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Acute Toxicity of Heavy Metals, Tributyltin, Ammonia and Polycyclic Aromatic Hydrocarbons to Benthic Amphipod *Grandidierella japonica*

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Abstract – Benthic amphipod, *Grandidierella japonica* widely inhabits the Korean coastal waters and is developed as a standard test species for sediment toxicity tests. We exposed *G. japonica* to various pollutants including 4 kinds of inorganic metals (Ag, Cd, Cu and Hg), tributyltin [TBT], ammonia and 7 polycyclic aromatic hydrocarbon (PAH) compounds (acenaphthene, chrysene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene) to estimate the no observed effect concentration (NOEC) and the median lethal concentration (LC50) of each pollutant during the 96-hour acute exposure. Among all tested pollutants, TBT was most toxic to *G. japonica*, and Hg was most toxic among inorganic metals. The toxicity of pyrene to *G. japonica* was greatest among PAH compounds, followed by fluoranthene, phenanathrene, acenaphthene, fluorene and naphthalene. The toxicity of PAH compounds was closely related to their physico-chemical characteristics such as K_{ow} and water solubility. *G. japonica* responded adequately to pollutant concentrations and exposure durations, and the sensitivity of *G. japonica* to various inorganic and organic pollutants was generally comparable to other amphipods used as standard test species in ecotoxicological studies, indicating this species can be applied in the assessment of environments polluted by various harmful substances.

Key words – *Grandidierella japonica*, amphipod bioassay, heavy metals, TBT, PAHs, acute

1. Introduction

The contamination of aquatic environments with various chemicals such as heavy metals, organotins, and polycyclic aromatic hydrocarbons (PAHs) has been a serious problem because of their persistence and toxicity to most aquatic organisms. Chemical analysis was often adopted in the monitoring of contamination levels of pollutants in various environments. However, chemical analysis alone may not provide adequate information concerning the biological effects of most pollutants (Long *et al.* 1990).

In order to overcome the inadequacy of the chemical analysis, various bioassays have been developed for decades because bioassays can assess the adverse biological effects of chemicals and also quantitatively measure the toxicity exerted by pollutants in environmental media on organisms. Consequently, bioassays as well as chemical analysis have been widely used in the biomonitoring and also in the integrative assessment of environmental quality in developed countries.

Recently, there has been a substantial increase in research and regulatory activity to assess the pollution of coastal environments in Korea. However, only a few studies concerned with the toxicity of pollutants are available due to the lack of available testing protocols and test species in Korea (Lee *et al.* 2005). Therefore, it is necessary to develop bioassay testing protocols and indigenous test organisms for the quantitative assessment of pollutant effects on aquatic organisms.

Grandidierella japonica is one of the candidate test species for sediment bioassays in Korea. It is indigenous amphipod species that inhabit tidal and subtidal sediments along the West and South coasts of Korea (Kim 1991). They are also found along the Pacific coastal areas located around Japan, Australia and North America (Moore 1986; Nipper 1989). *G. japonica* was suggested as a test species for sediment toxicity tests in the USA, because it could tolerate a variety of sediment types (sands, silts, or clays) and other environmental conditions such as temperature and salinity, and also it showed the relevant sensitivity to the reference toxicant, Cd and some field contaminated sediments (Nipper 1989; Kohn *et al.* 1994). *G. japonica*

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was included as a test species in a standard guide published by ASTM (American Society for Testing and Materials), which contains the procedures of acute sediment and water-only toxicity tests using amphipods as well as some important ecological and toxicological attributes of test species including *Ampelisca abdita*, *Eohaustorius estuarius*, *Rhephoxius abronius*, *Leptocheirus plumulosus* and *G. japonica* (ASTM 1999).

The sensitivity of test species to specific pollutants should be determined to identify which pollutant is more responsible for the observed toxic response of test organisms exposed to pollutant mixtures as well as to construct the environmental quality criteria of each chemical to protect the species. However, the sensitivity or responsiveness of *G. japonica* to many important pollutants is not systematically evaluated. Some previous studies determined the acute toxicity of pollutants such as Cd, ammonia and fluoranthene to *G. japonica* (Kwon *et al.* 1994; Boese *et al.* 1997), while the toxicity of other various pollutants to this species remained unknown.

