Bull. Environ. Contam. Toxicol. (2004) 72:632–638

DOI: 10.1007/s00128-004-0290-7

Environmental Contamination and Toxicology

Acute Toxicity of Bunker C Refined Oil to the Japanese Littleneck Clam *Ruditapes philippinarum* (Bivalvia: Veneridae)

K. Ara, D. Aoike, J. Hiromi, N. Uchida

Department of Marine Science and Resources, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-8510, Japan

Received: 31 March 2003/Accepted: 4 December 2003

Marine pollution caused by oil spills is deadly to many aquatic organisms and their habitats. Once an oil spill happens in the sea and the oil reaches the coastal region, the intertidal zone (e.g. rocky shore, tidal flat) and shallow, estuarine and neritic waters are more susceptible to oil pollution, due to the specific gravity of oil being greater than that of seawater. In these areas, oil permeates the sandy and muddy bottom and sediment, and it remains there for a long time.

The Japanese littleneck clam *Ruditapes philippinarum* (Adams and Reeve) is widely distributed in the sandy bottom, from the intertidal zone to ca. 10 m deep, in Japan, Taiwan, the Korean Peninsula and China (Okutani 2000), and is an important fishery resource in these countries. In shallow-water environments, this clam plays an important role, purifying seawater by effective filter-feeding of suspended organic and inorganic particles down to 1 μm in size (Nakamura 2001), while regenerating dissolved inorganic nutrients (N, P) by excretion (Magni et al. 2000). Although it is quite important to examine the toxic effects of oil on *R. philippinarum*, until now there has been no study available on it. For toxicity testing (bioassay) of oil, it is more realistic and important to examine the toxicity of oil with dispersant rather than oil only (without dispersant), because dispersant makes oil soluble in seawater and enhances the toxicity of oil and because some dispersants are more toxic than oil. Actually, in many cases, dispersants have been scattered in order to hasten the dilution and dispersion of oil in seawater, and they will be used more for future oil spills in the sea. In the present study, we present the acute toxicity, expressed as the LC_{50} (the lethal concentration to 50% of the test organisms), highest non-lethal concentration and lowest LC_{100}, of Bunker C refined oil with dispersant to *R. philippinarum*.

MATERIALS AND METHODS

Ruditapes philippinarum was collected by hand from the sandy sediments at the intertidal zone of Nojima Sandy Beach Park (35°19'N, 139°38'E), Yokohama, Kanagawa. Collected clams were introduced into a bucket containing surface water and sand from an oil-uncontaminated sampling spot and taken to the laboratory. These clams were acclimated in a tank (volume: 20 liters) containing 16 liters of surface water (salinity: 25 psu) and sand at the bottom (ca. 3 cm thick)

Correspondence to: K. Ara

for 3 days, prior to use, in an incubator (temperature: 20±1°C) with aeration.

Although methods used for the acute toxicity test were mostly based on Ara et al. (2002), an outline is briefly given as follows. Seawater (salinity: 25 psu) filtered through a glass-fiber filter (Whatman GF/F), Bunker C refined oil and dispersant "*D-1128*" (Taiho Tech. Co., Ltd.) were mixed at the volume ratio of 50:5:1. This mixture was sufficiently shaken for 4 hrs at 200 cycles/min (amplitude: 20 mm) on a shaker platform (Heidolph Promax 2020), and was left for 20 hrs to separate the aqueous phase from the oil. The aqueous phase was diluted to 0.016–50% with filtered seawater, as the test solution. Filtered seawater was prepared as the control.

