Bisphenol A and nonylphenol bioconcentration in spotted halibut *Varaspar variegates*

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Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, BPA) and 4-nonylphenol (isomers mixture, NP) are synthetic chemicals used in a wide variety of plastics and other products, and many scientists have reported that these chemicals have an endocrine-disrupting effect.1–4 High concentrations of BPA have been detected in industrial waste effluent.5 In Japan, endocrine-disrupting chemicals have been detected in river water, seawater, fish and other aquatic organisms.6,7 However, little information is available about uptake and accumulation of these chemicals from the environment on marine fish. In the present study, spotted halibut (*Varaspar variegates*) were exposed to waterborne BPA and NP in seawater for several days, and time dependence of BPA and NP concentrations in these fish was investigated.

Spotted halibut (2 months old) were maintained in a water tank at our laboratory under natural photoperiod at a constant temperature of 14.0 ± 0.2°C for 2 weeks before the exposure experiment. The fish were starved during this pre-experimental period. At the start of the experiment, 140 fish (body weight, 0.06–0.12 g; body length, 8–15 mm) were randomly selected, distributed into four groups of 35 fish, and placed into four glass tanks (30 L). During the 1-week experimental period, the fish were starved and exposed to waterborne BPA (Tokyo Kasai Kogyo Co., Ltd, Tokyo, Japan) and NP (isomers mixture; Kanto Chemical Co., Inc., Tokyo, Japan). The groups were exposed to the following concentration mixtures: group 1 (control), 0 µg/L BPA and 0 µg/L NP; group 2, 7 µg/L BPA and 8 µg/L NP; group 3, 70 µg/L BPA and 80 µg/L NP; group 4, 700 µg/L BPA and 800 µg/L NP. BPA and NP were dissolved in ethanol prior to being added to the tank water. The final concentration of ethanol in the water was 10 µL/L. Every 24 h during the experimental period, all of the water in each experimental tank was changed. Seven fish were taken out of each tank for analysis after 0, 1, 2, 4 and 7 days of exposure. To the samples were added the internal standards, BPA-d8 and NP-d4 (Kanto Chemical Co., Inc.), and they were homogenized with methanol. Then, the extracts were concentrated using flowing N2 gas and cleaned using a silica-gel column by hexane. The chemicals, which were eluted with acetone : hexane (30% : 70%) were then derivatized using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; SUPELCO, Inc., Bellefonte, PA, USA). Trimethylsilylated BPA (BPA-TMS) and NP (NP-TMS) were analyzed by gas chromatography/mass spectroscopy (GC/MS).

GC/MS analysis was performed using a Varian CP-3800 GC and SATURN 2000 MS in EI auto mode with a scan range of 40–600 a.m.u. (Varian, Palo Alto, CA, USA). Chromatographic separations were performed using a 30 m x 0.25 mm i.d. (film thickness, 0.25 µm) DB-5 capillary column (J & W Scientific, Palo Alto, CA, USA). The injector temperature was maintained at 280°C, and the injection volume was 2.0 µL. The oven temperature was maintained at 50°C for 1 min, and was then increased to 280°C at a rate of 10°C/min. Detection limits of BPA and NP were 0.01 µg/L and 0.04 µg/L in this method, respectively.

Time dependence of whole-body concentrations of BPA and NP in spotted halibut is shown in...
BCF of BPA and NP in spotted halibut

Fig. 1. In group 4 (700 mg/L BPA and 800 mg/L NP), whole-body concentrations of BPA and NP were 9.34 ± 3.60 mg/g and 112.94 ± 14.20 mg/g, respectively, after 1 day of exposure, and NP concentration was almost 100 mg/g higher than BPA concentration. On the second day of exposure, 95% of the fish in group 4 had died. Figure 1b shows the data for group 3 (70 mg/L BPA and 80 mg/L NP). In group 3, whole-body concentrations of BPA and NP ranged from 2.30 to 8.11 mg/g and 0.92–2.44 mg/g, respectively, over the course of exposure. Whole-body concentrations of BPA and NP in group 3 had increased after 1 day of exposure, and then remained nearly constant during the rest of the exposure period, except for BPA concentration on the fourth day. Figure 1c shows the concentrations in group 2 (7 mg/L BPA and 8 mg/L NP). In group 2, NP was not detected after 1 day, but the NP concentration was 0.25 mg/g after 2 days and remained nearly constant after that. BPA was not detected in group 2 during the exposure period. BPA and NP were not detected in group 1 (Fig. 1d). In the groups exposed to BPA and NP, chemical concentrations in the fish had increased after 1 or 2 days of exposure. This indicates that these chemicals are absorbed easily, and rapidly accumulated in the body of a fish. Although the chemicals were absorbed continuously into the fish from the seawater, their concentrations remained nearly constant after the initial increase. This indicates that spotted halibut metabolize and/or depurate BPA and NP. The chemical concentrations in the fish were dependent on the concentrations in the seawater. Table 1 shows bioconcentration factors, which are calculated as the ratio of chemical concentration in the fish body to concentration in the seawater. The logarithm of the octanol-water partition coefficients, log Pow, of BPA and NP are at the same levels (3.32 and 3.28 in water, respectively). The bioconcentration factors had a mean of approximately 30, a range of 17–38, and did not show a clear correlation with concentrations of BPA and NP. Okumura and Nishikawa reported BPA and NP bioconcentration factors for carp in a river of about 10 and 120, respectively. Tsuda et al. reported NP bioconcentration factors of 167 in kililfish and 21 in ayu fish. Our results are in the same order as for these previous findings. These facts suggest that bioconcentration factors of BPA and NP are similar for a wide variety of fish species, in both freshwater and seawater fish.

REFERENCES


