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ACCUMULATION AND BODY DISTRIBUTION OF PARABENS AND THEIR METABOLITES AMONG ORGAN SAMPLES COLLECTED FROM MARINE MAMMALS IN KOREA

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Introduction

Parabens are esters of p-hydroxybenzoic acid, with alkyl substituents such as methyl- (methyl paraben, MeP), ethyl- (ethyl paraben, EtP), propyl- (propyl paraben, PrP), butyl- (butyl paraben, BuP), heptyl- (heptyl paraben, HeP) and benzyl (benzyl paraben, BzP) groups^{1,2}. Parabens have been used in wide ranges of cosmetic products, food additives, and pharmaceuticals as preservatives³⁻⁵, since the mid-1920s. Among parabens, lower molecular esters such as MeP and PrP are the most widely used compounds due to their synergetic effects^{1,3}. Previous studies have reported the endocrine and reproductive toxicities associated with exposure to parabens⁵⁻⁷. In biota, parabens are metabolized by hydroxylation and esterase hydrolysis^{6,8,9}, resulting in the formation of protocatechuate (3,4-dihydroxybenzoic acid, 3,4-DHB), methyl protocatechuate (OH-MeP), ethyl protocatechuate (OH-EtP), and 4-hydroxybenzoic acid (4-HB)^{1,6}. Among metabolites, 4-HB is known as the major compound in laboratory animals and humans^{6,9}. Several studies have reported the presence of parabens and their metabolites in human fluids or tissues such as serum¹⁰, adipose tissue¹¹ and urine samples¹², implying human exposure of these contaminants. However, limited studies are available concerning the occurrence and body distribution of parabens and their metabolites in wildlife. To date, only one study has reported the occurrence and accumulation potentials of parabens and their metabolites in marine mammals from United States. In the present study, we analyzed parabens and their metabolites in various internal organs including blubber, muscle, melon, stomach, kidney, liver, brain, testis, ovary, uterus, and cord from two major cetacean species collected from Korean coastal waters, to understand the body distribution, partitioning, and metabolic mechanisms of parabens and their metabolites in marine mammals.

Materials and methods

-Sample collection

A total of 94 tissue samples of common dolphins (*Delphinus capensis*) and finless porpoises (*Neophocaena asiaeorientalis*) were collected from Korean coastal waters between 2012 and 2015. Collected samples were transported to the laboratory of the Cetacean Research Institute (CRI) and were then dissected after biometric measurement. Six individual species, comprising of immature male (n=1), immature female (n=1), mature male (n=2), and mature female (n=2), were investigated for both species in our study (Table 1).

-Sample preparation

Details of the sample preparation procedures were similar to the methods used in a previous study¹. In our study, six parabens (MeP, EtP, PrP, BuP, BzP and HeP) and four metabolites (OH-MeP, OH-EtP, 3,4-DHB and 4-HB) were analyzed. Briefly, tissue samples (200–300 mg) were weighed and spiked with 50 ng of internal standards (¹³C₆-MeP, ¹³C₆-BuP, d₄-HeP, d₄-BzP, and ¹³C₆-4-HB). After equilibration for 30 min, 3 mL of acetone were added to tissues. The mixture was homogenized in a mortar and transferred to a 15 mL polypropylene tube by washing with mixture of methanol and acetonitrile (1:1, v/v). The homogenized samples were shaken in an oscillator shaker for 60 min and then centrifuged for 5 min. The supernatant was transferred to new tube and extract were concentrated to 1 mL. To remove lipid, extracts were stored in a refrigerator (-20°C) overnight. After storage, samples were centrifuged for 5 min, and the supernatant was transferred to the vial for analysis.

-Instrumental analysis

Agilent 1100 Series HPLC system coupled to Applied Biosystems API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS) was used to quantify the parabens and their metabolites. The MS/MS system was operated in the negative ion multiple reaction monitoring mode. A Zorbax SB-Aq column was used for separation of target compounds.