In the present study, *G. japonica* was exposed to concentrations of various inorganic and organic chemicals known as important pollutants (such as Cd, Cu, Hg, ammonia, tributyl-tin and PAH compounds) in coastal environments, to compare its sensitivity to other test species and also to evaluate whether it showed the relevant responsiveness to the pollutants under varying pollutant concentrations and exposure durations.

2. Materials and Methods

The amphipod *Grandidierella japonica* was collected from a mudflat located in Yeongjong Island, Korea (37°29'N, 126°24'E). Upon returning to the laboratory, the amphipod was maintained in the aerated seawater (30 psu) at 20°C, and fed diatom *Phaeodactylum tricornutum* and ground fish meal (TetraMin®). After the 1-wk acclimation period, healthy individuals of *G. japonica* were exposed to 5 or 6 different concentrations of Ag, Cd, Cu, Hg, tributyltin (TBT), ammonia and 7 PAH compounds (acenaphthene, chrysene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene) for 96 h. Spiked media was prepared followed by ASTM (1999). Briefly, spiked test media was made by dissolving the adequate amount of stock solution for each chemical in 0.45-μm of filtered seawater. Deionized water was used as a solvent for the stock solution of inorganic metals and ammonia, and acetone was used for TBT and PAHs. Chemical analysis (n=3) revealed that the measured concentrations of all stock solutions for test chemicals were within the 10% variation for nominal concentrations; therefore, nominal concentrations were used to estimate the effect concentration of test chemicals. Since ammonia concentration can vary after dissolving stock solution, concentration of ammonia in each spiked media was confirmed by ammonia-specific electrodes before conducting the toxicity test. The measured concentration of ammonia in each media was >80% of the nominal level. However, the measurement of other chemicals in spiked media could not be performed due to logistical limitations.

Individuals of *G. japonica* were exposed for 96 hours to 0.05, 0.1, 0.25, 0.5 and 1.0 mg/L of Ag, 0.5, 1.0, 2.5, 5 and 10 mg/L of Cd, 0.1, 0.25, 0.5, 1.0 and 2.5 mg/L of Cu, 0.01, 0.025, 0.05, 0.1 and 0.2 mg/L of Hg, 1.0, 2.5, 5, 10 and 20 μg/L of TBT, 10, 25, 50, 100 and 200 mg/L of total ammonia, 0.2, 0.5, 1.0, 2.0 and 4.0 mg/L of acenaphthene, 0.1, 0.2, 0.5, 1 and 2 μg/L of chrysene, 0.025, 0.05, 0.1, 0.15 and 0.25 mg/L of fluoranthene, 0.1, 0.2, 0.5, 1.0 and 2.0 mg/L of fluorene, 0.05, 0.1, 0.25, 0.5 and 1.0 mg/L of phenanthrene, 1, 2.5, 5, 10 and 20 mg/L of naphthalene, and 0.01, 0.025, 0.05, 0.1 and 0.135 mg/L of pyrene in 30-psu seawater. The highest concentration of Ag, Cu and PAHs and was decided by considering the water solubility limits in seawater media.

Uncontaminated water was used as a negative control factor, which was included in each batch of toxicity tests and a solvent control (acetone 0.2%) was included to evaluate the effect of acetone in acetone-based stock solutions added to the highest concentrations of chemicals. All survival rates in controls were >90% in the present study.

Three replicate beakers and thirty individuals were allocated to each treatment. Test seawater was exchanged everyday following the observation of dead individuals. Test media were exchanged everyday and water quality of test seawater was checked at the beginning and end of water exchange. Salinity and temperature were maintained at 30 psu and 20°C, respectively, and dissolved oxygen concentrations were always over 80%.

