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ORIGINAL PAPER

TOXICITY OF ANTIMONY, GALLIUM, AND INDIUM TOWARD A TELEOST MODEL AND A NATIVE FISH SPECIES OF SEMICONDUCTOR MANUFACTURING DISTRICTS OF TAIWAN

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Abstract

Antimony (Sb), gallium (Ga), and indium (In) compounds are released into the aquatic environment during manufacturing processes such as wafer processing, epitaxy, and cleaning operations in making high-speed semiconductor and light emitting diodes (LEDs). In certain cases, it is possible that significant levels of these compounds are continually being discharged into aquatic environments, which could increase the hazardous effects. This study was conducted to determine adverse effects of antimony, gallium, and indium on a teleost model and a native fish species. Acute toxicity tests and the median lethal concentration (LC_{50}) could provide objective information for establishing water quality criteria for certain toxic substances. We discussed the acute toxic effects of antimony, gallium, and indium on zebrafish (*Danio rerio*) and rosy bitterling (*Rhodeus ocellatus*). The 96-h 50% lethal concentration (LC_{50}) values of antimony ranged within 2.46~5.87 mg l^{-1} for *D. rerio*, and 4.09~10.85 mg l^{-1} for *R. ocellatus*. With regard to gallium toxicity, the 96-h LC_{50} values ranged within 7.25~11.42 mg l^{-1} for *D. rerio*, and 9.47~12.11 mg l^{-1} for *R. ocellatus*. The 96-h LC_{50} values of indium ranged within 12.84~18.35 mg l^{-1} for *D. rerio*, and 12.28~15.95 mg l^{-1} for *R. ocellatus*. The toxicity of antimony was higher than that of gallium and indium metal compounds toward both the teleost model (*D. rerio*) and the native fish (*R. ocellatus*). Comparing the antimony and gallium toxicity tolerance of *D. rerio* with those of *R. ocellatus* when exposed to these metals, it is obvious that *D. rerio* is more sensitive than *R. ocellatus*.

Keywords: antimony, gallium, indium, acute toxicity, *Danio rerio*, *Rhodeus ocellatus*.

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INTRODUCTION

The metals antimony, gallium, and indium belong to groups IV and V of the periodic table. They are essential transition metals which are widely used in electroplating, and for the manufacture of computers, peripheral devices, telecommunication devices, and LEDs with integrated circuit (IC) products (Chen, Huang 2004). With the exception of ore exploitation, such threats can be due to traditional manufacturing, as 500,000 tons of antimony are consumed every year in a variety of industries, including the battery, rubber, cement, glass, paint, medicine, steel, and leather industries, and more than 1,800 tons of antimony waste and scrap were exported to Southeast Asia from 1997 to 2000 (Hsing et al. 2004, Guo et al. 2009). Antimony compounds are important materials in the semiconductor industry. Manufacturing processes devoted to the fabrication of Sb-based semiconductor devices generate large volumes of waste, with aqueous waste streams of the wet--polishing process containing 200~400 mg l^{-1} of dissolved antimony metal (Bustamante et al. 1997, Gebel et al. 1998, Sturgill et al. 2000). In addition, levels of antimony in groundwater and surface waters normally range within 0.1~0.2 μg l^{-1}, and approximately 6400 tons of antimony is transported annually to the oceans, with marine antimony concentrations being approximately 0.15 μg l^{-1} (Andreae et al. 1981, Filella et al. 2009). GaAs and InAs are important materials used in the electronic industries for their excellent electronic properties compared to silicon-based materials (Robinson 1983). The Foregs Geochemical Atlas (2006) reported that gallium values in stream water range <0.002~0.16 μg l^{-1}. Gallium contents in stream sediments range <1~36.0 mg kg^{-1}. Indium values in stream water range <0.002~0.015 μg l^{-1}. Accidental spills might lead to higher levels of toxic materials in aquatic ecosystems. Gallium and indium are also found in the groundwater under semiconductor manufacturing districts of northern Taiwan. Metal levels in well water were 7.90~41.39 μg l^{-1} for gallium and 0.95~105.45 μg l^{-1} for indium. Gallium and indium contents in ditch sediments range within 2.1~2.52 mg kg^{-1} and 3.1~3.6 mg kg^{-1}, respectively (Chen 2006, Hsu et al. 2011).