Result and discussion

- Concentrations of parabens and their metabolites in marine mammals

The concentrations of parabens and their metabolites in various internal organs of common dolphins and finless porpoises collected from Korean coastal waters (Figure 1). Among analyzed compounds for parabens, MeP and 4-HB were predominantly detected in all of the organ samples, while the other parabens showed the lower detection rates (<5%). The concentrations of MeP and 4-HB in common dolphins ranged from 1.30 to 224 ng/g wet weight (ww) and 54.0 to 3510 ng/g ww, respectively. The highest median concentration of MeP was found in liver (147 ng/g ww), followed by kidney (133 ng/g ww) and stomach (86.4 ng/g ww). For 4-HB, stomach (2132 ng/g ww) showed the highest median concentration followed by liver (1175 ng/g ww) and kidney (1090 ng/g ww). For finless porpoises, the concentrations of MeP ranged from 6.42 to 570 ng/g ww and 73.3 to 10538 ng/g ww for 4-HB. Similar to the results of common dolphin, liver (413 ng/g ww) showed highest median concentration of MeP, followed by kidney (288 ng/g ww) and stomach (128 ng/g ww) in finless porpoises. The highest median concentration of 4-HB in finless porpoises was found in stomach (6881 ng/g ww), followed by kidney (3751 ng/g ww) and liver (3504 ng/g ww). Concentrations of MeP and 4-HB were higher in organs from finless porpoises than those found in common dolphins ($p < 0.01$), indicating that habitat could be an important factor for bioaccumulation of parabens and their metabolites. No age- and sex-dependent accumulation patterns were found for parabens and their metabolites in both cetacean species.

- Body distribution of MeP and 4-HB in marine mammals

Concentrations of MeP and 4-HB for both species were higher in liver, kidney, stomach and gonad than blubber and melon with high lipid contents. This might be affected by their lower K_{ow} value, 1.96 for MeP and 1.58 for 4-HB⁴. In both species, the highest concentrations of MeP were found in liver and kidney which are internal organs related with metabolism. Considering the fast excretion rate of parabens in animals^{2,14}, the high concentrations of parabens could be due to the lower metabolic capacity of marine mammals with on-going exposure of MeP from human activities. Stomach also showed relatively higher concentrations of MeP, indicating that diet might be a major exposure pathway of MeP to marine mammals. Our findings were supported by the higher concentration of MeP in stomach contents of finless porpoises. In contrast, the highest concentration of 4-HB in stomach in both species can be explained by the high concentration of 4-HB in their diets and/or hydrolysis of MeP by digestion process. A previous study confirmed the presence of 4-HB by hydrolysis of MeP⁶. The higher 4-HB concentration was 3373 ng/g ww with the highest 4-HB concentration in stomach sample (10538 ng/g ww). Lactating common dolphin showed high concentration of 4-HB in uterus (3507 ng/g ww) and ovary (1653 ng/g ww), comparable to 4-HB concentration in stomach. Our finding suggests that delivery and lactating are the major factor for the re-distribution of MeP and 4-HB in bodies of marine mammals.

- Correlations between MeP and 4-HB among organ samples

Although most of parabens are synthesized by industry, some organisms can naturally produce them including 4-HB². In contrast, anthropogenic MeP can be also metabolized to 4-HB via hydrolysis in organism^{2,13}. Therefore, the correlation analysis between MeP and 4-HB provide information on the similarity of exposure sources (Figure 2). We divided the internal organs of cetaceans into three groups depending on the regression trends. In both species, liver and kidney showed relatively higher MeP concentrations than the other organs while stomach showed relatively higher 4-HB concentrations. The correlations between the concentrations MeP and 4-HB in liver and kidney were significant. However, no significant correlations were found between the concentrations of MeP and 4-HB in stomach samples. Our findings suggest the high levels of 4-HB in their diets and/or selective hydrolysis of MeP by digestive process.

In the present study, we investigated the body distributions of MeP and 4-HB in marine mammals. Our result showed the different body distribution of parabens and metabolites, depending on physiological characteristics of species. Further studies are needed to understand the body burden and body distribution of parabens and their metabolites between marine mammals and their diets.

