transporting oxygen to and carbon dioxide from body tissues, and teleost erythrocytes are oval, nucleated cells with abundant pale eosinophilic cytoplasm[19]. Heavy metal ions, such as copper, cadmium, and mercury ions, induce lysis of erythrocytes and may cause the accelerated destruction of erythrocytes[16].

The purposes of this study were to investigate the effects of antimony on acute toxicity, serum metabolic enzyme activities as well as on blood cell morphological changes of the zebrafish. The results could then be used to evaluate the possible adverse effects of antimony, and provide essential information which can be used to objectively institute measures to minimize the pollution by antomony and its impacts on aquatic ecosystems.

EXPERIMENTAL

Zebrafish (*Brachydanio rerio*) were obtained from the local commercial suppliers. Fish were transported to the glass aquarium in our laboratory which was equipped with a water-cycling device, and dechlorinated tap water (pH 7.5-8.1; dissolved oxygen concentration 8.0-8.9 mg/l; hardness 22-32 mg $CaCO_3$/L, ammonia < 0.5 mg/L, and nitrite 0.05-0.1 mg/L) was used. Fish were acclimated for 2 weeks and fed aquarium fish mixture everyday. The temperature was maintained at 23.0 ± 0.6°C, and the photoperiod was set at 8 h of light and 16 h of dark during the entire experiment. Zebrafish (4 months old, 2.4 ± 0.2 cm in body length, 0.37 ± 0.11 g in body weight) were used for acute and chronic toxicity tests in the initial experiments. Antimony chloride (III) (purity 99.999%) was purchased from Alfa Aesar (Ward Hill, MA, USA). A stock solution was prepared in deionized water (1000 mg/L Sb in 0.1% nitric acid).

Laboratory static renewal test were conducted to determine the median lethal concentration (LC_{50}) for zebrafish. 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 antimony concentrations (0.1, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 mg/L) for 96 h or more. The control and each treated group were run in duplicate. During the experiment, dead fish were removed, and mortality was recorded after 24, 48, 72, and 96 h. The LC_{50} of antimony and its 95% confidence limits for fish were calculated using a Basic program from the probit analysis described by[4].

Zebrafish for sublethal toxicity tests were randomly placed in 100 L glass aquaria. Every aquarium contained 10 fish which were exposed to the following concentrations: 0.5, 1.5 and 3.0 mg/L Sb test solutions and a control, respectively. Sublethal levels of antimony were equivalent to approximately 10%, 30%, and 65% of the 96-h LC_{50} value (4.65 mg/L) according to acute toxicity tests.

Six fish per exposure concentration were anesthetized with MS-222 (Sigma Chemical, St. Louis, MO, USA) and sacrificed after 2 weeks of exposure. Blood samples were taken from each fish by puncture of the caudal vessel. Blood was allowed to coagulate at room temperature for 0.5 h. Serum was obtained by centrifugation of an amount of blood at 1500 xg (for 10 min at 4°C) and metabolic enzyme activities were measured using a Johnson and Johnson Ektachem 250 biochemical analyzer (New York, NY, USA). Assays were run in triplicate. Test kits from Johnson and Johnson were used for determinations. Aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) activities were measured according to the method of[11], and alkaline phosphatase (ALP, EC 3.1.3.1) activities were determined by the method of[25].

Blood smears were made in duplicate in accordance with the description by[19]. Smears made from blood samples were air dried for 1 hour and then fixed in 95% methanol at 4°C. Slides were stained with a modified Wright stain (Sigma Chemical, St. Louis, MO), and a cover slip was placed on top using glycerol.

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

RESULTS

Physicochemical factors (temperature, pH, and dissolved oxygen) were measured throughout each experiment (TABLE 1). All physicochemical parameters remained constant throughout the experimental period.

According to the static renewal method for acute toxicity testing[1], median lethal concentrations (LC_{50}) of antimony for zebrafish (Brachydanio rerio) were obtained. Values for the 48-, 72-, and 96-h LC_{50} are presented in TABLE 2. It is clear that the higher the concentration, the shorter the LC_{50} of the animals.