Dunnett's test or t-test with Bonferroni adjustment was conducted to compare means of control and treatments and median lethal concentrations (LC50s) were estimated using Probit analysis or the trimmed Spearman-Karber method according to the established standard procedure (USEPA 1994). Unionized ammonia concentration was calculated using an equation from USEPA (1999).

3. Results and Discussion

Toxicity of metals, TBT and ammonia

Survival rates of *G. japonica* generally decreased with exposure duration and concentration of test chemicals (Fig. 1). *G. japonica* showed significantly reduced survival rates when exposed to >0.5 mg/L of Ag, >1 mg/L of Cd and Cu, >0.025 mg/L of Hg, >2.5 μg/L of TBT, and >100 mg/L of total ammonia for 96 h. The NOECs of Ag, Cd, Cu, Hg, TBT and total ammonia were 0.25, 0.5, 0.5, 0.01, 0.001 and 50 mg/L, respectively (Table 1). Temporal

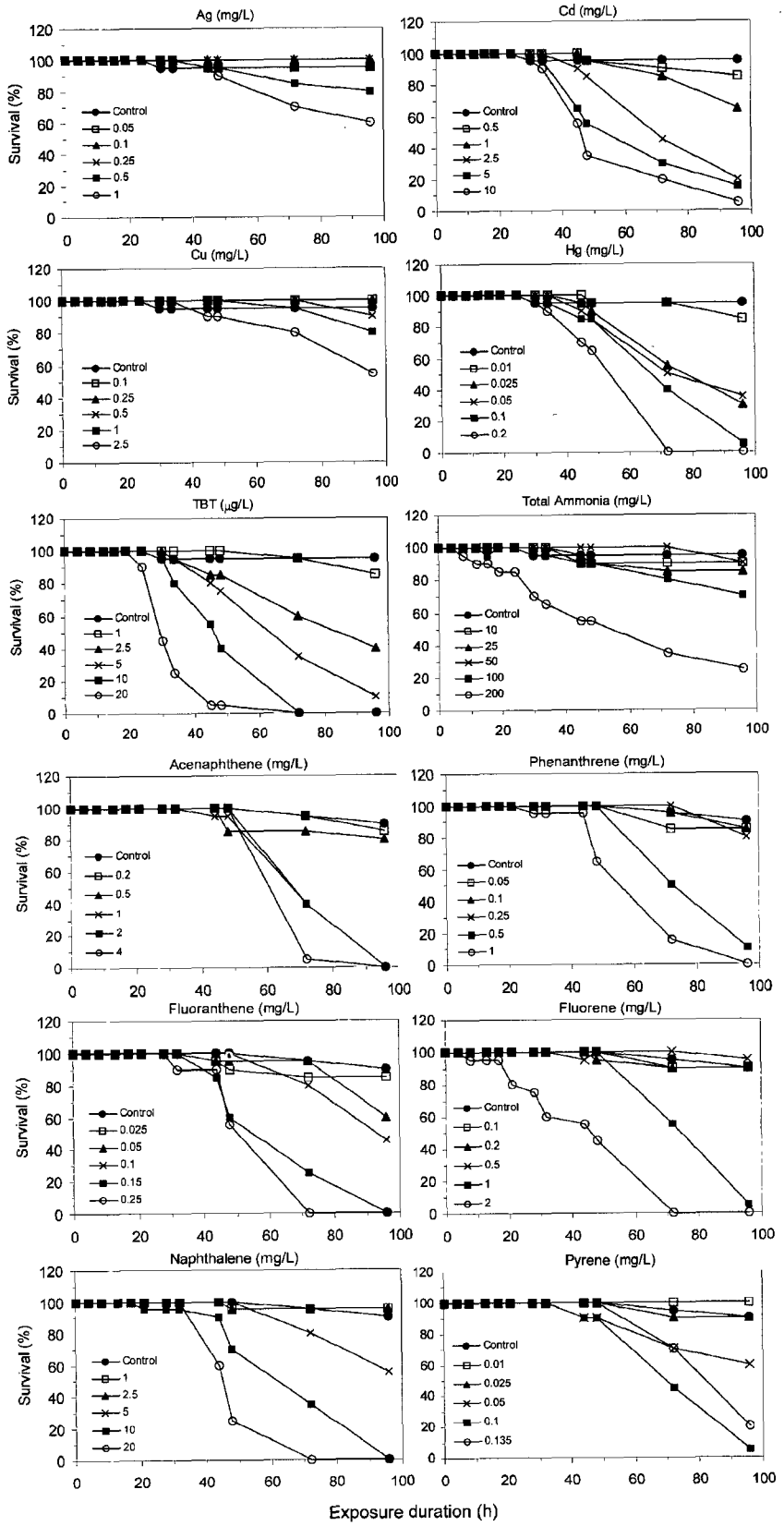

Fig. 1. Temporal change of survival rates of *Grandidierella japonica* to various concentratwns exposed Ag, Cd, Cu, Hg, TBT, ammonia and PAH compounds including acenaphthene, phenanthrene, fluoranthene, fluorene, naphthalene and pyrene for 96 hours.

Table 1. The 96-h LC50s and 95% confidence interval (CI) of various pollutants for *Grandidierella japonica* in the present study.

Chemical	Unit	NOEC	LOEC	96-LC50	96-LC50 CI	
					LL[1]	UL[2]
Ag	mg/L	0.25	0.5	>1.0		
Cd	mg/L	0.5	1.0	1.47	0.54	3.97
Cu	mg/L	0.5	1.0	>2.5		
Hg	mg/L	0.01	0.025	0.024	0.0095	0.062
TBT	μg/L	1.0	2.5	2.18	1.00	4.75
Total ammonia	mg/L	50	100	141.4	74.2	269.4
acenaphthene	mg/L	0.5	1.0	0.66	0.43	1.02
chrysene	μg/L	>2.0	>2.0	>2.0		
fluoranthene	μg/L	25	50	74.1	37.2	147.7
fluorene	mg/L	0.5	1.0	0.73	0.59	0.92