Two individuals of *R. philippinarum* (shell length: ca. 22 mm) were introduced into each lidded glass bottle (volume: 240 ml, covered with aluminum foil to keep in the dark), containing 140 ml of test solution and previously 0.25 mm-sieved and autoclaved (120°C in temperature; 2 atmosphere, 20 minutes) sand at the bottom. The experiment, with six replicates and one control on each concentration of test solution, was run for 96 hrs in an incubator (temperature: 20±1°C). The clams were not fed during the experiment. Test solutions were renewed every 48 hrs, although the oil (hydrocarbon) concentration of the test solution did not decrease considerably during the first 24–96 hrs (Ara et al. 2002). After exposure, the survival of *R. philippinarum* in each bottle was checked: non-reactive individuals were regarded as dead, when touched on their soft tissue. Additionally, clams whose valves were easily opened by cutting ligament using a fine knife were regarded as dead. The oil (hydrocarbon) concentration of the test solutions was determined by the IGOSS method (Hydrographic Department 1998): hydrocarbon from the test solutions was extracted by adding *n*-hexane, and fluorescent intensity of the solutions was measured with excitation at 315 nm and emission at 360 nm by a JASCO Spectro-fluorometer FP-777.

Regression analysis was done to determine the relationships between the survival rate of *R. philippinarum* and hydrocarbon concentration of the test solutions and between acute toxicity of Bunker C refined oil and exposure time. These relationships were converted to linearized equations and solved by the least-squares method.

RESULTS AND DISCUSSION

All clams in control bottles were always alive during the experiment. At each exposure time (i.e. 24, 48, 72 and 96 hrs), the survival rate (*SR*) of *Ruditapes philippinarum* decreased with increasing hydrocarbon concentration (*HC*) (Fig. 1), and the relationships were expressed by:

A: $SR = -48.54 \times \ln HC + 267.25$ ($r^2=0.843$, $p<0.01$),
B: $SR = -25.18 \times \ln HC + 127.3$ ($r^2=0.892$, $p<0.001$),
C: $SR = -38.98 \times \ln HC + 97.09$ ($r^2=0.874$, $p<0.001$), and
D: $SR = -51.19 \times \ln HC + 83.12$ ($r^2=0.891$, $p<0.001$).

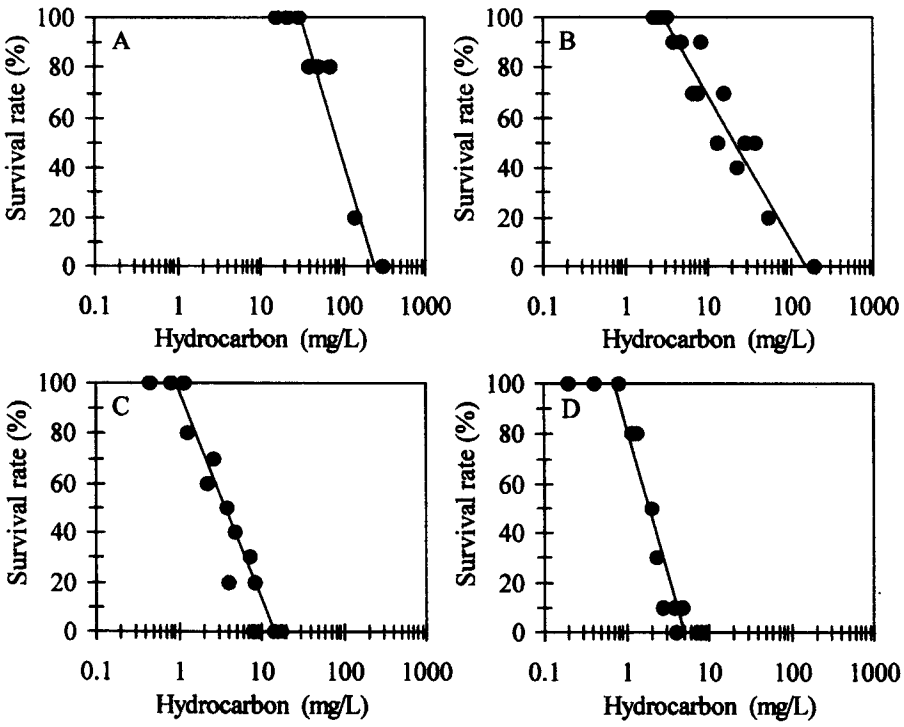

Figure 1. Relationships between the survival rate of *Ruditapes philippinarum* and hydrocarbon concentration of Bunker C refined oil. A: 24 hrs; B: 48 hrs; C: 72 hrs; D: 96 hrs.

