Bull. Environ. Contam. Toxicol. (2002) 69:104–110

DOI: 10.1007/s00128-002-0015-8

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity of Bunker A and C Refined Oils to the Marine Harpacticoid Copepod *Tigriopus japonicus* Mori

K. Ara, K. Nojima, J. Hiromi

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

Received: 31 December 2001/Accepted: 1 March 2002

Spilt oil from ships, naval accidents and establishments on shore is provably the most dramatic marine pollution, and is one of catastrophic disasters that happened frequently in aquatic environments. For evaluating the toxic effects of oils on marine ecosystem when a significant amount of oils is spilt in the sea, it is necessary to examine experimentally the degree of toxic impact of oils on marine organisms. A lot of extensive studies have been performed to examine the toxic effects of crude, refined and fuel oils and petroleum products on marine organisms such as commercially important fishes, crabs and shellfishes (e.g. Capuzzo 1987; Koyama et al. 1998). However, there is yet little information on the toxic effects of oils on small marine organisms such as copepods, that are important prey items for various aquatic animals. In the present study, we present here the acute toxicity (LC_{50}: the concentration resulting 50% of the exposed organisms being died) of Banker A and C refined oils on the demersal harpacticoid copepod *Tigriopus japonicus,* which is widely distributed along the coast of Japan, attaining dense populations in tide pools.

MATERIALS AND METHODS

Tigriopus japonicus was collected using a hand-net (mesh size: 100 μm) at tide pools situated in Enoshima Island (35°16'N, 139°29'E), Fujisawa, Kanagawa. They were transferred into a bottle filled with surface seawater and taken to the laboratory. These copepods were acclimated at least for 7 days prior to experiment under laboratory condition (temperature: 23±1 °C; salinity: 34 psu). The raphidophycean flagellate *Heterosigma akashiwo* was fed prior to experiment.

Seawater (salinity: 34 psu) filtered through a glass-fiber filter (Whatman GF/F),

Correspondence to: K. Ara

Banker A or C refined oils and dispersant "*D-1128*" (Taiho Tech., Ltd.) were mixed at the volume ratio of 9:1:0.2. This mixture was shaken for 5 min at 100 cycles min^{-1} on a shaker platform, and was left for 24 hrs to separate aqueous layer from the oils. The aqueous layer diluted to 0.01–6.25% with filtered seawater was prepared as test solution, and the filtered seawater was also prepared as control. Twenty ovigerous females or adult males of *T. japonicus* were introduced into each glass tube (volume: 50 ml) containing 20 ml of test solution, and these tubes were covered to keep dark for preventing the decomposition of dissolved hydrocarbon by ultraviolet. On each concentration of test solution, triplicates were incubated for 96 hrs at temperature of 23 ± 1°C. To compare the effects of hydrocarbon concentration changing with time on the survival rate of ovigerous females, we conducted experiments in unrenewed test solutions and in ones renewed every 24 hrs. After the exposure, the survival of the copepods in each tube was checked under a microscope. Oil (dissolved hydrocarbon) concentration of the test solutions was determined by the IGOSS method (Hydrographic Department 1998), which extracts with *n*-hexan and then determines fluorometrically, using chrysene as the standard substance of oil.

RESULTS AND DISCUSSION

Control survival was always 100% for ovigerous females and adult males. For ovigerous females, the survival rate, no-effect and lethal concentrations of hydrocarbon of Bunker A and C refined oils in test solutions renewed every 24 hrs were much lower than those in unrenewed test solutions (Figs 1, 2). In any case, the survival rates of ovigerous females decreased with increasing hydrocarbon concentration. There were significant correlations between survival rates of ovigerous females and hydrocarbon concentration of Bunker A and B, respectively (Figs 1, 2). From obtained regression equations of hydrocarbon concentration-survival rate relationships, the 96-hr LC_{50} values of Bunker A and C were calculated to be 0.95 and 12.84 ppm in unrenewed test solutions, whereas the values were 0.12 and 2.52 ppm in test solutions renewed every 24 hrs, respectively. All adult males died at extremely low concentrations in unrenewed test solutions; 0.06 and 0.69 ppm of Bunker A and C, respectively.

