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Dietary uptake kinetics of polychlorinated biphenyls from sediment-contaminated sandworms in a marine benthic fish (*Pseudopleuronectes yokohamae*)

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ABSTRACT

To evaluate the dietary uptake of polychlorinated biphenyls (PCBs) from live food, we investigated the dietary uptake and depuration kinetics of PCBs in a marine benthic fish (marbled sole, *Pseudopleuronectes yokohamae*) by using as food live sandworms (*Perinereis nuntia*) that were laboratory-exposed to field-collected PCB-contaminated sediment. Marbled sole were fed the PCB-contaminated sandworms for 28 d and then uncontaminated sandworms for 56 d. The assimilation efficiencies (AEs) of 84 PCB congeners via the gastrointestinal tract (GIT) to the muscle of the fish ranged from 0.21 to 0.78; whole-body AEs would be lower than those of muscle because of the lower PCB concentrations, on a lipid basis. The AEs determined in this study were lower than those in other studies that used PCB-spiked commercial pelletized food. The lower AEs found in this study might be attributable to differences in the food administered (live sandworms vs. commercial pellet food), possibly because of low digestibility of sandworm lipids by marbled sole. In addition, the AEs in this study tended to increase with increasing log octanol–water partition coefficients (K_{OW}) up to about seven, although AEs in the other studies using commercial pelletized food did not increase with increasing log K_{OW}. This result suggests the co-transport of highly hydrophobic PCB congeners along with lipids and fatty acids from the digested sandworms into the GIT epithelium cells. The growth-corrected half-lives of 26 PCB congeners in the muscle of fish ranged from 20 to 107 d.

1. Introduction

In aquatic environments, several persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), tend to accumulate in the bottom sediment because of their hydrophobicity (Kobayashi et al., 2010a). Bottom sediment therefore may play an important role as a source of these compounds to overlying water and aquatic organisms (Kitamura et al., 2009; Sakurai et al., 2009). In particular, benthic fish may be exposed to these PCBs via ingestion of sediment-dwelling prey and bottom sediment (Moermond et al., 2004; Sakurai et al., 2009; Kobayashi et al., 2010b).

The transfer of chemicals to aquatic organisms in the marine environment deserves further investigation. Marine fish account for more than half of global fishery production (capture + aquaculture) and dietary caloric intake from aquatic organisms (FAO, 2010). However, most biomagnification studies in aquatic environments have used small freshwater fishes (e.g., guppy and goldfish) (Bruggeman et al., 1981; Sijm et al., 1992; Gobas et al., 1993). The size of fish studied would affect the kinetic parameters for chemical uptake and depuration (Sijm and van der Linde, 1995). Therefore, biomagnification studies using larger, edible marine fish are necessary to predict the concentrations of chemicals in edible fish in the marine environment, and to assess human exposure to, and the risks posed by, chemicals via the ingestion of marine fish.

The assimilation efficiency (AE) of chemicals via the gastrointestinal tract (GIT) is an essential parameter for determining biomagnification, but AEs vary widely among fish species and among studies (Barber, 2008). The high variability of AE among studies may be due to differences in the composition and digestibility of the diet, differences in food digestibility among fish species, variations in sorption coefficients of chemicals with diet, permeability of chemicals through the gut membrane, and feeding rates (Niimi and Oliver, 1988; Clark and Mackay, 1991; Burreau et al., 1997; Arnot and Gobas, 2004; Liu et al., 2010). Therefore, the AE for chemicals in fish may be affected by differences between live and commercial pelletized food (Burreau et al., 1997; Liu et al., 2010) because of differences in the composition and digestibility of the diet and in the sorption coefficients of the chemicals. However, most biomagnification studies to date have used commercial food

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pellets spiked with target chemicals, and relatively little is known about the biomagnification of chemicals in edible marine or benthic fish through live food sources.

The aim of this study was to evaluate the uptake and depuration kinetics of PCBs in a marine benthic fish (marbled sole, *Pseudopleuronectes yokohamae*) by using as food live polychaetes (sandworm, *Perinereis nuntia*) that were exposed in the laboratory to PCB-contaminated sediment. We compared our results to those of other biomagnification studies that used PCB-spiked commercial pellets or live food. Marbled sole lives on and in sediment or sand and is commonly caught and eaten in Japan. We selected a polychaete as a model food for this study because polychaetes constitute a major part of the diet of marbled sole (Park, 1988) and are the predominant food consumed in some habitats, such as in Tokyo Bay, Japan (Park, 1988).

2. Materials and methods

2.1. Materials

Young fish (5 months after hatching) were obtained from the Kanagawa Sea Farming Association (Kanagawa, Japan) and then cultured at our institute (NIES) until the start of the experiments (water temperature 17 °C), at which time the fish were aged 2 years (3 years old by the end of the experiment). Fish were fed a commercial pellet food (Otohime EP2, Marubeni Nisshin Feed Co., Ltd., Tokyo, Japan) at 1% of body weight daily until 2 weeks before the start of the experiment. Cultured adult sandworms were purchased from a commercial bait shop.