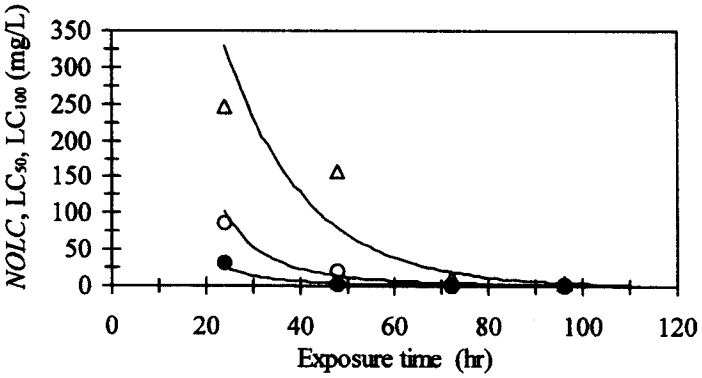

Figure 2. Relationships between acute toxicity of Bunker C refined oil and exposure time. Closed circle: the highest non-lethal concentration (*NOLC*); open circle: LC_{50}; open triangle: the lowest LC_{100}.

From the obtained regression equations of hydrocarbon concentration-survival rate relationships, the LC_{50} value was calculated to be 87.8, 21.6, 3.3 and 1.9 mg/L for 24, 48, 72 and 96 hrs, respectively. The estimated values of the highest non-lethal concentration (*NOLC*), LC_{50} and lowest LC_{100} decreased exponentially with lengthening exposure time (*T*) (Fig. 2), and the relationships were expressed by:

$$NOLC = 2.17\times10^{5}\ T^{-2.827}\ (r^2=0.973, p<0.05),$$
$$LC_{50} = 9.77\times10^{5}\ T^{-2.881}\ (r^2=0.969, p<0.05), \text{ and}$$
$$LC_{100} = 1.37\times10^{3}\ e^{-0.05927T}\ (r^2=0.929, p<0.05).$$

Acute toxicity studies have generally measured LC_{50} values at 48 and/or 96 hrs. These are calculated from the survival rates of a test organism in relation to the concentrations of a chemical substance. However, for evaluating the essential acute toxic effects of the chemical substance to the test organism, it is necessary to estimate the highest *NOLC* and lowest LC_{100} values in addition to the LC_{50} value. Additionally, the present study showed that the values of the highest *NOLC*, LC_{50} and lowest LC_{100} declined considerably with lengthening exposure time (Figs 1, 2). From the obtained regression equations of the highest *NOLC*-, LC_{50}- and lowest LC_{100}-exposure time relationships, the time-dependent acute toxic effects of Bunker C refined oil on the survival of *R. philippinarum* can be estimated, although these have not been determined in most acute toxicity studies.

Acute toxicity tests of Bunker C oil have been done for some marine invertebrates (Table 1). It is difficult to compare strictly the LC_{50} values obtained in the present study with those in other studies, because of differences in method for determining hydrocarbon concentration, loss of hydrocarbons through volatilization and degradation, and test solutions (water soluble fraction) with or without dispersant. As mentioned above, we employed the IGOSS method, which is recommended for determining petroleum hydrocarbon concentration in toxicity tests (J. Koyama, Kagoshima University, personal communication), because this method determines principally polycyclic aromatic hydrocarbons that consist of very toxic components (e.g. naphtalene, benzopyrene). By this method, the calibration curves, i.e. the linear regression equations of fluorescent intensity-hydrocarbon concentration (range: 0–1000 mg/L) relationships, using chrysene as the standard substance of oil, were highly significant (coefficients of correlation: >0.99). We confirmed, preliminarily, that the toxic effects of the dispersant "*D-1128*" were very low and ignorable, because the dispersant is nonionic and ethylene-glycolic and because small amount of the dispersant (volume ratio: <0.1% of test solution) was utilized in the present study. Nonethless, judging from the LC_{50} values obtained, the 96hr-LC_{50} value for a polychaete, *Perinereis brevicirris*, was much higher than that obtained for other animals. One note on the acute toxicity of Bunker C oil to marine organisms was the greater tolerance of *R. philippinarum* during the 24–48hr exposure periods than other animals, although the 72hr- and 96hr-LC_{50} values obtained for these animals were similar to each other (Table 1). This might be due to the characteristic feature of *R. philippinarum*, which is adapted to disturbed habitat conditions, because this clam is widely distributed in intertidal and shallow waters where environmental conditions are considerably variable due to the influence of tidal cycle and freshwater inflow.

