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Effects of antimony on aquatic organisms (Larva and embryo of *Oryzias latipes*, *Moina macrocopa*, *Simocephalus mixtus*, and *Pseudokirchneriella subcapitata*)

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ABSTRACT

Antimony is widespread in aquatic environment. Trivalent forms of antimony are known to be more toxic than other chemical species of antimony. In the present study, antimony potassium tartrate (APT), the trivalent inorganic forms of antimony, was selected as a test antimony compound due to its high water solubility. The effects of antimony on Japanese medaka (*Oryzias latipes*), planktonic crustacea (*Moina macrocopa* and *Simocephalus mixtus*), and green algae (*Pseudokirchneriella subcapitata*) were evaluated. Larval survival and the embryonic development were measured for fish assay. APT was less toxic to larval medaka (24-h LC50, 261; 48-h LC50, 238 mg L^{-1}). *Simocephalus mixtus* was killed by very low concentrations of APT (24-h LC50, 4.92 mg L^{-1}), and antimony was also toxic to *Moina macrocopa* (24-h LC50, 12.83 mg L^{-1}). Toxicities of APT to *S. mixtus* and *Moina macrocopa* were about 50 and 20 times more toxic to *Oryzias latipes* larvae, respectively, in terms of 24-h LC50 value. Growth inhibition of *Pseudokirchneriella subcapitata* was observed in the presence of APT (72-h EC50, 206 mg L^{-1}). This study demonstrated that APT is more toxic to planktonic crustacea than fish and green algae, and planktonic crustacea appears a better indicator of antimony pollution in aquatic environment.

1. Introduction

Antimony (Sb) is a naturally occurring element in environmental media, and it also releases to the environment from anthropogenic sources such as mining and processing of ores and the production of alloys (CEPA, 1997). Concentrations of antimony are generally less than 1 μg L^{-1} in unpolluted water (Filella et al., 2002). A range of 0.1–27 μg L^{-1} for antimony was detected in Korean four main rivers in the years 2004–2005 (An et al., 2008).

Antimony is a silvery white metal between arsenic and bismuth, in the group 15 periodic table of the elements (ATSDR, 1992; Filella et al., 2002; Filella et al., 2007). Antimony can exist in four oxidation states (−3, 0, +3, and +5), but it is primarily found in Sb(+3) and Sb(+5). The trivalent inorganic forms of antimony are the most common species, and are known to be more toxic than pentavalent one (Filella et al., 2002). Antimony potassium tartrate (APT) is one of the trivalent antimony. Although the carcinogenicity of antimony was not assessed under the integrated risk information system (IRIS) of US Environmental Protection Agency (US EPA), it is reported that APT of 5 mg L^{-1} led to alter the cholesterol levels in male and female rats, and it caused the decrease of heart weight in male rats (IRIS, 1991).

The influence of antimony on aquatic organisms, including bluegill *Lepomis macrochirus* (Buccafusco et al., 1981), fathead minnow *Pimephales promelas* (LeBlanc and Dean, 1984), water flea *Daphnia magna* (Khangarot and Ray, 1989), ciliated protozoa *Tetrahymena pyriformis* (Sauvant et al., 1995), and tubificid worm *Tubifex tubifex* (Khangarot, 1991) has been reported. In addition, the toxicity of antimony chloride ($SbCl_3$) on tilapia (*Oreochromis mossambicus*) larvae was assessed (Lin and Hwang, 1998). Scud *Hyalella azteca* was tested for antimony as sodium antimonite ($NaSbO_3$) (Borgmann et al., 2005). However, little information is available about the effect of ATP on aquatic organisms, and there is few toxicity data for antimony on planktonic crustacea.

In the present study, the effects of antimony on *Oryzias latipes* (Japanese medaka), *Moina macrocopa* (crustacea), *Simocephalus mixtus* (crustacea), and *Pseudokirchneriella subcapitata* (green algae) were evaluated to investigate the adverse effect of antimony in aquatic environment. Embryos and larvae of *Oryzias latipes* were used to reduce sacrifice of adult vertebrates as alternative methods. Antimony in the form of APT was used due to its high water solubility relative to other antimony compounds such as antimony chloride ($SbCl_3$) and sodium antimonite ($NaSbO_3$). To the best of our knowledge, this study is the first to use *Oryzias latipes*, *Moina macrocopa*, *S. mixtus* and *Pseudokirchneriella subcapitata* to assess the effect of APT.

2. Materials and methods

2.1. Chemical

Antimony was obtained from Sigma (Louise, MO, USA) as antimony potassium tartrate (APT, $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, ⩾99.9%)

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and used without further purification. Stock solution of APT was prepared in distilled water. Stock solution was diluted to obtain suitable concentrations of APT in dechlorinated tap water, embryo rearing solution, moderately hard water (MHW) medium of USEPA (2002), and algal medium for fish, fertilized eggs, planktonic crustacea, and green algae, respectively.

2.2. Fish assay

Oryzias latipes (Japanese medaka, orange-red type) was obtained from National Institute of Environmental Research (NIER, Incheon, Korea). The fish culture was maintained in dechlorinated tap water (pH 7.0 ± 0.2, hardness 65 ± 4.5 mg L^{-1}) at a constant temperature of 24 ± 2 °C with a photoperiod of 16:8 h (light:dark). Tetra Min (Teta Werke, Germany) and brine shrimp (OSI PRO80™, USA) were used as a food source. Seven day old-larvae were used for toxicity test. Fish assay was conducted following the method described in the OECD guideline for testing of chemicals No. 203 (OECD, 1992a). Fifty milliliters of dechlorinated tap water was placed in a test unit. Each test unit contained ten larvae, and test was done with three replicates. Test duration was set as 24, 48, 72, and 96 h. The test unit was placed in temperature-controlled incubator (25 °C), and the test species were not fed and test solution was not aerated. The experiment was done in a static procedure. The dead individuals were immediately removed and median lethal concentration (LC50) was calculated at 24-h intervals.

