Acute Toxicity Responses of Two Crustaceans, *Americamysis bahia* and *Daphnia magna*, to Endocrine Disrupters

Masashi Hirano,[a] Hiroshi Ishibashi,[a] Naomi Matsumura,[a] Yukiko Nagao,[a] Naoko Watanabe,[a] Akiko Watanabe,[b] Norio Onikura,[b] Katsuyuki Kishi,[b] and Koji Arizono*,[a]

[a]*Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, 3–1–100 Tsukide, Kumamoto 862–8502, Japan and* [b]*Japan Pulp and Paper Research Institute Inc., 5–13–11 Tokodai, Tsukuba, Ibaraki 300–26, Japan*

(Received September 12, 2003; Accepted September 17, 2003; Published online October 7, 2003)

The acute toxicity of endocrine disrupters in two crustaceans, *Americamysis bahia* (*A. bahia*) and *Daphnia magna* (*D. magna*), was investigated and the toxicological responses compared. Bisphenol A had the lowest toxicity to *D. magna*, the 48 hr median lethal concentrations (LC_{50}) values was 12.8 mg/l. However, the toxic sensitivity of *A. bahia* to bisphenol A was approximately 10-fold higher than for *D. magna* (48 hr LC_{50}; 1.34 mg/l). The 48 hr LC_{50} of estradiol-17β was 2.97 and 1.69 mg/l in *D. magna* and *A. bahia*, respectively. Nonylphenol had the highest lethal toxicity against both *A. bahia* (48 hr LC_{50}; 0.051 mg/l) and *D. magna* (48 hr LC_{50}; 0.18 mg/l). However, ecdysteroids, and ecdysteroidal activity insecticides, such as tebufenozide, and juvenile hormone analog, had no toxic effects against either *A. bahia* or *D. magna* at the concentrations tested in this study. These results suggest that *A. bahia* is a more suitable toxicity test organism for endocrine disrupters than *D. magna* because of the higher sensitivity of *A. bahia* to the toxicological effect.

Key words —— *Americamysis bahia*, *Daphnia magna*, endocrine disrupter, acute toxicity

INTRODUCTION

Recently, a number of global studies of endocrine disrupters (EDs) have reported interactions with the development and functioning of endocrine systems in animals and humans.[1,2] Many of these chemicals may also adversely affect the reproductive health of aquatic organisms. Therefore, various screening and testing systems for EDs have been established in the OECD (Organization for Economic Cooperation and Development) and U.S. EPA (Environmental Protection Agency).[3,4]

Under laboratory culture, the mysid shrimp *Americamysis bahia* (*A. bahia*), reaches sexual maturity in 12 to 20 days, depending on water temperature and diet.[5] Normally, the female will have eggs in the ovary at approximately 12 days of age. The short life span of *A. bahia*, ease of culture and sensitivity to toxic substances has meant they have been widely used in toxicity testing.[6,7] However, most studies have focused on the affects of heavy metals or agriculture chemicals, and little is known about the relative toxic effects of EDs on *A. bahia*. As invertebrates are present in about 90% of the ecosystem, evaluation of the adverse effects on invertebrates of ED exposure is important, in order to develop systems to test the health of any given environment.

We evaluated and compared the acute toxicity of various EDs to the crustaceans *A. bahia* and *Daphnia magna* (*D. magna*), which are widely used in aquatic toxicology. The lethality of the EDs was used as the endpoint in an aquatic acute toxicity testing system. In general, determination of lethal concentrations, such as the median lethal concentration (LC_{50}), is recognized as the first step for risk assessment of synthetic and natural chemicals. We also discuss the utility of this toxicity testing system.

MATERIALS AND METHODS

Test Chemicals —— Estradiol-17β, ponasterone A, and ecdysone-α and -β were obtained from Sigma Chemical Industries, Ltd. (St. Louis, MO, U.S.A.). Nonylphenol was obtained from Aldrich Chemical Company Inc. (Tokyo, Japan). Bisphenol A and

*To whom correspondence should be addressed: Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, 3–1–100 Tsukide, Kumamoto 862–8502, Japan. Tel.: +81-96-383-2929 (ext. 486); Fax: +81-96-383-6062; E-mail: arizono@pu-kumamoto.ac.jp

Table 1. Effects of Endocrine Disrupters on Viability of *A. bahia* and *D. magna*