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Table 1. Sample information on common dolphins (*Delphinus capensis*) and finless porpoises (*Neophocaena asiaorientalis*) collected from Korean coastal waters

	Common dolphins	Finless porpoises
Blubber	6	6
Muscle	6	6
Melon	6	6
Stomach (Stomach contents)	6	6 (2)
Kidney	6	6
Liver	6	6
Brain	5	-
Gonad/Ovary	6	6
Uterus	2	-
Cord	1	-

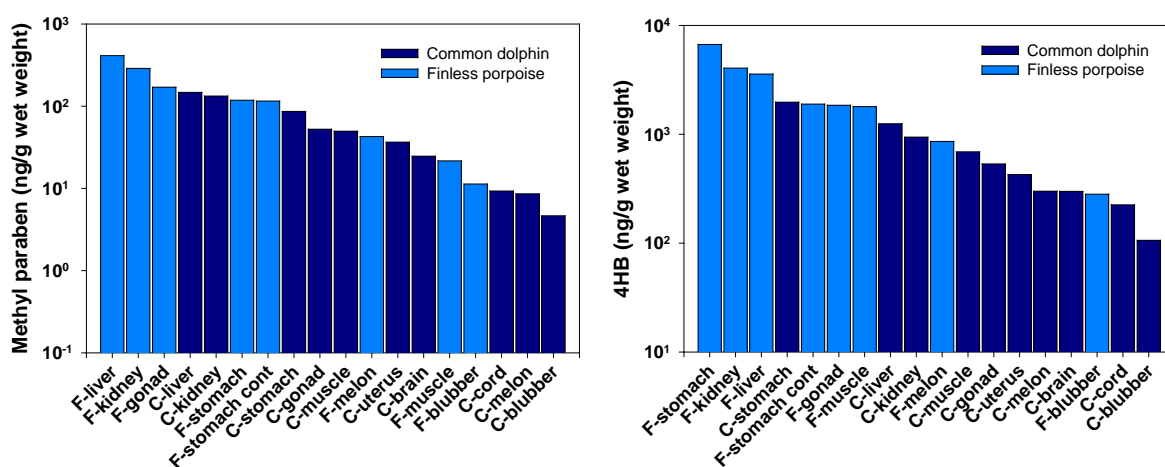


Figure 1. Concentrations of MeP and 4-HB in various organs of common dolphin and finless porpoises collected from Korean coastal waters

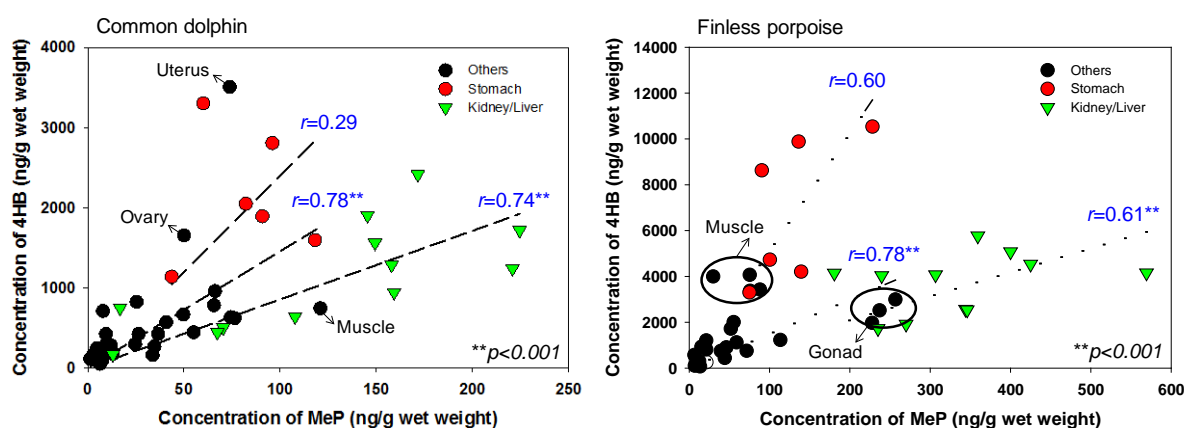


Figure 2. Correlations between concentrations of MeP and 4-HB in various organs in common dolphins and finless porpoises collected from Korean coastal waters.