2.3. Embryotoxicity assay

Newly fertilized medaka eggs were collected from the broodstock in the morning of the experimental day (day 0). Fertilized eggs were exposed to a range of concentrations of Sb dissolved in embryo rearing solution under static non-renewal condition. The embryo rearing solution contains NaCl, KCl, $CaCl_2$, $MgSO_4$, and methylene blue was added to prevent fungal growth. Embryotoxicity assay was conducted following the method described in the OECD guideline for testing of chemicals No. 210 (OECD, 1992b). One milliliter of embryo rearing solution was placed in a test unit. Each test unit contained one egg. There were 10 vials per treatment, which consisted of 0, 5, 10, 25, 50, 100, 125, 150, 200, and 300 mg Sb L^{-1} concentrations. The capped exposure test units were incubated at 25 °C. The embryos were examined daily under a dissection microscope for mortality, hatchability, and abnormality. The observed abnormalities included retarded swimming after hatching, ocular breakage, and blastula edema with hemorrhage.

2.4. Cladoceran assay

Test species were *Moina macrocopa* and *S. mixtus*. *Moina macrocopa* was obtained from the Korea Institute of Toxicology (KIT, Daejeon, Korea). *S. mixtus* was collected from the farm pond in Namyangju city, Korea. Cultures of *Moina macrocopa* and *S. mixtus* were kept in MHW medium at 21 and 24 °C, respectively, with a photoperiod of 16:8 h (light:dark). They were fed daily with green algae *Pseudokirchneriella subcapitata* with the concentration of 2×10^4 cells mL^{-1}. Acute toxicity test was performed following the method described in the OECD guideline for testing of chemicals No. 202 (OECD, 2004). Ten milliliters of MHW medium was placed in 35-mL flat-bottomed glass vial. Each test unit contained five neonates (less than 24 h), and test was done with three replicates. Test duration was set as 48 and 24 h for *Moina macrocopa* and *S. mixtus*, respectively. Survival and immobilization were measured for *Moina macrocopa*. Toxicity endpoint selected for *S. mixtus* was heart beating under microscope because this species tends to stay at the surface of test unit and its movement is not active enough to measure the conventional immobilization.

2.5. Algal assay

Pseudokirchneriella subcapitata was obtained from the Korea Institute of Toxicology (KIT, Daejeon, Korea), and it was cultured in our laboratory for several months before the test. Growth inhibition test with *Pseudokirchneriella subcapitata* was performed in algal medium according to the OECD guideline for testing of chemicals No. 201 (OECD, 2006). The initial algal density of 2×10^4 cells mL^{-1} was inoculated in 3.8-mL 24 well microplate containing 2 mL of exposure solution in triplicate. The microplates were placed on a algal growth chamber under continuous fluorescent illumination (approximately 4000 lux), and incubated at 25 ± 1 °C. The cell density was measured using the spectrophotometer at 685 nm, and median effective concentration (EC50) were calculated at 24-h intervals for 72 h.

2.6. Statistical analyses

LC50, EC50 values and corresponding 95% confidence limits for the test species were calculated by computer software, Trimmed–Spearman–Karber (TSK) Program (USEPA, 1999). No-observed-effective concentration (NOEC) values were estimated using the Dunnett's Program (Dunnett, 1955). The average specific growth rate of algae for the exposure duration was calculated from the logistic equation (1):

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} (\text{day}^{-1}) \quad (1)$$

where μ_{i-j} is the average specific growth rate from time i to j; X_i is the optical density (at 685 nm) at time i; X_j is the optical density (at 685 nm) at time j.

3. Results and discussion

3.1. Effect on survival of Oryzias latipes larvae

Larval survival of *Oryzias latipes* in each antimony treatment, expressed as a percentage of the mean of the control treatment, is presented in Fig. 1. Survival of larval fish was inhibited in the presence of antimony. Antimony caused a concentration-related decrease in

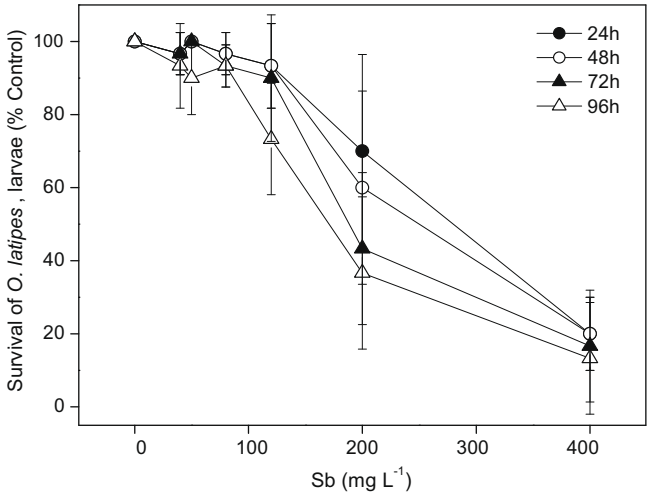

Fig. 1. Survival of *Oryzias latipes* as a percentage of the mean of the control (no Sb added) treatments for 24, 48, 72, and 96 h. Bars represent one standard deviation of the mean of three replicates. Error bars smaller than the symbols are not shown.