Chemical	*D. magna*	*A. bahia*	
	48 hr-LC_{50} (mg/l)	48 hr-LC_{50} (mg/l)	96 hr-LC_{50} (mg/l)
Estradiol-17β	2.97 (2.76–4.11)	1.69 (1.37–2.25)	0.89 (0.71–1.13)
Bisphenol A	12.8 (11.57–14.37)	1.34 (1.09–1.67)	1.03 (0.83–1.33)
Nonylphenol	0.18 (0.15–0.22)	0.051 (0.042–0.063)	0.045 (0.037–0.057)
Methoprene	1.04 (0.89–1.18)	0.30 (0.25–0.35)	0.23 (0.21–0.26)
Ecdysone-α	> 1	> 1	> 1
Ecdysone-β	> 1	> 1	> 1
Tebufenozide	> 10	> 10	> 10
Ponasterone A	> 5	> 5	> 5

Values in parenthesis mean 95% confidence limits.

tebufenozide were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Methoprene was obtained from Labor Dr. Ehrenstorfer, Augsburg, Germany. All chemical compounds were dissolved in dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries) in order to prepare the test solutions.

Acute Toxicity Test ——

Americamysis bahia: Mysids (Chesapeake Cultures, Inc., Virginia, U.S.A.) were originally obtained from the Japan Pulp and Paper Research Institute, Inc. (Tsukuba, Japan), and a breeding stock of these mysids has been maintained at our laboratory. The culture originated at the U.S. EPA, Gulf Breeze, Florida, U.S.A. An acute toxicity test was performed according to a procedure of the U.S. EPA using 24-hr-old larvae.[8] *A. bahia* were tested in 300 ml glass beakers containing 200 ml test solution. The salinity of the test solution was 25‰. Testing was performed in a climate chamber at 25 ± 1°C and with a 16 : 8 hr light : dark photoperiod. All chemicals were dissolved in DMSO as the carrier solvent. In addition, we established a control group maintained in artificial seawater only and also a solvent control group maintained in 0.1% DMSO for the duration of the test. The test solution in each chamber was renewed daily during the experimental period. *A. bahia* were fed twice daily with *Artemia* nauplii (< 24 hr after hatching) at approximately 100 nauplii per mysid. The criteria for death were no visible movement and no visible response to stimuli with a dropper. Acute effects are reported as LC_{50} values calculated by probit analysis in daily observations on mortality for the exposure period (*i.e.*, 48 hr and 96 hr LC_{50}).

Daphnia magna: Daphtoxkit F magna (Creasel BVBA; Deinze, Belgium), were also subjected to the acute toxicity assay. The acute immobilization tests were performed using a commercial test kit according to the manufacturer's instructions, which adheres fully to OECD Guideline 202. The 24 hr and 48 hr LC_{50} for the test chemicals were determined based on the immobilization of *D. magna*.

RESULTS AND DISCUSSION

There is growing concern that EDs, such as estradiol-17β, nonylphenol and bisphenol A, may affect several physiological systems, development, growth and reproduction of all organisms in the ecosystem. In this study, we assessed and compared the acute toxicity of these EDs against the two crustaceans, *A. bahia* and *D. magna*.

Control survival was ≥ 90% for both bioassays, and remained within the guidelines established by the U.S. EPA. The 48 hr and/or 96 hr LC_{50} of the chemicals to *A. bahia* and *D. magna* are summarized in Table 1. The 48 hr LC_{50} values of ecdysone-α and -β were > 1 mg/l in *D. magna* and *A. bahia*, respectively, tebufenozide and ponasterone A 48 hr LC_{50} values were > 10 and 5 mg/l, respectively, while the juvenile hormone analog (JHA) methoprene, 48 hr LC_{50} was 0.3 mg/l in *A. bahia*. On the other hand, in *D. magna*, the 48 hr LC_{50} of methoprene was 1.04 mg/l. These results suggest that ecdysteroids, ecdysteroidal activity insecticides and JHA analog were not toxic against *A. bahia* and *D. magna* at the concentrations tested in this study. However, Baldwin *et al.* demonstrated that 20-hydroxyecdysone elicited no significant effect on molting frequency in *D. magna*, and its significant effects on reproduction were only at concentrations (260 nM) associated with premature death caused by incomplete ecdysis. Ponasterone A also elicited

effects similar to those of 20-hydroxyecdysone at approximately 10-fold lower concentrations.[9] Therefore, in the future, the effects of ecdysteroids, ecdysteroidal activity insecticide and JHA on the molting and/or reproduction of *A. bahia* must be assessed. Olmstead and LeBlanc reported that the insecticidal JHA methoprene mimics the action of the crustacean juvenoid hormone methyl farnesoate, resulting in inappropriate production of male offspring in *D. magna*.[10,11] They also demonstrated that methoprene had significant toxicity against endocrine-related processes in the 5–50 nM (1.6–16 μg/l) concentration range, and that molting and reproduction were affected at significantly lower methoprene concentrations, with a distinct concentration response and a threshold of < 0.2 nM (62.1 ng/l).[10,11] Those results indicated that the JHA methoprene has the potential to interfere with metabolism or clearance of the hormone by competitively binding to the enzyme or active transporters that modulate activity or levels of the hormone. In the present study, ecdysteroids, ecdysteroidal activity insecticide and JHA did not have extensive toxic effects. Although these chemicals have the potential for cause adverse effects during chronic exposure tests, other endpoints, such as growth, reproduction or molting, are required in order to estimate the toxicological and/or endocrine disrupting effects on *A. bahia* in the future.