naphthalene	mg/L	2.5	5.0	5.3	3.3	8.6
phenanthrene	mg/L	0.25	0.5	0.35	0.20	0.59
pyrene	μg/L	25	50	58.2	28.8	117.6

[1]lower limit, [2]upper limit

Table 2. Comparison of 96-h LC50s of Cd, Hg, tributyl-tin (TBT), total ammonia (T-NH_3) and various PAH compounds including Acenaphthene (ANT), Fluoranthene (FRT), Fluorene (FLR), Naphthalene (NPT), Phenanthrene (PNT) and Pyrene (PYR) for various marine and estuarine amphipods obtained from the literature and this study.

Species	Cd	Hg	TBT	T-NH_3	ANT	FRT	FLR	NPT	PNT	PYR
Ampelisca abdita	0.2-0.58[a], 0.33[b], 0.63[c], 0.94[c], 1.3[c]	-[1]	-	50[c], 100[d]	-	0.067[e]	-	-	-	-
Eohaustorius estuarius	7.4[f], 9.3[b], 12.5[g]	-	0.002[h]	126[c]	0.71[i]	-	-	-	0.16[i]	-
Leptocheirus plumulosus	0.36-1.88[j], 1.06[b], 1.45[g]	-	-	44[k], 89[k]	0.68[l]	-	-	-	0.18[l]	-
Rhepoxinus abronius	0.53[c], 0.76[f], 0.92[b], 1.5[g], 1.9[c]	-	0.014[h], 0.032[h]	79[c]	-	0.011[m], 0.023[m]	-	-	-	-
Grandidierella japonica	0.34[g], 1.17[b], 3.14[c]	-	-	148[c]	-	0.036[g]	-	-	-	-
Monocorophium acherusicum[2]	0.7-1.4	0.026	0.0095	155	-	-	-	-	0.23	-
Grandidierella japonica (This study)	1.47	0.024	0.0020	141	0.66	0.074	0.73	5.3	0.35	0.058

[1]no available data, [2]all LC50 data for *M. acherusicum* were cited from Lee *et al.* (2005) [References] [a]Redmond *et al.* (1994), [b]ASTM (1999), [c]Kohn *et al.* (1994), [d]Ho *et al.* (1999), [e]Spehar *et al.* (1999), [f]DeWitt (1989), [g]Boese *et al.* (1997), [h]Meador (1993), [i]Swartz *et al.* (1995), [j]McGee *et al.* (1998), [k]Moore *et al.* (1997), [l]DeWitt *et al.* (1992), [m]Swartz *et al.* (1990)

changes in survival rates of *G. japonica* exposed to various concentrations of these chemicals during the 96-h exposure period was shown in Fig. 1.

