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Comparative acute toxicity of copper(II), cadmium(II), and gallium(III) on freshwater shrimp(*macrobrachium nipponense*) and reference values for five aquatic organisms

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ABSTRACT

The purposes of this study were to evaluate acute lethal concentrations of copper, cadmium, and gallium on freshwater shrimp (*Macrobrachium nipponense*). The static renewal test method of acute toxicity test was used, and water temperature was maintained at 25.0±0.50C. Data of individual metal obtained from acute toxicity tests were determined using probit analysis method by Finney. The median lethal concentration (96-h LC₅₀) of copper, cadmium, and gallium for *M. nipponense* were estimated as 0.0313, 0.0539, and 2.8266mg/l, respectively. Comparing the tolerance of *M. nipponense* with other species which exposed to these metals from our former acute toxicity tests, it is obviously that the *M. nipponense* is more sensitive than that of various other aquatic animals.

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KEYWORDS

Cu;

Cd;

Ga;

Shrimp;

Macrobrachium nipponense;LC₅₀;

Acute toxicity.

INTRODUCTION

Semiconductor industry has become one of the leading manufacturing in almost every developed country. Copper, cadmium, and gallium are essential transition metals which are widely being used for the manufacture of integrated circuits and electroplating appliances. These metals are released into the environments by manufacturing processes, such as etching, wet polishing, and cleaning operations may be produce many potentially hazardous wastes^[3,4]. Industrial accidental spillages might be lead to high concentration metal com-

pounds in the water and effects on aquatic organisms will be associated with acute and chronic toxic effects. Because the heavy metals are not degraded and accumulate in ecosystems, and toxic effects may be found at the molecular, cellular, histological level even to homeostasis in organisms^[2]. In the last decade, many reports also revealed that these metal compounds potential possesses toxicological, apoptotic, and carcinogenic properties^[27,17,13,8].

Industrial wastewater discharges contain various metallic compounds, toxicity of processes is further complicated by the presence of mixture, and the combined

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effects have to be carried out^[16]. Each metal generates variable pollution issues in freshwater environments, and therefore metals have to be considered separately and in combination as well^[24]. Individual components of heavy metal have been reported by different authors to have varying toxicological effects on aquatic organisms, death of animal have also been reported at various concentrations^[7]. Both acute and chronic toxicity tests were found more detail information to assess environmental stresses, for establishing water quality criteria and in regulating wastewater discharges. However, the results of acute toxicity will provide practical critical values that can be used for establishing tentative water quality criteria of novel toxicants. Fish and shrimp are particularly sensitive to environmental contamination of water, and are recognized as a useful model for detection of water quality^[21]. Freshwater fish can be used in three ways in pollution control, involving three different time frames: (1) the determination of water quality criteria from which standards can be established, (2) monitoring the health of populations of aquatic animals in the field or in a hatchery, (3) providing an early warning system of potential harm to the environment^[1].

Searching for suitable model species has become a need to evaluate the water quality in aquatic environment. Stream fish, *Cyprinus carpio* and *Acrossocheilus paradoxus* are important fish species in local stream. Japanese eel (*Anguilla japonica*), a seawater prawn geographically distributed from Japanese, Taiwan, to southern Pacific Ocean in Far East Asia, was one of important aquacultural species in Taiwan. White shrimp *Litopenaeus vannamei*, a tropical seawater prawn geographically distributed from Sonora, Mexico, to northern Peru in Mid- and Southern America^[9], was one of worldwide important prawn species in aquaculture, not only in North, Central, and South America but also in Asia as an exotic culture species. The aquacultural water used to keep and rear fish in farms always came directly from the coastal water without any other processes. However, the coastal part of the seawater was very easy to be contaminated by many kinds of pollutants, such as chemical residues and human pathogens, and heavy metals^[10]. Especially, these streams and fish farm are near semiconductor manufacturing districts in Taiwan. There were some risks to use the natural coastal water directly in aquaculture, which provided a large part of aquatic products to human beings.