Bisphenol A had the lowest toxicity to *D. magna*, the 48 hr LC_{50} values was 12.8 mg/l. However, the toxic sensitivity of *A. bahia* to bisphenol A was approximately 10-fold higher than for *D. magna* (48 hr LC_{50}; 1.34 mg/l). The 48 hr LC_{50} of estradiol-17β was 2.97 and 1.69 mg/l in *D. magna* and *A. bahia*, respectively. Nonylphenol had the highest lethal toxicity against both *A. bahia* (48 hr LC_{50}; 0.051 mg/l) and *D. magna* (48 hr LC_{50}; 0.18 mg/l). Yokota *et al*. indicated that hatchability in flow-through exposure of medaka *Oryzias latipes* to 183 μg/l 4-nonylphenol was significantly decreased.[12] Gray and Metcalfe also reported that the embryo-larval LC_{50} of *p*-nonylphenol for medaka using a static assay was 460 μg/l.[13] Our results suggest that different toxic mechanisms of nonylphenol may be observed between invertebrates and fish. Therefore, *A. bahia* may be potentially sensitive to EDs such as nonylphenol, in many cases at levels that are likely to occur in the environment. The acute toxicity of nonylphenol has been previously assessed using invertebrates, such as daphnia (*D. magna* and *Ceriodaphnia dubia*),[14–16] mysids (*Mysidopsis capricornutum* and *Mysidopsis bahia*),[17] am amphipod (*Hyalella azteca*),[18] sandworms, univalve shells, and odonates.[19] The nonylphenol 96 hr LC_{50} values determined in those studies range from 43 to 774 μg/l, with mysid shrimp again reacting to the lowest levels among those invertebrates. The no-observed-effect concentration (NOEC) of 4-nonylphenol in mysid shrimp has been estimated to be 18 μg/l. In the present study, the 96 hr LC_{50} of nonylphenol in *A. bahia* was 45 μg/l, while the NOEC ranged from 12.5 to 25 μg/l (Fig. 1). These toxicity profiles are similar to those determined in previous studies. Our results suggest a difference in the susceptibility of *D. magna* and *A. bahia* to lethally toxic chemical compounds. Moreover, the toxic sensitivity of *A. bahia* to all chemicals tested was higher than that of *D. magna*.

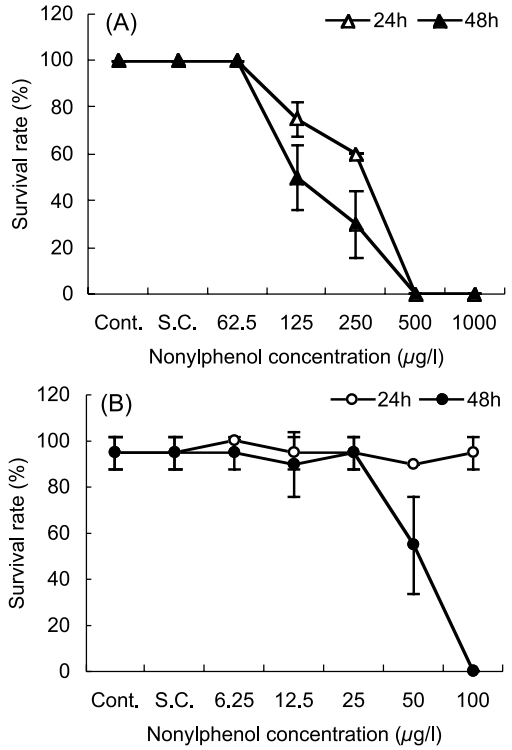

Fig. 1. Effect of Nonylphenol on Viability of *D. magna* (A) and *A. bahia* (B)

In the present study, *A. bahia* was shown to have high sensitively, in an acute toxicity test, to environmental xenobiotic chemicals, whereas natural hormones had low toxicity. Furthermore, *A. bahia* was more sensitive to the toxicity of all chemicals tested than *D. magna*. Presently, a popular aquatic invertebrate test organism for ecological risk assess-

ment by the OECD and Japan is the daphnid *D. magna*. However, the common freshwater test crustacean, *D. magna*, reproduces primarily by asexual means. Thus, the effects of toxicants on genetic recombination cannot be fully assessed as they can using mysids. The present results suggest that *A. bahia* is a more suitable toxicity test organism for EDs than *D. magna* because of the higher sensitivity to the toxicological effect. Thus other endpoints, such as growth, reproduction and molting, need to be assessed in the future.