The 96-h LC50 was lowest for TBT, and followed by Hg, Cd and Cu among inorganic and organic metals (Table 1). The LC50 for TBT was 10–1000 times lower than the inorganic metals. The severe toxicity of TBT may be related to the different uptake pathways of organometals, which can directly penetrate the lipid membrane, while the passage of inorganic metals is usually limited by the capacity of ion channels on the membrane. The sensitivity of *G. japonica* to TBT was very comparable with *Eohaustorius estuarius*, of which 96-h LC50 was 2 μg/L, but the LC50 of TBT for *G. japonica* was 5–10 times lower than that of *Monocorophium acherusicum* and *Rhepoxinus abronius* (Table 2). The LC50s of Ag and Cu could not be determined since the highest concentration levels of both metals adopted in this study was close to the solubility of these metals in seawater (Philips 1980; Irwin *et al.* 1998).

Among inorganic metals, Hg was most toxic to *G. japonica*. The well-known toxicity of Hg is probably related to its strong binding affinity to sulfur, which is an important constituent for most enzymes. The sensitivity of *G. japonica* to Hg was very comparable to that of *M. acherusicum*. Unfortunately, no LC50 data of Hg and Cu was available from literature for other benthic amphipod species.

The toxicity of Cd to amphipods has been most extensively tested among pollutants (Table 2). The 96-h LC50 of Cd for *G. japonica* in the present study lies within the range of the LC50s for the same species studied in the literature, which varied in the range from 0.3–3 mg/L (Kohn *et al.* 1994; Boese *et al.* 1997; ASTM 1999). The most Cd LC50s for *A. abdita* were lower than those for other amphipods, and the sensitivity of *G.*

japonica to Cd was generally comparable to *R. abronius*, *L. plumulosus* and *M. acherusicum*. However, *Eohaustorius estuarius* (7–13 mg/L) and *Corophium volutator* (2–14 mg/L) were more tolerable to Cd than other benthic amphipods (DeWitt 1989; Boese *et al.* 1997; ASTM 1999; Kater *et al.* 2001).

The 96-h LC50 of total and unionized ammonia for *G. japonica* in the present study was 141 and 3.5 mg/L, respectively. Results showed that *G. japonica* was relatively tolerable to ammonia when compared to other amphipods including *A. abdita*, *R. abronius*, *L. plumulosus* and *E. estuarius* (Table 2). Only *M. acherusicum* (155 mg/L) had higher LC50 of total ammonia than *G. japonica*. Although ammonia is the important pollutant in benthic environments, it is usually attributed to being a confounding factor for most sediment bioassays, which is aimed at assessing the toxicity of conservative pollutants such as inorganic and organic metals, chlorinated hydrocarbons, alkyl-phenols, and so on (Burton 1992; Ankley *et al.* 1994; U.S.EPA 1994). Therefore, relatively high tolerance of *G. japonica* to ammonia can be advantageous as test species in sediment bioassay.

Toxicity of PAH compounds

G. japonica showed significantly reduced survival rates when exposed to >1 mg/L of acenaphthene, >0.05 mg/L of fluoranthene, >1 mg/L of fluorene, >5 mg/L of naphthalene, >0.5 mg/L of phenanthrene and >0.05 mg/L of pyrene for 96 h. The NOECs of acenaphthene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene were 0.5, 0.025, 0.5, 2.5, 0.25 and 0.025, respectively (Table 1). No significant mortality of *G. japonica* was observed for all concentrations of chrysene during the 96-h exposure period. Temporal variation of survival rates of *G. japonica* exposed to various concentrations of these compounds during the 96-h exposure period was shown in Fig. 1.