Freshwater shrimp (*Macrobrachium nipponense*) is a common aquatic invertebrate widely distributed in downstream of rivers throughout eastern Asia-Pacific area and constructs a primary connection in the freshwater ecological chain. The purposes of this study were to assess under laboratory conditions the acute lethal toxicity of concentrations of copper, gallium, and cadmium on juvenile *M. nipponense*, to determine individual safety concentrations. We also compiled 96-h LC₅₀ values of copper, cadmium, and gallium to aquatic animals from our laboratory. Search for suitable model species which can be used as a practical species to reveal toxicological information.

EXPERIMENTAL

Freshwater shrimp (*Macrobrachium nipponense*) were obtained from the local commercial suppliers. *M. nipponense* were transported to the glass aquarium in our laboratory which was equipped with a water-cycling device; dechlorinated tap water (pH 7.4-8.1; dissolved oxygen concentration 7.3-7.8mg/l; hardness 38-45 CaCO₃mg/l) was used during the entire experiment. The temperature was maintained at 25.0±0.5°C, and the photoperiod was set at 12h of light and 12h of dark. They were acclimated for 2 weeks and fed aquarium shrimp mixture everyday. Juveniles (4.2±1.7mm in body length) were used in the initial experiments. Copper sulfate (II) and cadmium chloride (II) were purchased from Sigma (St. Louis, MO). Gallium sulfate (III) was purchased from Alfa Aesar (Ward Hill, MA). All metal compounds had a purity of 99% or greater. Stock solutions were prepared in deionized water (1000mg/l test chemical in 0.1% nitric acid).

Laboratory static renewal tests were conducted to determine the median lethal concentration (LC₅₀) for *M. nipponense*. Ten animal of similar size were randomly sampled and placed in 20-l glass beakers. After 24h of acclimatization, *M. nipponense* were exposed to different copper (0, 0.005, 0.01, 0.05, 0.1, and 0.2 mg/l), cadmium (0, 0.005, 0.01, 0.05, 0.1, 0.2 and 0.4 mg/l), and gallium (0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0mg/l) concentrations for 96h or more, respectively. The control and each treated group were run in duplicate. During the experiment, dead animal were removed, and mortality was recorded after 24, 48, 72, and 96h. The LC50 of every test chemicals and their 95% con-

fidence limits for *M.nipponense* were calculated using a Basic program from the probit analysis described by finney^[6].

RESULTS AND DISCUSSION

Median lethal concentrations(48-h and 96-h) of three metals to freshwater shrimp (*Macrobrachium nipponense*) are present in TABLE 1. Results demonstrated that mortality rate of the exposed *M.nipponense* and the concentrations of the testing solution are positively related. It is clear that the higher the concentration, the shorter the LC₅₀ of the *M.nipponense*. Based on LC₅₀ values, the rank order of metals from most toxic to least toxic was: copper, cadmium, gallium.

From the toxicity testing of gallium, no mortality was observed in the group of *M.nipponense* exposed to 2.0mg/l within the first 48 hours, the 96-h LC₅₀ of the *M.nipponense* was determined to be 2.8266mg L⁻¹ with upper and lower limits of 1.9292 and 4.1414mg/l, respectively. Obviously, the toxicity of gallium to *M.nipponense* is less than those of other metal in present study. Nevertheless, there are numerous studies carried out on the gallium compounds for use in semiconductor manufacturing has been accompanied by increasing amounts of toxic materials and harmful to health, such as bone marrow depression, hemorrhagic nephritis, and testicular toxicity in vertebrates^[23,29,22]. As to aquatic animals, Lin and Hwang^[12] showed that the 96-h LC₅₀ of gallium for tilapia larvae(*Oreochromis mossambicus*) was estimated to be 14.32mg/l; revealing *M.nipponense* is more sensitive to gallium exposure than is the tilapia. Betoulle^[26] reported that gallium(III) accumulates in head kidney and blood in the juvenile common carp(*Cyprinus carpio*). Gallium also acts as a hepatotoxicity and causes renal damage in treated *C.carpio*^[18,19].