Acknowledgements This study was supported in part by funds from the Environment Agency, Japan.

REFERENCES

1) Colborn, T., vom Saal, F. S. and Soto, A. M. (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect.*, **101**, 378–384.
2) Colborn, T., Dumanoshi, D. and Myers, J. P. (1996) *Our Stolen Future*, Dutton, NY, U.S.A.
3) Organization for Economic Cooperation and Development (1999) *Final report of the fish expert consultation meeting, London, UK, October 28-29*, Environmental Health and Safety Division, Paris, France.
4) U.S. Environmental Protection Agency (1998) Endocrine Disruptor Screening Program: Proposed Statement of Policy. *Fed. Regist.*, **248**, 71542–71568.
5) Nimmo, D. R., Bahner, L. H., Rigby, R. A., Sheppard, J. M. and Wilson, A. J., Jr. (1977) *Mysidopsis bahia*: an estuarine species suitable for life-cycle toxicity tests to determine the effects of a pollutant. In *Aquatic Toxicology and Hazard Evaluation* (Mayer, F. L. and Hamelin, J. L., Eds.), ASTM STP 634, American Society for testing and Materials, Philadelphia, Pennsylvania, pp. 109–111.
6) Gentile, S., Gentile, J., Walker, J. and Heltshe, J. F. (1982) Chronic effects of cadmium on two species of mysid shrimp: *Mysidopsis bahia* and *Mysidopsis bigelowi*. *Hydrobiologia*, **93**, 195–204.
7) Lussier, S. M., Gentile, J. H. and Walker, J. (1985) Acute and chronic effects of heavy metals and cyanide on *Mysydopsis bahia* (Crustacea: Mysidacea). *Aquat. Toxicol.*, **7**, 25–35.
8) U.S. Environmental Protection Agency (1993) *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th ed.*, EPA/600/4–90–027F, Cincinnati, OH.
9) Baldwin, W. S., Bailey, R., Long, K. E. and Klaine, S. (2001) Incomplete ecdysis is an indicator of ecdysteroid exposure in *Daphnia magna*. *Environ. Toxicol. Chem.*, **20**, 1564–1569.
10) Olmstead, A. W. and LeBlanc, G. L. (2001) Low exposure concentration effects of methoprene on endcrine-regulated processes in the crustacean *Daphnia magna*. *Toxicol. Sci.*, **62**, 268–273.
11) Olmstead, A. W. and LeBlanc, G. L. (2003) Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean *Daphnia magna*. *Environ. Health Perspect.*, **111**, 919–924.
12) Yokota, H., Seki, M., Maeda, M., Oshima, Y., Tadokoro, H., Honjo, T. and Kobayashi, K. (2001) Life-cycle toxicity of 4-nonylphenol to medaka (*Oryzias latipes*). *Environ. Toxicol. Chem.*, **20**, 2552–2560.
13) Gray, M. A. and Metcalfe, C. D. (1997) Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to *p*-nonylphenol. *Environ. Toxicol. Chem.*, **16**, 1082–1086.
14) England, D. E. (1995) *Chronic toxicity of nonylphenol to Ceriodaphnia dubia*, Report No. 41756, ABC Laboratories Inc., Columbia, MO.
15) Huls (1992) *Determination of the effects of nonylphenol on the swimming behaviour of Daphnia magna* (in accordance with EC 84/449), Final report DK-522.
16) Comber, M. H. I., Williams, T. D. and Stewart, K. M. (1993) The effects of nonylphenol on *Daphnia magna*. *Water Res.*, **27**, 273–276.
17) Ward, T. J. and Boeri, R. L. (1990) *Acute flow through toxicity of nonylphenol to the mysid (Mysidopsis bahia)*, Study No. 8974-CMA, EnviroSystems, Hampton, NH.
18) England, D. E. and Bussard, J. B. (1994) *Toxicity of nonylphenol to the amphipod Hyalella azteca (Saussure)*, Report No. 41569, ABC Laboratories Inc., Columbia, MO.
19) Brooke, L. T. (1993) *Acute and chronic toxicity of nonylphenol to ten species of aquatic organisms*, Report to the USEPA for the Work Assignment No. 02 of Contact No. 68-Cl-0034, Lake Superior Research Institute, University of Wisconsin-Superior, Superior, WI.