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Chemical sensitivity of the male daphnid, *Daphnia magna*, induced by exposure to juvenile hormone and its analogs

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Abstract

It was reported that males daphnid *Daphnia magna* that have been induced by methyl farnesoate exposure exhibit higher tolerance to chemical compounds such as potassium dichromate and pentachlorophenol than females. Male neonates are also known to be induced by exposure to juvenile hormone analogs, such as fenoxycarb and pyriproxyfen. If these analogs can be used to produce male progeny, the biological and physiological studies of daphnid male would be progressed since the effects of these analogs were several hundred times higher than that of methyl farnesoate. Therefore, in the present study, it was investigated that the chemical sensitivity of male neonates induced by exposure to juvenile hormone (methyl farnesoate) and its analogs. The minimum concentrations of methyl farnesoate, fenoxycarb and pyriproxyfen to induce 100% male-reproduction were 200 nM (50 μg/l), 0.23 nM (70 ng/l) and 0.31 nM (100 ng/l), respectively. In addition, no reduction of relative reproduction was observed at the juvenoid concentrations in 24 h exposure producing 100% male progeny. The median effective concentrations (EC_{50}) of potassium dichromate for immobility of male neonates, established by a standardized method for investigating sensitivity to chemicals, were significantly higher (12–29%) than that of females at least after 24 h exposure in all the male neonates induced by juvenoids used in this study ($P < 0.05$). This study demonstrated that the male daphnids induced by exposure to juvenile hormone and its analogs exhibit similar chemical tolerance.

Keywords: Acute toxicity test; Fenoxycarb; Methyl farnesoate; Potassium dichromate; Pyriproxyfen; Sex difference

1. Introduction

The cladoceran crustacean *Daphnia magna*, a cyclical parthenogen, are formed asexually as female neonates under favorable environmental conditions. Male neonates appear under unfavorable environmental conditions such as decreasing photoperiod, decreasing food concentration, and increasing population density (Hobaek and Larsson, 1990; Kleiven et al., 1992). These female and male neonates are genetically identical to their mothers. Daphnids enter a sexual phase of reproduction by producing males and sexually responsive females. The female daphnids mate with male counterparts, and the fertilized diapause eggs, encased in a protective ephippium, are released into the environment. Diapause eggs are highly resistant to factors associated with severe environments such as desiccation and freezing, and can even hatch decades after release (Hebert, 1978).

D. magna have a long and productive history in aquatic toxicity testing. This is attributable to several beneficial traits such as the fact that the complete life cycle is spent in water, prolific breeding, ease of handling, and comparatively short longevity (Tatarazako and Oda, 2007). In addition, since daphnids are known to be quite sensitive to a wide range of chemicals, including heavy metals (Tatarazako and Oda, 2007), there have been many studies investigating the sensitivity of females to chemical compounds (Klein, 2000; Yeh and Chen, 2006; Park and Choi, 2007). As a result, this species is highly recommended as a test

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animal for the OECD test guideline (TG) 202 (OECD, 2004) and TG 211 (OECD, 1998).

Although it is phenomenologically well known that male is necessary to produce diapause eggs at gamogenesis phase, little is known concerning the biological and physiological characteristics of male daphnid except for a report of the toxicity of heavy metals to male of *D. magna* (Saika et al., 2004). The biological and physiological studies of daphnid males require a technique for routinely producing male neonates. Olmstead and LeBlanc (2002) reported that the exposure of daphnid oocytes to the crustacean hormone, methyl farnesoate, during late ovarian development caused the oocytes to develop into male progeny, whereas only females were produced in daphnids that remained unexposed. A similar phenomenon has been observed in other cladoceran taxonomic groups, for example, in the genera *Moina* and *Ceriodaphnia* (Oda et al., 2005a). Several studies have demonstrated that the production of male neonates in *D. magna* can be induced by exposure to not only insect or crustacean juvenile hormones but also pesticides designed as juvenile hormone analogs (Olmstead and LeBlanc, 2002, 2003; Tatarazako et al., 2003; Oda et al., 2005b). However, Tatarazako et al. (2003) and Oda et al. (2005b) found that most of these juvenoids also reduced the total number of neonates considerably in 21 days reproduction tests, based on OECD TG 211 (OECD, 1998). Recently, Oda et al. (2006) exposed females to 5–10 μg/l fenoxycarb or pyriproxyfen for over as short time period (12 h), and found that these treatments induced a production of male neonates, but also a small reduction in the total number of neonates.