Soluble antimony salts are more toxic than similar lead (Pb) or arsenic (As) compounds, and trivalent antimony salts are 10-times more toxic than pentavalent salts (Wey et al. 1997, Smichowski 2008). Teleost studies have shown that exposure to gallium and indium compounds induces changes in gene expressions and immune responses. Toxicity to the immune system has frequently been related to lymphocyte apoptosis (Betoulle et al. 2002). Aquatic organisms can be used for providing an early warning system for potential harm to the aquatic environment, monitoring the health of fauna and flora in the field, and determination of water quality standards. Instantaneous and prolonged effects provide detailed information for assessing environmental stress. Acute effect results in particular provide practical critical values, and suitable model species are needed to evaluate the water quality in aquatic ecosystems (Stephenson 1982, Belanger et al. 2013). Previous studies were conducted to examine the adverse effects of antimony, gallium, and indium on cultured fish, i.e., tilapia and common carp, near local semiconductor science-based industrial parks of Taiwan (Yang 2014). However, zebrafish (*Danio rerio*) is not only a popular tropical fish pet but also an important model organism for toxicity studies (Spitsbergen, Kent 2003). Zebrafish have certain characteristics, including a large population and a relatively low cost of maintenance and production, which make them convenient to manipulate in the laboratory (Majewski et al. 2017). The rosy bitterling (*Rhodeus ocellatus*) is frequently encountered in shallow ponds and streams near semiconductor manufacturing districts in Taiwan. They may be practical model species for assessing aquatic environmental safety. Therefore, the purpose of this study was to investigate the acute toxic effects of antimony, gallium, and indium on zebrafish and rosy bitterling.

MATERIAL AND METHODS

Zebrafish (*D. rerio*) and rosy bitterling (*R. ocellatus*) were obtained from local suppliers in Taipei, Taiwan. They were transported separately to a 50-l glass aquarium in our laboratory, which was equipped with a water-cycling device; dechlorinated tap water (with a pH of 7.4~8.1, dissolved oxygen (DO) of 7.0~7.7 mg l^{-1}, and hardness of 38~45 $CaCO_3$ mg l^{-1}) was used during the entire experiment. The temperature was maintained at 23.5~25.5°C, with a 12-h light and 12-h dark photoperiod.

Danio rerio (2 weeks old, 0.064 ± 0.018 g in body weight) and *R. ocellatus* (2 weeks old, 0.077 ± 0.015 g in body weight) were used for the tests in the initial experiments. Antimony chloride (III) and gallium sulfate (III) were purchased from Alfa Aesar (Ward Hill, MA). Indium chloride (III) was purchased from Sigma (St. Louis, MO). All metal compounds had purity of 99% or greater. Stock solutions were prepared in deionized water (1000 mg l^{-1} test chemical in 0.1% nitric acid).

Toxicity test methods for *D. rerio and R. ocellatus* were based on guidelines of the Fish Acute Toxicity Test (OECD 1992). Ten animals of similar size were randomly sampled and placed in 10-l glass beakers. After 24 h of acclimatization, the fish were exposed to different Sb (0, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 14.0, 16.0, and 20.0 mg l^{-1}), Ga (0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, and 24.0 mg l^{-1}), and In (0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, and 24.0 mg l^{-1}) concentrations for 96 h or more. The control and each treated group were run in duplicate. During the experiment, dead animal were removed, and the mortality was recorded after 48, 72, and 96 h. LC_{50} values of the three metals and their 95% confidence limits for the two species were calculated using a Basic program from the probit analysis described by

Finney (2009). The data were analyzed with a one-way analysis of variation (ANOVA) to assess differences in toxicity among antimony, gallium, and indium, and used the Tukey's multiple-comparison test to determine the toxicity of individual metals among antimony, gallium, and indium.

RESULTS AND DISCUSSION

This study recorded mortality in the whole exposure duration for *D. rerio* and *R. ocellatus*, exposed to varying concentrations of antimony (Sb), gallium (Ga), and indium (In). No mortality was observed in the control groups during the acute tests. Results demonstrated a positive relationship between the mortality rates of the exposed two fishes (*D. rerio* and *R. ocellatus*) and the concentrations of the three metals (antimony, gallium, and indium) in the test solutions.

In toxicity testing of antimony, no mortality was observed in the group of *D. rerio* exposed to 0.5 mg l^{-1} within 96 h (Figure 1*a*), or in the group of *R. ocellatus* exposed to 2.0 mg l^{-1} for the same duration (Figure 1*b*). The 96-h LC_{50} of *D. rerio* was determined to be 3.80 Sb mg l^{-1}, and the 96-h LC_{50} of