Sediment for exposure experiments was collected from the estuary of a river in the Tokyo Metropolis in September 2008 by using an Eckman-Birge grab sampler. Then the sediment was wet-sieved (1-mm mesh). The preparation and character of the sediment sample are described in Supplementary material. Gravel for control treatments (diameter about 1–2 cm) was purchased from an aquarium supply store and well washed with seawater. We used seawater collected from the deep waters (about 330 m) of Sagami Bay, Japan, between October 2008 and January 2009.

2.2. Control and PCB-exposed sandworms

Sandworms for control treatments were maintained in polypropylene containers (370 × 300 × 210 mm [depth]) that contained gravel and seawater (hereafter, "control sandworms"). A commercial pelletized food (Otohime EP1, Marubeni Nisshin Feed Co., Ltd.) was added each morning, and any food remaining that evening was removed. Sandworms for exposure treatments were maintained for at least 10 d in a polypropylene container (i.d. 200 mm, depth 150 mm) containing the field-collected sediment (hereafter, "PCB-exposed sandworms"). No food was added to this tank because the sandworms ingest organic matter in the sediment. Seawater in both containers was aerated, and was drained each morning, leaving only wet sediment or gravel, and replaced in the evening.

About 2 g wet weight of sandworms (usually four or five individuals) was taken from each rearing container daily and stored at −20 °C for later PCB analysis. The sandworm samples were combined for every 2-week period (days 0–14, 15–28, 29–56, 57–70, and 71–84) and analyzed. The PCB-exposed sandworms were collected by sieving, and sediment particles adhering to the outer surfaces of the sandworms were washed off with seawater or removed directly using forceps. The amount of sediment inside the sandworms was measured by the calcination method (Mount et al., 1999) using different sediment collected from Tokyo Bay.

2.3. Design of exposure tests

The fish for PCB exposure tests (control treatment [mean ± standard deviation]: total length 165 ± 13 mm, weight 70 ± 23 g, n = 30; exposure treatment: total length 165 ± 21 mm, weight 70 ± 20 g, n = 30) were held in glass aquaria that had glass overflow tubes (7–9 fish per aquarium; aquarium size 600 × 300 × 450 mm [depth] or 750 × 430 × 450 mm [depth]). The glass aquaria held 60 or 100 L, respectively, of seawater. Aerated seawater in a head tank was circulated 25 or 42 times per day through each aquarium, depending on the aquarium size. Seawater was maintained at 17 °C and treated with a UV lamp (UVF 1000, REI-SEA Co. Ltd., Tokyo, Japan) and an external filter (EHEIM Professional 3, EHEIM GmbH & Co. KG., Deizisau, Germany). The same seawater was circulated through both control and treatment tanks to ensure equal uptake of any PCBs dissolved in the water.

All fish were acclimated by being fed with the control sandworms for 2 weeks before the start of the experiment, and then the fish were fed sandworms for either the control or the exposure treatment for 28 d; this was followed by a 56-d depuration period during which they were fed the control sandworms. Three fish were sampled on day 0, and then three fish were sampled from each treatment on days 3, 7, 14, and 28 of the uptake period and days 3, 7, 14, 28, and 56 of the depuration period. Sampled fish were stored at −20 °C until dissection. The daily rate of feeding was approximately 1% of the mean wet-weight of the fish. During feeding, individual fish were isolated by a partition board and then fed sandworms of the appropriate weight to ensure a consistent feeding rate for each individual fish. The fish were weighed every 28 d (i.e., days 0, 28, 56, and 84), and the feeding rate was corrected on the basis of the mean weight of all fish in each treatment. The fish samples were dissected and muscle tissue samples were analyzed. We used the muscle tissue of the fish to assess the exposure of, and risk to, humans through consumption of the edible parts (i.e., muscle tissue) of the fish.

Feces from fish was collected daily in a stainless-steel container by using a siphon tube before and after feeding, and then the contents of the container were filtered through a nylon screen (400 mesh, 38 μm opening size). The feces collected on the nylon screen was transferred to a glass bottle in a small amount of seawater and frozen at −20 °C until final filtration through a glass–fiber filter. One-liter water samples were collected twice per week throughout the uptake period (day 0–28). The feces and water samples were combined for every 2-week period and analyzed.

2.4. PCB analysis

The extraction and cleanup procedures and the measurement conditions using gas chromatography – high-resolution mass spectrometry for fish and water samples generally followed previously reported procedures (Kobayashi et al., 2010b). All 209 PCB congeners were identified and quantified. Recovery rates for PCB congener internal standards are given in Supplementary material. In this paper "ΣPCB concentrations" refers to the sum of the concentrations of all 209 congeners. PCB congeners are numbered in accordance with the BZ numbering system (Ballschmiter and Zell, 1980).

2.5. Lipid content

Lipids in fish muscle tissue were extracted by diethyl ether and measured gravimetrically (Hygiene test method-annotation, Kanehara and Co., 2000). Because the weight of fecal samples was limited, the lipid content of feces was measured by using a subsample (20% by weight) of the extract prepared for PCB analysis. Details of the measurement of the lipid content in the fecal samples are described in Supplementary material.