Effect of Bisphenol A on the Feeding Behavior of *Caenorhabditis elegans*

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We observed and evaluated the feeding behavior of the free-living nematode *Caenorhabditis elegans* (*C. elegans*) after exposure to bisphenol A (BPA) and nonylphenol (NP). Exposed organisms were transferred to chemical-free culture medium and their attainment levels (the number of worms reaching the food source divided by the total number of worms on the Petri plate) were recorded after 2, 4, 6, 8, and 24 hr. Results showed a significant decrease in the attainment level of worms exposed to 10 μM and 0.1 μM BPA. However, there was a slight increase in the attainment level of nematodes treated with 1 μM NP. These results differ from previous studies showing NP as being more lethal to nematodes than BPA.

Key words —— feeding behavior, *Caenorhabditis elegans*, bisphenol A, nonylphenol, biosensor

INTRODUCTION

Experiments have been carried out in order to evaluate the toxicity of chemical substances using various animals and toxicological endpoints. *C. elegans* is a free-living nematode with a completely mapped genome that has become a commonly used laboratory model organism for molecular biology, cellular biology, *etc*.[1,2] Recently, *C. elegans* has also been used for the toxicity studies of the chemical substances and environmental samples.[3–8] Biosensors using *C. elegans* represent a more complex level of biological organization and a higher trophic level compared to bacterial, yeast and cell culture biosensors.[9–11] *C. elegans* is also easily grown in the laboratory compared to other organisms such as fish and mice,[12,13] which are often used in *in vivo* assays.

Toxicant evaluation has several endpoints, with lethality and reproduction often used.[3] Recently, we utilized *C. elegans* in the toxicity evaluation of chemical substances.[14,15] In this study, we examined the feeding behavior of the nematode as a biological parameter after exposure to bisphenol A (BPA) or nonylphenol (NP).

MATERIALS AND METHODS

Wild type *C. elegans* were grown and maintained as described by Brenner (1974), and routinely cultured at 20°C on glass, acid-washed petri plates containing nematode growing media (NGM) agar and *Escherichia coli* (*E. coli*) as food source.[1]

The ages of the nematodes were initially determined. Two and half-day old worms were appropriate because they moved smoothly to the food source unlike the younger and older ones. Adult worms often do not migrate when ovulation begins.

The testing procedure is as follows. Eggs were placed on *E. coli*-free NGM agar plates and incubated for 15–20 hr at 20°C. Juvenile worms (first stage) were then transferred to each NGM agar plate containing the chemicals and a lawn of *E. coli*. The experimental plates consisted of two concentrations (10 μM and 0.1 μM) of BPA, two concentrations (1 μM and 0.1 μM) of NP, and two controls, one containing only medium and the other containing dimethyl sulfoxide (DMSO) (5.0 g/l), which was used as the solvent for BPA and NP. The set-ups were then incubated at 20°C for 46 hr, and then the worms transferred to chemical-free 9 cm plates for the feeding behavior assay. On the latter, *E. coli* was grown circularly within a 0.5 cm radius from the center. The transfer of the exposed worms was carried out as follows. Each exposed worm was rinsed with M9 buffer in 15-ml glass centrifuge tubes and centrifuged at 800 rpm for 30 sec. After the supernatants were removed, the pelleted worms were rinsed thrice with M9 buffer as described earlier to eliminate bacteria. The washed worms were then resuspended in M9 buffer and then transferred one by one with 2 μl

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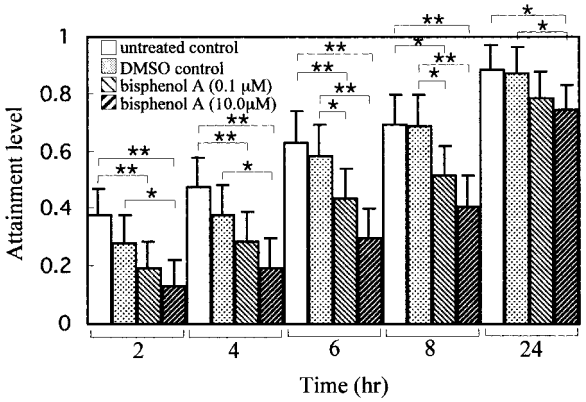

Fig. 1. Influence of BPA in Attainment Level of *C. elegans*
$^{*}p < 0.05$, $^{**}p < 0.01$ statistically significant. Error bar represents SEM.

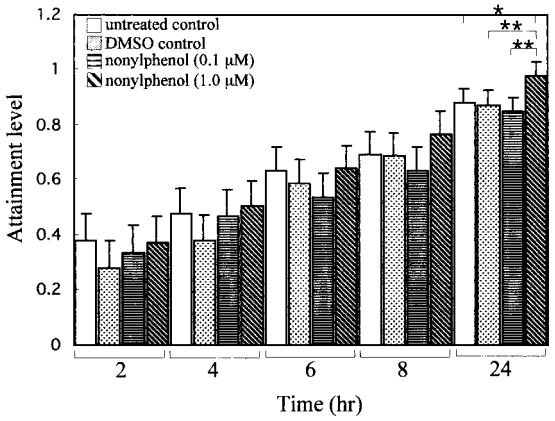

Fig. 2. Influence of NP in Attainment Level of *C. elegans*
$^{*}p < 0.05$, $^{**}p < 0.01$ statistically significant. Error bar represents SEM.

M9 onto the NGM agar. The worms were evenly placed 4 cm from the center. Each test plate was loaded with 12 worms and five replicates were set up for each treatment (60 worms total per treatment). The testing was replicated five times (300 worms total per treatment). After 2, 4, 6, 8, and 24 hr of incubation at 20ºC, the number of nematodes that have reached the *E. coli* colony was counted in each plate using a dissecting microscope. The attainment level of *C. elegans* was obtained by dividing the number of worms that reached the food source by the total number of worms on the plate.

RESULTS AND DISCUSSION

All worms survived during the experiment. As shown in Fig. 1, there was no evidence ($p > 0.05$) of altered attainment levels between the untreated control and the DMSO control. However, when the *C. elegans* were exposed to 10 μM BPA, the attainment levels decreased significantly ($p < 0.05$) compared to the untreated control in all observation points. The decrease was similarly significant ($p < 0.05$) using 0.1 μM BPA at 2, 4, 6, and 8 hr. At the higher concentration, the difference was more significant ($p < 0.01$) between 2 to 8 hr. However, a comparison between the attainment levels of the organism treated with 0.1 μM and 10 μM BPA showed no significant difference. Results using NP as shown in Fig. 2 do not reveal any significant differences between treatments and controls at 2, 4, 6, and 8 hr. A 24 hr exposure to 1 μM NP led to an increase ($p < 0.05$) in the attainment level of *C. elegans*. Considering that NP is generally more lethal to *C. elegans* than BPA, these results are interesting. After exposure to these compounds, the activity of the worms appeared no different from the controls as observed under a dissecting microscope. There were no significant differences either in their respiration volumes when the oxygen consumption of approximately 200 worms in M9 buffer was measured using a disposable oxygen electrode sensor and multichannel dissolved oxygen meters (DOX-96).[16] In this study, we indicated that *C. elegans* have a good responsibility for BAP using the feeding behavior as a biological parameter. Thus it seems that this parameter will be able to become interesting test endpoint for chemical hazard assessment.

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