Compared to copper, higher cadmium dose are needed to obtain 48-hr and 96-h LC₅₀. Copper has a stronger toxicity than cadmium in the present study. Both cadmium and copper are highly toxic for aquatic organism; effects of short and long term of exposure to two metals are already reported in considerable studies^[7,11,25,5]. Liver and kidney of Japanese eel (*Anguilla japonica*) are of high bioaccumulative affinity towards cadmium ion^[15]. Karan et al.^[28] reported significant changes in metabolic enzymes in gill, liver, and blood of

TABLE 1: Median lethal concentrations(LC₅₀)of copper, cadmium, and gallium to freshwater shrimp(*Macrobrachium nipponense*)

	LC ₅₀ (mg/l)	
	48 h	96h
Copper(II)	0.0959(0.0686-0.1343)	0.0313 (0.0174-0.0562)
Cadmium(II)	0.1120(0.0824-1.1523)	0.0539 (0.0284-0.1021)
Gallium(III)	7.0307(4.6778-10.5671)	2.8266 (1.9292-4.1414)

The 95% confidence limits are given in parentheses

TABLE 2 : 96-h LC₅₀ values(mg/l) of copper, cadmium, and gallium to aquatic organism

species	Cu (II)	Cd (II)	Ga (III)	citation
<i>Cyprinus carpio</i> (j)			19.78	Yang and Chen 2003b
<i>Cyprinus carpio</i> (f)			12.55	Yang and Chen 2003b
<i>Macrobrachium nipponense</i> (j)			2.827	This study
<i>Anguilla japonica</i> (l)		3.77		Yang 1995
<i>Litopenaeus vannamei</i> (l)		1.07		Wu and Chen 2004
<i>Acrosscheilus paradoxus</i> (l)		0.29		Yuan 1994
<i>Macrobrachium nipponense</i> (j)		0.054		This study
<i>Anguilla japonica</i> (l)	0.31			Yang 1995
<i>Macrobrachium nipponense</i> (j)	0.031			This study
<i>Acrosscheilus paradoxus</i> (l)	0.026			Yuan 1994

Life stages of the test animals (f: fry; l: larva; j: juvenile)

C.carpio exposed to sublethal concentration of copper(96-h LC₅₀ value: 0.64mg/l). Acute lethal effects of these metals were attributed to excess mucous covering gill tissues and lead to the breakdown of respiratory function.

There have some difficulties to compare our 96-h LC₅₀ values with other published papers because the physical parameters and the life stages of the test animals were different. We have compiled 96-h LC₅₀ values of gallium, cadmium, and copper to aquatic animals from our laboratory(TABLE 2). Our results are in good agreement with the reports using fish and shrimp species; i.e. the acute 96-h LC₅₀ values are in the mg/l range. Comparing the tolerance of *M.nipponense* jenvile with those of other species investigated such as *C.carpio* jenvile and fry, adult zebrafish(*Brachydanio rerio*), freshwater fish larvae(*Acrosscheilus paradoxus*), white

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shrimp larvae (*Litopenaeus vannamei*), and Japanese eel larvae (*Anguilla japonica*) exposed to same metals, it is clear that the *M. nipponense* is more sensitive than that of other species. For example, the 96-h LC₅₀ value of cadmium for white shrimp, *L. vannamei*, was estimated to be 1.07 mg/l^[20], indicating that the shrimp species are more tolerant to cadmium exposure than is the *M. nipponense*. Even though considerable species might be used as bioindicators, the selection of species and tests depend abundance on the location and purpose of the assessment. It is a practice to use most sensitive species in such test so that even a minute stress of environment is detected. And larger aquatic vertebrates are more tolerant to invertebrate in similar laboratory conditions. *M. nipponense* can be considered as suitable species when its susceptibility to such metals is compared with other aquatic animals.

CONCLUSIONS

The regulatory approach pertaining to different activities on heavy metals does exist in an industrial country like Taiwan, however upon screening the existing data base on surveillance studies in high risk population which mainly involve the aquatic fauna and flora. The natural habitats of *M. nipponense* are also frequently polluted by industrial input of wastewater. To maintain the balance of the local river ecosystems in such water it is essential to know its susceptibility to these pollutants. And we found the fact that *M. nipponense* seems to be a promising candidate for the evaluation of freshwater quality as their sensitive to these metal toxicants.