Table 1. The LC_{50} values (mg/L) of Bunker C oil to marine invertebrates. WSF: water soluble fraction without dispersant; DWSF: water soluble fraction with dispersant; *T*: exposure time (hr); ♀: adult female; ♂: adult male; *SS*: shell size.

Test animal (life stage/size)	Oil type	*T* (hr)	LC_{50}	Reference
Mysid				
Mysidopsis almyra	*WSF	24	2.0	1
	*WSF	48	1.8	1
Grass shrimp				
Palaemonetes pugio	*WSF	24	1.4	1
	*WSF	48	1.4	1
	*WSF	96	1.4	1
Brown shrimp				
Penaeus aztecus (postlarvae)	*WSF	24	1.3	1
	*WSF	48	1.2	1
	*WSF	96	1.9	1
Hippolytid shrimp				
Heptacarpus futilirostris	**DWSF	48	17.2	3
	**DWSF	96	13.4	3
Copepoda				
Tigriopus japonicus (?)	**DWSF	96	0.041–0.86	3
Tigriopus japonicus (♀)	**DWSF	96	12.84	4
	**DWSF	96	2.52***	4
Tigriopus japonicus (♂)	**DWSF	96	<0.69	4
Polychaeta				
Perinereis brevicirris	**DWSF	96	>123	3
Quahog clam				
Mercenaria sp. (embryo)	*WSF	48	1.0	2
Mercenaria sp. (larvae)	*WSF	48	3.2	2
	*WSF	144	1.8	2
	*WSF	240	1.6	2
Japanese littleneck clam				
Ruditapes philippinarum	**DWSF	24	87.8	5
(*SS*: 22 mm)	**DWSF	48	21.6	5
	**DWSF	72	3.3****	5
	**DWSF	96	1.9****	5

* Venezuelan Bunker C residual oil; ** Bunker C refined oil taken from the Russian tanker "*Nakhodka*" in Japan Sea; *** in test solutions renewed every 24 hrs; **** in test solutions renewed every 48 hrs; 1: Anderson et al. (1974); 2: Byrne and Calder (1977); 3: Koyama et al. (1998); 4: Ara et al. (2002); 5: this study.

This clam can resist and be alive for 2–3 days by strongly closing their shells under serious conditions, e.g. exposure to air, extremely low salinity and chemical

pollutants (T. Okutani, personal communication). On the basis of laboratory experimentation, Okubo and Okubo (1965) observed that *R. philippinarum* (formerly named "*Venerupis japonica*") continued to close their shells for a maximum duration of 72–96 hrs under low-salinity conditions.