The results of metabolic enzyme activity analysis are presented in TABLE 3. No significant changes occurred in the activities of the 3 enzymes (ALT, AST, and ALP) in the 0.5 mg/L Sb group compared with the control group. In treatment with 1.5 mg/L Sb, there was no significant difference in serum AST and ALP activities compared with the control group. On the other hand, ALT activities in serum of treated fish were significantly higher than those of the control group at the same level of antimony administration. Statistically significant increases in all enzyme activities were recorded at the highest (3.0 mg/L) antimony concentrations; values recorded were 64% to 117% higher than those of the control group.

TABLE 1: Physicochemical parameters monitored over the experimental period

Parameter	Control	0.5 mg/l	1.5 mg/l	3.0 mg/l
Temperature (°C)	24.9±0.3	24.8±0.5	25.1±0.3	24.8±0.6
pH	7.5±0.09	7.5±0.39	7.5±0.21	7.5±0.28
Dissolved oxygen(mg/l)	7.3±0.61	7.3±0.33	7.4±0.10	7.4±0.09

All values are given as the mean ± SD; n = 15

TABLE 2: Median lethal concentrations (LC_{50}) of antimony to zebrafish

LC_{50} (mg/l Ga)		
48 h	72 h	96 h
6.54	5.26	4.65
(5.08-8.41)	(4.22-6.57)	(3.37-6.42)

The 95% confidence limits are given in parentheses

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TABLE 3: Metabolic enzyme activities of zebrafish exposed to antimony

	ALT (U/l)	AST (U/l)	ALP (U/l)
Control	38.80±6.25[a]	43.48±4.61[a]	22.39±5.96[a]
0.5 mg/l Sb	37.57±3.46[a]	46.25±15.58[a]	24.00±2.52[a]
1.5 mg/l Sb	52.19±6.91[b]	46.01±12.02[a]	23.21±3.45 [a]
3.0 mg/l Sb	83.54±10.03[c]	71.24±12.75[b]	48.75±7.51[b]

According to the peripheral blood smear examination, normal erythrocytes of untreated fish have an oval shape with a rounded to oval central nucleus with densely packed chromatin (Figure 1). The present study shows erythrocyte morphological alterations in zebrafish exposed to antimony (Figure 2-4). Obliviously, high ratios of red blood cells were in the process of losing their normal outline and cytoplasm at higher exposure levels (1.5 and 3.0 mg/l Sb).

Heavy metals are some of the most-active polluting substances as they can cause serious impairment to circulatory, metabolic, physiological, and even structural systems when high concentrations are present in aquatic ecosystems[21]. Although heavy metals are often assorted to as a common group of pollutants, individual metals pose different characters in water mass, and therefore they have to be considered separately[24].

The 96-hr LC_{50} value of antimony (III) for larval tilapia (Oreochromis mossambicus) was estimated to be 18.9 mg/L[9] and 96-hr LC_{50} of Sb to juvenile sheepshead minnows (Cyrinodan variegatus) to be 6.2-8.3 mg/L[22], indicating that zebrafish were as sensitive to antimony as the much tilapia and sheepshead minnows. Further, almost no toxic effect was seen at 0.5 mg/L Sb which is equivalent to 10% of the 96-h LC_{50} value. Less than 0.5 mg/L is proposed as a biologically safe concentration which can be used for establishing tentative water quality criteria concerning of same size zebrafish.