For ovigerous females and adult males of *Tigriopus japonicus*, the acute toxicity of Bunker A was much stronger than that of Bunker C, as generally known on the

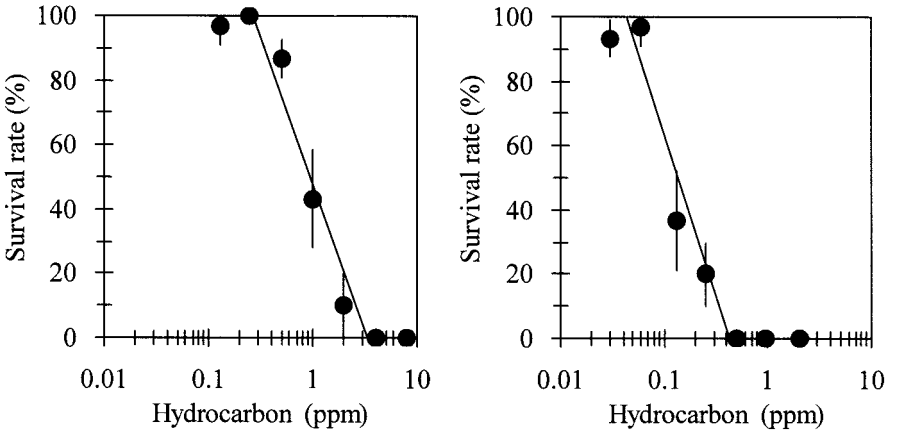

Figure 1. Relationships between survival rate (*SR*) of *Tigriopus japonicus* adult females and hydrocarbon concentration (*HC*) of Bunker A refined oil. Survival rate is expressed as mean (●) and SD (vertical bars). Left: in unrenewed test solution (SR=-39.88×lnHc+48.02, r^2=0.960, p<0.001); right: in test solutions renewed every 24 h (SR=-37.51×lnHc-28.98, r^2=0.918, p<0.05).

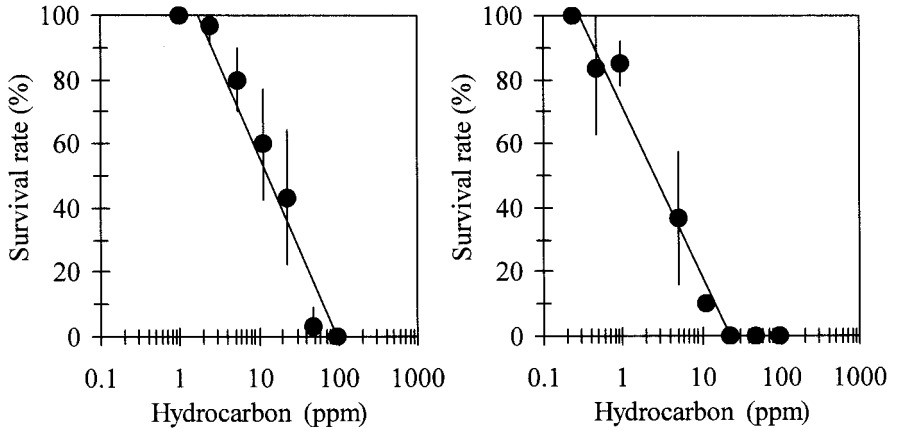

Figure 2. Relationships between survival rate (*SR*) of *Tigriopus japonicus* adult females and hydrocarbon concentration (*HC*) of Bunker C refined oil. Survival rate is expressed as mean (●) and SD (vertical bars). Left: in unrenewed test solution (SR=-24.52×lnHc+112.59, r^2=0.940, p<0.001); right: in test solutions renewed every 24 h (SR=-22.72×lnHc+70.98, r^2=0.974, p<0.001).

basis of toxicity tests for the prawn *Penaeus japonicus* Bate (Iwata, Shintani and Deshimaru, unpublished data). The 96-hr LC_{50} values of Bunker A and C refined oils to *T. japonicus* ovigerous females in unrenewed test solutions were ca. 7- and 5-fold higher than those in test solutions renewed every 24 hrs, respectively. As similarly observed by Anderson et al. (1974), this might be due to a significant loss in hydrocarbon concentration with time: ca. 9 and 36% of initial hydrocarbon concentrations of Bunker A decreased during the first 24 and 96 hrs, respectively. However, no large decline in hydrocarbon concentration was found for Bunker C.

The dispersant "*D-1128*" used in the present study was a nonionic and ethylene-glycolic. On the basis of the acute toxicity test of "*D-1128*" to marine fish, Koyama (unpublished data) regarded it as a dispersant whose toxicity is very low. In the present study, the "*D-1128*" concentration in test solutions was very low (volume ratio: $\leqq$0.1% of test solution). Therefore, we assured that the acute toxicity of the dispersant utilized in the present study would be very low and ignorable in comparison with that of Bunker A and C refined oils, although the toxicity of "*D-1128*" to *T. japonicus* was not tested. The dispersant can reduce a loss of hydrocarbon concentration rather than test solution (WSF) without dispersant since the concentration of oil dispersed in test solutions can increase due to adding a dispersant (Koyama et al. 1998).