Petroleum hydrocarbon concentrations in estuarine and coastal waters of Japan have reached 115 μg/L (usually ca. 1–10 μg/L) (Hydrographic Department 1998), which can be assumed the "safety range" (non-lethal concentration) for *R. philippinarum*, because of the concentration being 10-to-1000-fold lower than its LC_{50} values (Howarth 1989). However, much higher concentrations (ca. 3–10 mg/L) of hydrocarbon of Bunker C oil have been observed in coastal waters of Japan, when a large amount of oil has been spilled (e.g. Ochi and Okaichi 1979; Yamaguchi 1997). These concentrations are actually within a range that has acute toxic effects on the survival of *R. philippinarum*. Larval and/or early life stages of organisms are generally more sensitive and much less tolerant to environmental stress than adult and/or later stages, although the toxic effects of Bunker C refined oil on the *R. philippinarum* larvae were not measured in the present study. Thus, petroleum hydrocarbon concentrations in natural shallow waters of Japan can be serious regionally and temporally for the survival of *R. philippinarum*. The catch of *R. philippinarum* in Japan, especially in Tokyo, Ise and Mikawa Bays, has decreased gradually from the 1960s (Sasaki 1999). The toxic effects of spilled oil can be one of the principal reasons for death and/or decrease in the *R. philippinarum* population, as well as, e.g. rapid rise and/or decline in water temperature, low salinity, ground-level movement by wave, deficiency in dissolved oxygen and food (Kakino 1996). In natural environments, even if there is no serious toxic effects of oil on the survival of *R. philippinarum*, the clams are easily oil-polluted by exposure to low oil concentration. In consequence, they can have an oil-taste and/or oil-smell, which damage the *R. philippinarum* fishery, due to the loss of its commercial value.

Acknowledgments. We thank Professor Jiro Koyama (Kagoshima University), Dr. Kazuyoshi Hashizume (National Institute for Environmental Studies) and Dr. Takashi Okutani for technical advice and valuable comments.

REFERENCES

Anderson JW, Neff JM, Cox BA, Tatem HE, Hightower GM (1974) Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar Biol 27:75–88

Ara K, Nojima K, Hiromi J (2002) Acute toxicity of Bunker A and C refined oils to the marine harpacticoid copepod *Tigriopus japonicus* Mori. Bull Environ Contam Toxicol 69:104–110

Bryne CJ, Calder JA (1977) Effect of the water-soluble fractions of crude, refined and waste oils on the embryonic and larval stages of the quahog clam *Mercenaria* sp. Mar Biol 40:225–231

Howarth RW (1989) Determining the ecological effects of oil pollution in marine ecosystems. In: Levin SA, Harwell MA, Kelly JR, Kimball KD (eds)

Ecotoxicology: Problems and Approaches. Springer-Verlag, New York, p 69–97

Hydrographic Department (1998) Report of Marine Pollution Surveys. No. 24. Marine Safety Agency of Japan, Tokyo

Kakino J (1996) 2. Fishery tendency and recent research on Japanese littleneck clam in Tokyo Bay. Bull Japan Soc Fish Oceanog 60:265–268

Koyama J, Kakuno A, Okumura Y, Ikeda K, Shimizu A, Yamada H (1998) Toxicity of spilt crude oils to marine organisms. Kaiyo Monthly 30:622–630

Magni P, Montani S, Takeda C, Tsutsumi H (2000) Temporal scaling and relevance of bivalve nutrient excretion on a tidal flat of the Seto Inland Sea, Japan. Mar Ecol Prog Ser 198:139–155

Nakamura Y (2001) Filtration rates of the Manila clam, *Ruditapes philippinarum*: dependence on prey items including bacteria and picocyanobacteria. J Exp Mar Biol Ecol 266:181–192

Ochi T, Okaichi T (1979) Drift of oil residues in sea water of Bisan Seto after oil spill accident in Mizushima, as assessed by fluorescence spectrophotometry. Tech Bull Fac Agr Kagawa Univ 30:157–162

Okubo K, Okubo T (1965) Influence of diluted sea water on the physiological activity of baby-neck clam, *Venerupis japonica*, and the toxic effect of a herbicide PCB, pentachlorophenate. Bull Tokai Reg Fish Res Lab 44:31–40

Okutani T (2000) Marine Mollusks in Japan. The Tokai University Press, Tokyo, 1173 p

Sasaki K (1999) Material circulation and production in estuary and tidal flat–29. Tidal flat and fishery resources 3. Japanese littleneck clam of Buzen Sea. Aquabiology 21:61–66

Yamaguchi H (1997) Outflow of crude oil in Japan Sea. J Japan Soc Civil Eng 82:28–34