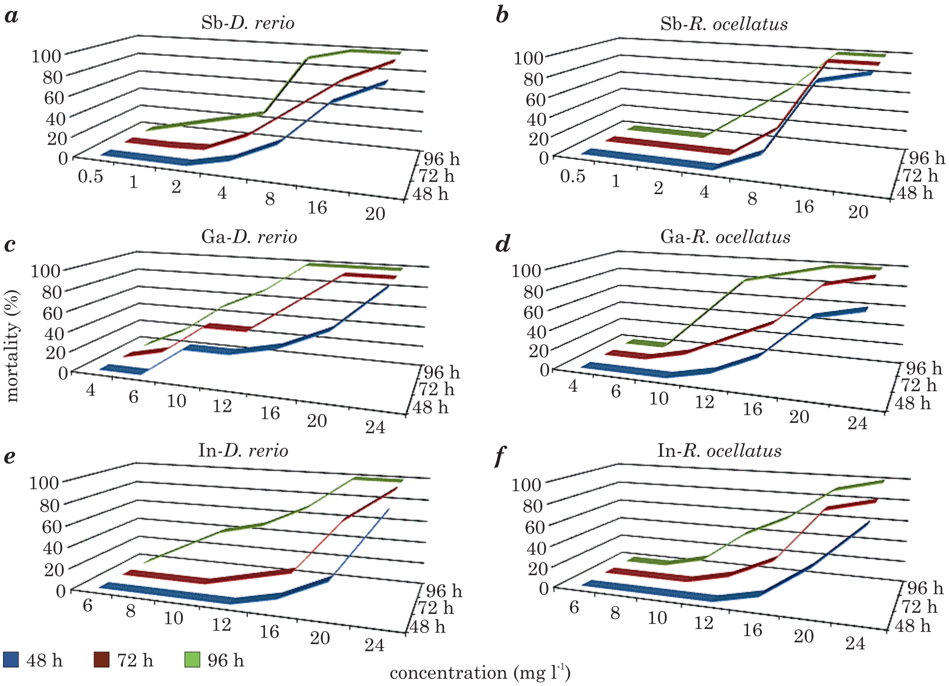

Fig. 1. Lethality curves: *a* – antimony to *Danio rerio*, *b* – antimony to *Rhodeus ocellatus*, *c* – gallium to *D. rerio*, *d* – gallium to *R. ocellatus*, *e* – indium to *Danio rerio*, *f* – indium to *R. ocellatus*

R. ocellatus was determined to be 6.66 Sb mg l^{-1}. In toxicity testing of gallium, no mortality was observed in the group of *D. rerio* exposed to 4.0 mg L^{-1} within 96 h (Figure 1*c*), or in the group of *R. ocellatus* exposed to 6.0 mg l^{-1} for the same duration (Figure 1*d*). The 96-h LC_{50} of *D. rerio* was determined to be 9.10 Ga mg l^{-1}, and the 96-h LC_{50} of *R. ocellatus* was determined to be 10.71 Ga mg l^{-1}. In toxicity testing of indium, no mortality was observed in the group of *D. rerio* exposed to 6.0 mg L^{-1} within 96 h (Figure 1*e*), or the group of *R. ocellatus* exposed to 8.0 mg l^{-1} for the same duration (Figure 1*f*). The 96-h LC_{50} of *D. rerio* was determined to be 15.35 mg l^{-1}, and the 96-h LC_{50} of the *R. ocellatus* was determined to be 13.99 mg l^{-1}. It is clear that the higher the concentration, the shorter the median lethal concentration (96-h LC_{50}) of these metals to *D. rerio* and *R. ocellatus*, as summarized in Table 1. Based on 96-h LC_{50} values, the ranking of the 3 metals from most toxic to least toxic was: antimony > gallium > indium.

As for the results of the acute toxicity tests for antimony performed on *D. rerio* and *R. ocellatus*, this metal was found to be toxic in the concentration range of 2.46~13.57 mg l^{-1} and exhibited stronger toxic effects on *D. rerio*. Regarding the results of the acute toxicity tests for gallium performed on *D. rerio* and *R. ocellatus*, this metal was found to be toxic in the range of 7.25~28.4 mg l^{-1} and exhibited stronger toxic effects on *D. rerio*. With respect

Table 1

Median lethal concentrations (LC_{50}) of antimony, gallium, and indium to zebrafish (*Danio rerio*) and rosy bitterling (*Rhodeus ocellatus*)

Specification	48-h LC_{50} (mg l^{-1})	72-h LC_{50} (mg l^{-1})	96-h LC_{50} (mg l^{-1})
Antimony (III)*			
Danio rerio	10.05 (7.45~13.57)	7.99 (5.34~11.94)	3.80 (2.46~5.87)
Rhodeus ocellatus	11.38 (9.77~13.26)	9.85 (8.23~11.77)	6.66 (4.09~10.85)
Gallium (III)			
Danio rerio	17.70 (11.04~28.40)	12.18 (9.44~15.70)	9.10 (7.25~11.42)
Rhodeus ocellatus	18.02 (15.72~20.66)	14.54 (12.73~16.60)	10.71 (9.47~12.11)
Indium (III)			
Danio rerio	21.84 (19.73~22.53)	18.28 (15.36~21.75)	15.35 (12.84~18.35)
Rhodeus ocellatus	20.62 (18.67~22.78)	17.07 (15.14~19.25)	13.99 (12.28~15.95)