It was reported that the minimum concentration of methyl farnesoate used over a 24 h exposure period to produce 100% male progeny was 50 μg/l (Ikuno et al., 2008). The report also showed that male daphnids induced by exposure to 100 μg/l methyl farnesoate over a 24 h period exhibited higher tolerance to chemical compounds such as potassium dichromate and pentachlorophenol than the females (Ikuno et al., 2008). Although it is possible for males to be induced by juvenile hormone analogs, as described above, the differences in chemical tolerance between males induced by different endocrine molecules such as juvenile hormone and its analogs has yet to be ascertained. If these analogs can be used to produce male as in the case of methyl farnesoate, male production will be more effective and easier. Therefore, in the present study, the minimum concentration of fenoxycarb and pyriproxyfen to produce 100% males progeny over a 24 h exposure period was determined, and subsequently the sensitivity of these male to potassium dichromate was compared with those of other groups and females.

2. Materials and methods

All parts of this study were conducted in the Department of Marine Science and Resources, Nihon University.

2.1. Daphnid culture

The *D. magna* Straus were originally derived from the National Institute for Environmental Studies of Japan (NIES), and then had been maintained at the Mitsubishi Chemical Safety Institute Ltd. for over 10 years. The daphnids were cultured in an incubator at a density of 20 adults in 1 l of the Elendt M4 medium (Elendt and Bias, 1990) at 20 °C with a natural photoperiod. Culture medium was renewed and offspring were removed two times weekly. Cultured daphnids were fed daily with approximately 0.15 ng carbon of microalga *Chlorella vulgaris* (Super Fresh Chlorella V-12, Chlorella Industry Co., Ltd., Tokyo, Japan) per daphnid. These culture conditions maintained the daphnids in the parthenogenic reproductive phase, with virtually no males produced.

2.2. Chemicals

The chemicals used for male offspring reproduction were methyl farnesoate (Echelon Biosciences Inc., Salt Lake, UT, USA), which is a crustacean juvenile hormone (Laufer et al., 1993), fenoxycarb [ethyl 2-(4-phenoxyphenoxy) ethylcarbamate] (Wako Pure Chemical Industries Ltd., Osaka, Japan) and pyriproxyfen [4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether] (LKT Laboratory Inc., MN, USA). The latter two chemicals are juvenile hormone analogs (Olmstead and LeBlanc, 2003; Tatarazako et al., 2003; Gorr et al., 2006). Potassium dichromate (analytical reagent grade; Wako Pure Chemical Industries Ltd.) was used as the chemical for the toxicity bioassay. Ethanol and dimethylformamide were used as solvents for the preparation of stock solutions of methyl farnesoate and the analogs, respectively, and were obtained from Wako Pure Chemical Industries Ltd.

2.3. Reproduction tests

Mature females >13 days old were used for male progeny production in the exposure tests involving methyl farnesoate, fenoxycarb or pyriproxyfen. Daphnids were exposed to various concentrations of methyl farnesoate (4–400 nM, 1–100 μg/l), fenoxycarb (0.03–3.32 nM, 10–1000 ng/l) or pyriproxyfen (0.03–3.11 nM, 10–1000 ng/l) for 24 h, including the 60–72 h period after the last molt. The same procedures were followed using a solvent control (0.005% v/v ethanol, 0.01% v/v dimethylformamide) and a normal control (Elendt M4 medium only). Glass beakers (500 ml), each containing ten mature *D. magna* in 500 ml of media were used for each concentration. After 24 h exposure, daphnids were rinsed and transferred to new Elendt M4 medium. Thereafter the medium was not renewed until the next phase of reproduction. All tests were conducted for 5 days in an incubator at a temperature of 20 ± 1 °C with a natural photoperiod. Daphnids were fed daily with approximately 0.15 ng carbon of microalga per daphnid during the experiments. The sex of neonates was identified under a stereoscopic microscope (×20–40) using