REFERENCES

- [1] A.G.Heath; 'Water pollution and fish physiology', CRC Press; Boca Raton, New York, (1987).
- [2] A.L.Jr.Buikema, B.R.Niederlehner, J.Cairns Jr.; Water Res., **16**, 239-262 (1982).
- [3] A.L.Robinson; Science, **210**, 275-277 (1983).
- [4] C.F.Chelton, M.Glowatz, J.A.Mosovsky; IEEE Trans.Educ., **34**, 269-288 (1991).
- [5] C.K.C.Wong, M.H.Wong; Aqua.Toxicol., **48**, 517-527 (2000).
- [6] D.J.Finney; 'Probit Analysis', Cambridge University Press; London, (1971).
- [7] D.M.Woltering; Aqua.Toxicol., **5**, 1-21 (1984).
- [8] D.P.Marisa, A.R.Parrish; Mechan.Muta., **533**, 227-241 (2003).
- [9] F.Paez-Osuna, C.Ruiz-Fernandez; Environ.Pollu., **87**, 243-247 (1995).
- [10] F.Paez-Osuna, L.Tron-Mayen; Environ.Intl., **22**, 443-450 (1996).
- [11] G.De Boeck, A.Vlaeminck, R.Blust; Arch.Environ. Contam.Toxicol., **33**, 415-422 (1997).
- [12] H.C.Lin, P.P.Hwang; Bull.Environ.Contam.Toxicol., **60**, 931-935 (1998).
- [13] H.Huang, S.C.Shu, J.H.Shih, C.J.Kuo, I.D.Chiu; Toxicology, **129**, 113-123 (1998).
- [14] H.N.Yang; Acute Toxicity and Chronic Effects of Copper, Cadmium, and Zinc on the Japanese Eel (*Anguilla japonica*), PhD dissertation, National Taiwan University, Taiwan, (1995).
- [15] H.N.Yang, H.C.Chen; Bull.Environ.Contam.Toxicol., **56**, 670-676 (1996).
- [16] J.A.Sturgill, J.T.Swartzbaugh, P.M.Randall; Clean Prod.Proc., **2**, 18-27 (2000).
- [17] J.Bustamante, D.Lennart, V.Marie, F.Bruce, O.Sten; Toxicology, **118**, 129-136 (1997).
- [18] J.L.Yang, H.C.Chen; Zool.Stud., **42**, 455-461 (2003a).
- [19] J.L.Yang, H.C.Chen; Bull.Environ.Contam.Toxicol., **71**, 240-247 (2003b).
- [20] J.P.Wu, H.C.Chen; Chemosphere, **57**, 1591-1598 (2004).
- [21] J.Nemcsok, I.Benedeczky; 'Environmental and Ecological Biochemistry', Elsevier, Amsterdam, (1995).
- [22] M.Omura, A.Tanaka, M.Hirata, M.Zhao, Y.Makita, N.Inoue, K.Gotoh, N.Ishinishi; Fundamen.Appl. Toxicol., **2**, 13-26 (1996).
- [23] R.A.Newman, A.R.Brody, I.H.Krakoff; Cancer, **44**, 1728- 1740 (1979).
- [24] R.Lloyd; 'Pollution and Freshwater Fish', Blackwell, London, (1992).
- [25] R.N.Singhal, M.Jain; Bull.Environ.Contam.Toxicol., **58**, 456-462 (1997).
- [26] S.Betoulle; J.Toxicol.Environ.Health., **A65**, 603-615 (2002).
- [27] U.H.Riaz, J.P.Wereley, C.R.Chitambar; Exp. Hematol., **23**, 428-432 (1995).
- [28] V.Karan, S.Vitorvic, V.Tutundzic, V.Poleksic; Ecotoxicol.Environ.Safety, **40**, 49-55 (1998).
- [29] Y.Aoki, M.M.Lipsky, B.A.Fowler; Toxicol.Appl. Pharmacol., **106**, 462-468 (1990).
- [30] Y.G.Yuan; The Study of Using *Acrossocheilus Paradoxus* to be An Indicator of Contaminated River, PhD dissertation, National Taiwan University, Taiwan. (1994).