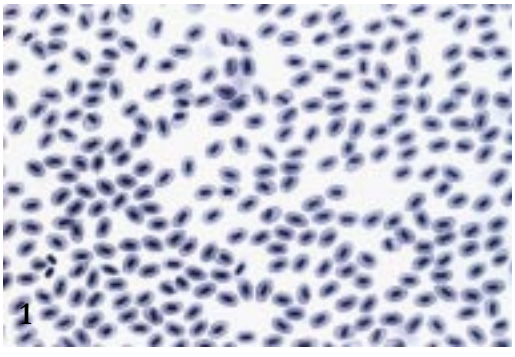

Figure 1: Blood smear of zebrafish exposed to Sb-free water for 14 days

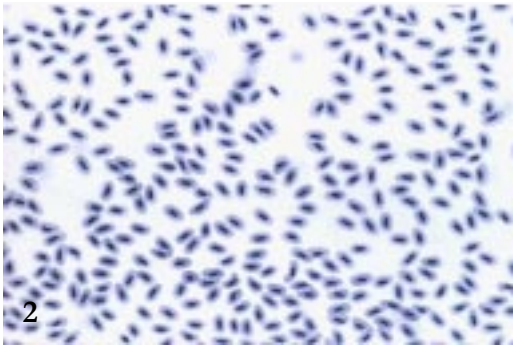

Figure 2: Blood smear of zebrafish to 0.5 Sb mg/L for 14 days

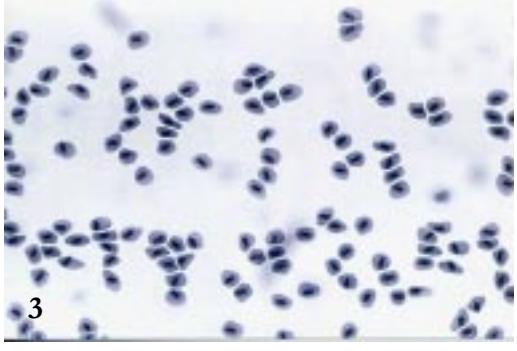

Figure 3: Blood smear of Zebrafish to 1.5 Sb mg/L for 14 days

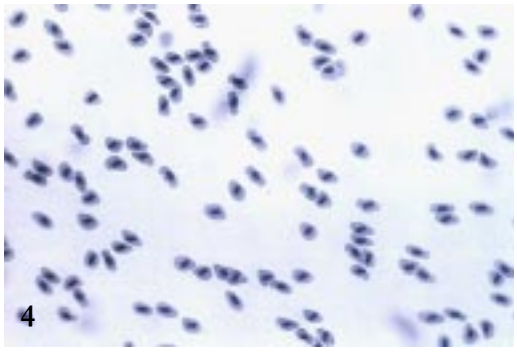

Figure 4: Blood smear of zebrafish to 3.0 Sb mg/L for 14 days. (modified Wright)

Much more extensive biochemical toxicological research has been conducted in mammals than in fish. However, it is not surprising that many biochemical similarities exist among vertebrate species (Hochachka and Mommsen, 1995). The increased transaminase (AST and ALT) activity in zebrafish

exposed to the highest concentration group may reveal possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver. Meanwhile, elevation of serum ALP also correlates with exposure levels of fish. Increased serum activities of ALP have been explained by pathological processes such as liver impairment, kidney dysfunction, and bone disease. Although its real biochemical functions that act in the organism are not known[5]. Antimony treatment increases serum AST, ALT, and ALP activities of fishes reflecting a situation of tissue damage and stress. Our results indicate that changes in metabolic enzyme activities in serum occurred in higher exposure groups; this fact should be confirmed by observations of damage to organelles within hepatocytes in future study. This awareness must include knowledge of its effect on fishes, more specifically of its cytopathology, histopathology, and influence on biochemical parameters, behavior, growth, reproduction, etc.

Morphological changes of erythrocytes in bloodstream suggest obstruction of gas exchange as an additional process of Sb exposure[23]. Suggested that the affinity of heavy metals for SH-groups in membrane proteins could affect membrane conformation and permeability. Peroxidation of membrane lipids may be a possible mechanism of damage to erythrocyte membranes treated with metals[27]. Erythrocyte morphological changes may be utilized as a practical approach for assessing the effects of antimony compounds on intoxicated fish, and it's also need to understanding of the toxicological mechanism in future studies.

CONCLUSIONS

The major findings of this study are that antimony is a toxic substance in zebrafish, with elevation enzyme activities in serum as well as erythrocyte deformation in bloodstream of fish exposed to various concentrations. Antimony is one of the intermetallic elements increasingly being used in semiconductor technology for making infrared detectors, diodes, hall-effect devices and other electronic manufacturing. Many wastewater discharges contain a mixture of pollutants, the combined effect of antimony with arsenic, gallium, cadmium, or indium has to be carried out, and these metals have to be considered separately for establishing of water quality criteria. And we must be aware of their potential hazard to the aquatic ecosystems, although no adverse effects following industrial exposure have been reported to date.

ACKNOWLEDGMENTS

We wish to thank Dr. PP Hwang at the Zoology Institute, Academia Sinica, Taipei for his valuable advice; and Mr. Chamberlin for correction of the English text.

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