There are some acute toxicity tests of Bunker A and C oils to estuarine and coastal crustaceans (Table 1). The LC_{50} values of Bunker C refined oil to *T. japonicus* obtained in the present study are similar to those obtained by Koyama et al. (1998), in which the LC_{50} values were estimated from the exposure experiment, checking the survival of *T. japonicus* every 24 h during the 96-h period, in 1 and 10% of water-soluble fraction with dispersant, corresponding to 0.041 and 0.86 ppm, respectively. For analyzing hydrocarbon concentration of oils, Anderson et al. (1974) employed the infrared spectrometry that measures absorbed band due to CH-linkage, and this method is useful to determine the total hydrocarbons including alkanes, which are regarded as weakly toxic to organisms. On the other hand, the IGOSS method employed in the present study determines principally polycyclic aromatic hydrocarbons that consist of strongly toxic components such as naphtalene and benzopyrene, and is recommendable for determining hydrocarbon concentration in toxicity tests of oils to organisms (Koyama, Kagoshima University, personal communication). Therefore, *T. japonicus* adult

Table 1. The LC_{50} values (ppm) of Bunker A and C oils to marine crustaceans. WSF: water-soluble fraction without dispersant; DWSF: water-soluble fraction with dispersant; *T*: exposure time (h); ♀: adult females; ♂: adult males

Test animal (life stage)	Oil type	*T*	LC_{50}	Reference
Mysid				
Mysidopsis almyra	C* WSF	24	2.0	1
	C* WSF	48	1.8	1
Grass shrimp				
Palaemonetes pugio	C* WSF	24	1.4	1
	C* WSF	48	1.4	1
	C* WSF	96	1.4	1
Brown shrimp				
Penaeus aztecus	C* WSF	24	1.3	1
(postlarvae)	C* WSF	48	1.2	1
	C* WSF	96	1.9	1
Copepoda				
Tigriopus japonicus (?)	C** DWSF	96	0.041–0.86	2
Tigriopus japonicus (♀)	A DWSF	96	0.95	3
	A DWSF	96	0.14***	3
	C DWSF	96	12.84	3
	C DWSF	96	2.52***	3
Tigriopus japonicus (♂)	A DWSF	96	<0.06	3
	C DWSF	96	<0.69	3

* Venezuelan Bunker C residual oil; ** Bunker C refined oil spilt from the Russian tanker "*Nakhodka*" in Japan Sea; *** in test solutions renewed every 24 hrs; 1: Anderson et al. (1974); 2: Koyama et al. (1998); 3: this study.

females represented higher tolerance to Bunker C oil than other organisms examined by Anderson et al. (1974). This can be explained by the difference of their habitats: *T. japonicus* is widely distributed in coastal waters, commonly occurring much densely in tide pools where hydrographic conditions (i.e. water temperature and salinity) are extremely serious, whereas the other organisms inhabit epipelagic and/or intertidal areas in coastal waters where environmental conditions are dependent mainly on the tidal phase.

Although the 96-hr LC_{50} values of Bunker A and C refined oils to adult males were not specified, their toxic susceptibility to oils was at least ca. 16–19-fold higher than ovigerous females. This can be explained by less tolerance to environmental stress of males than females (Davis 1984). This implies that *T.*

japonicus would be much less tolerant to oil pollution and cannot maintain its population because of inability of the reproduction due to lack of males.

Oil concentrations less than 10-to-1000-fold lower than the LC_{50} values are assumed to be a "safety range" that there would be no toxic effects to organisms (Department of Interior 1985; Howarth 1989). Oil (hydrocarbon) concentrations in principal estuarine and coastal waters of Japan have amounted to 115 ppb (usually ca. 1–10 ppb) (Hydrographic Department 1998). These concentrations are actually within a range that can reveal acute toxic effects on survival of *T. japonicus* adult males. The present study showed that the concentration of oils spilt and dispersed in seawater can be one of important factors affecting the survival, reproduction and population dynamics of *T. japonicus* in natural environments. Consequently, for evaluating the toxic effects of oils to organisms, this suggests strongly the necessity of chronic toxicity tests of oils at concentrations much lower than the LC_{50} values in addition to the acute toxicity tests.

Acknowledgments. The authors would like to thank Dr. Jiro Koyama (Kagoshima University) and Dr. Naoyuki Uchida (Nihon University) 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

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

Capuzzo JM (1987) Biological effects of petroleum hydrocarbons: assessments from experimental results. In: Boesch DF, Rabalais NN (eds) Long-term Environmental Effects of Offshore Oil and Gas Development. Elesevier, London, p 343–410

Davis CC (1984) Planktonic copepods (including Monstrilloida). In: Steidinger KA, Waker LM (eds) Marine Plankton Life Cycle Strategies. Boca Raton, Florida, p 67–91

Department of Interior (1985) Final Environmental Impact Statement, North Aleutian Basin Lease Sale #92, Bureau of Land Management (cited in Howarth 1989)

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

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