The LC50 was lowest for pyrene (0.058 mg/L), followed by fluoranthene (0.074 mg/L), phenanthrene (0.35 mg/L), acenaphthene (0.66 mg/L), fluorene (0.73 mg/L) and naphthalene (5.3 mg/L). The toxicity of PAHs was well explained by the log K_{ow} (octanol-water partitioning coefficient) of each compound, which is highest for fluoranthene (5.1), and followed by pyrene (4.9), phenanthrene (4.5), fluorene (4.2), acenaphthene (4.0) and naphthalene (3.5). The correlation coefficient (r) between log K_{ow} and log LC50 was very high (0.973), suggesting a close relationship between physicochemical property and the biological activity of PAHs. The relationship of LC50 (mM) of *G. japonica* and K_{ow} can be described below:

$$\log [LC50\ (mM)] = -1.3 \times \log [K_{ow}] + 3.1$$

of which the slope (–1.3) is steeper than the universal narcosis slope (–0.95) suggested by Di Toro *et al.* (2001). Similarly, the slope of the relationship between log-transformed LC50 of 5 PAH compounds for puffer fish *Takifugu obscurus* and log K_{ow} was –1.6, which is much steeper than the general narcosis slope (Lee *et al.* 2004). These results suggest that the toxicity of PAH compounds may not be explained by the general narcosis model alone. There can be other important modes of toxic action especially for PAH compounds with higher K_{ow} or where the degree of narcotic toxicity may not be linearly related to K_{ow}. Therefore, QSAR for PAHs based on a simple narcosis model should be adequately modified.

The LC50s of acenaphthene, fluoranthene and phenanthrene for *G. japonica* in the present study were generally comparable or slightly higher compared to those studied in the literature (Table 2). The LC50s of acenaphthene for *G. japonica* (0.66 mg/L) in the present study were very comparable with those of *E. estuarius* (0.71 mg/L) and *L. plumulosus* (0.68 mg/L) (DeWitt *et al.* 1992; Swartz *et al.* 1995).

Boese *et al.* (1997) reported the LC50 of fluoranthene for *G. japonica* was 36 mg/L, which was about a half of the LC50 value obtained in the present study. This intra-species variation might be due to the genetic variation or different experimental conditions such as body size of test individuals as well as seasonal changes. The more than 2-fold variation was often found in toxicity test results from studies even when using test individuals collected from the same site. For example, Cd LC50 of *C. volutator* collected from a site varied ~10 times depending on body size, sex and season (Kater *et al.* 2001).

The LC50s of fluoranthene for *G. japonica* in both studies were generally comparable to those for *A. abdita* and *R. abronius* studied in the literature (Table 2). *G. japonica* seemed to be slightly less sensitive to phenanthrene than other amphipods including *E. estuarius*, *L. plumulosus* and *M. acherusicum*; however, the difference of LC50 was within a factor of 2. Similarly, the LC50 of naphthalene for amphipod *Elasmopus pectenicrus* (2.8 mg/L) was also slightly lower than that for *G. japonica* (Irwin *et al.* 1998). Unfortunately, no previous LC50 results for fluorene and pyrene for amphipod species was available in the literature.

Our results showed that the survival of *G. japonica* significantly responded to the pollutant concentration and exposure duration, and also showed that the sensitivity of *G. japonica* to various pollutants including inorganic metals, organotin, ammonia and PAHs was relevant when compared to other standard amphipod test species. In terms of sensitivity to various water-born chemicals having a variety of toxic mechanisms, *G. japonica* can be used to assess the adverse biological effects of pollutants in aquatic environments. Test species used in sediment bioassays should respond to pollutants associated with both the dissolved and particulate phase. Since *G. japonica* is a

candidate test species for sediment bioassays, further studies would be needed to determine the sensitivity of *G. japonica* to particle-associated pollutants to evaluate the adequateness of this species in sediment bioassays.

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