* The 95% confidence limits are given in parentheses.
* Comparisons of the toxicity among antimony, gallium, and indium, according to an ANOVA test ($F_{2,15}$ = 13.7; p < 0.001) indicated a significant difference among these compounds, and the Tukey's multiple-comparison test showed that the toxicity of antimony significantly differed (indicated by an asterisk*) from that of gallium and indium compounds.

to the results of the acute toxicity tests for indium performed on *D. rerio* and *R. ocellatus*, this metal was found to be toxic in the range of 12.28~22.78 mg l^{-1} and exhibited less toxic effects toward *D. rerio*.

Median lethal concentrations of antimony, gallium, and indium as determined by acute toxicity tests performed on aquatic organisms in previous studies are shown in Table 2. These values indicate that the acute toxicity of antimony was higher thand those of gallium and indium towards both fish species, and were in close agreement with results of the present study. *Danio rerio* was found to be more susceptible than the other teleost fish exposed to

Table 2

Median lethal concentrations (LC_{50}) and 95% confidence intervals for antimony, gallium, and indium, as determined by performing acute toxicity tests on aquatic organisms

Species	Exposure time (h)	LC_{50} (mg l^{-1})	Confidence intervals	Reference
Antimony				
Oreochromis mossambicus (L)*	96	18.9		Lin, Hwang (1998*a*)
Pimephales promelas	48	18.8		Kimball (1978)
Daphnia magna	48	21.9		Kimball (1978)
Pargus major		12.4	7.8~25.7	Takayanagi (2001)
Brachydanio rerio (J)*	96	4.65	3.37~6.42	Chen et al. (2007)
Cyprinus carpio (J)	96	14.05	11.09~17.80	Chen, Yang (2007)
Macrobrachium nipponense (A)*	96	6.75	5.73~7.95	Yang et al. (2010)
Macrobrachium nipponense(J)	96	1.96	1.39~2.77	Yang (2014)
Gallium				
Oreochromis mossambicus (L)	96	14.32		Lin, Hwang (1998*b*)
Cyprinus carpio (A)	96	95.6		Betoulle et al. (2002)
Cyprinus carpio (J)	96	19.78	18.49~21.16	Yang, Chen (2003)
Cyprinus carpio (F)	96	12.55	10.82~14.54	Yang, Chen (2003)
Brachionus plicatilis	24	11.48		Onikura et al. (2005)
Americamysis bahia	96		12.76~22.47	Onikura et al. (2005)
Artemia salina	48		52.78~54.64	Onikura et al. (2005)
Macrobrachium nipponense (J)	96	2.77	1.82~4.23	Yang (2014)
Indium				
Oreochromis mossambicus (L)	96	37.6		Lin, Hwang (1998*c*)
Brachionus plicatilis	24	24.42	15.39~38.77	Onikura et al. (2008)
Artemia salina	48	51	45.60~56.90	Onikura et al. (2008)
Americamysis bahia	96	30.48	21.75~42.71	Onikura et al. (2008)
Macrobrachium nipponense (J)	96	6.89	3.65~12.30	Yang (2014)

* A – adult, J – juvenile, L – larva

antimony and gallium. The natural habitats of *R. ocellatus* are frequently polluted by industrial wastewater. Our results suggest that *D. rerio* and *R. ocellatus* may be promising candidates for evaluating water quality/criteria, as they are sensitive to these metals.

Table 2 also shows that the 96-h LC_{50} values of antimony, gallium, and indium were toxic in the concentration range of 1.39~12.3 mg l^{-1} for shrimp (*Macrobrachium nipponense*); this reveals that *M. nipponense* was more sensitive to exposure to these metals than were other species. *Macrobrachium nipponense* is a common freshwater swamp shrimp widely distributed in streams and wetlands throughout the Asia-Pacific region (Yang et al. 2007). *Macrobrachium nipponense* is a dominant species in freshwater environments near semiconductor manufacturing districts in Taiwan, and seems to be a potential invertebrate for evaluating freshwater quality (Yang 2014).

CONCLUSIONS

The toxicity of antimony was higher than that of gallium and indium metal compounds toward both the teleost model (*D. rerio*) and the native fish (*R. ocellatus*). Limited studies have revealed the content and dissemination of gallium and indium in wastewater effluents or aquatic systems. However, manufacturing processes devoted to the fabrication of superlattice based GaInSb system generate large volumes of waste. Although these metals are often referred to as trace elements, each metal produces different problems in water masses, and therefore they have to be considered separately.

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