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Accumulation profiles of parabens and their metabolites in fish, black bear, and birds, including bald eagles and albatrosses

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ABSTRACT

Although several studies have reported the ubiquitous occurrence of parabens in human specimens and the environment, little is known about the accumulation of these estrogenic chemicals in fish and birds. In this study, accumulation profiles of six parabens and their metabolites were determined in 254 tissue (including liver, kidney, egg, and plasma) samples from 12 species of fish and seven species of birds collected from inland, coastal, and remote aquatic ecosystems. In addition, liver and kidney tissues from black bears were analyzed. Methyl paraben (MeP) was found in a majority of the tissues, with the highest concentration (796 ng/g (wet weight [wet wt])) found in the liver of a bald eagle from Michigan. 4-Hydroxy benzoate (HB) was the major metabolite, found in 91% of the tissue samples analyzed at concentrations as high as 68,600 ng/g, wet wt, which was found in the liver of a white-tailed sea eagle from the Baltic Sea coast. The accumulation pattern of MeP and 4-HB varied, depending on the species. The mean concentrations of MeP measured in fishes from Michigan, New York, and Florida waters were <2.01 (fillet), 152 (liver), and 32.0 (liver) ng/g, wet wt, respectively, and the corresponding 4-HB concentrations were 39.5, 10,500, and 642 ng/g, wet wt. The mean hepatic and renal concentrations of 4-HB in black bears were 1,720 and 1,330 ng/g, wet wt, respectively. The concentrations of MeP and 4-HB were significantly positively correlated with each other in various tissues and species, which suggested a common source of exposure to these compounds in fish and birds. Trace concentrations of MeP and 4-HB also were found in the tissues of albatrosses from Midway Atoll, Northwestern Pacific Ocean, which suggested widespread distribution of these compounds in the marine environment.

1. Introduction

The esters of *p*-hydroxy benzoic acid (HB), commonly known as parabens, are widely used as preservatives in consumer products, including processed foods, personal care products, and pharmaceuticals (Liao et al., 2013; Soni et al., 2005). Methyl- (MeP), ethyl- (EtP), propyl- (PrP), butyl- (BuP), heptyl- (HeP), and benzyl- (BzP) parabens are some of the esters of HB in commerce. Owing to their synergistic antimicrobial action, MeP and PrP are the most commonly used parabens and are often used in combination. In recent years, however, there has been an increasing concern about the endocrine-disrupting potential of parabens. Estrogenic effects of parabens have been documented in vitro and in vivo, and the estrogenic potential of these compounds is known to be associated with alkyl chain length (Boberg et al., 2010; Byford et al., 2002; CIR, 2008; Darbre and Harvey, 2008). Adverse effects on sperm production and serum testosterone levels were reported in male rats following oral exposure to BuP and PrP (Oishi, 2001, 2002). Further, topical application of paraben-containing cosmetics has been linked to human breast cancer (Darbre, 2001; Darbre and Harvey, 2014; Harvey, 2003).

Although studies have reported the occurrence of intact parabens in human and marine mammal tissues and/or urine (Asimakopoulos et al., 2014, 2015; Jiménez-Díaz et al., 2011; Wang et al., 2015a; Xue et al., 2015a, 2015b), these compounds undergo metabolism by esterases (Aubert et al., 2012). 4-HB is considered to be the final metabolite of parabens in biota (Aubert et al., 2012; Jewell et al., 2007), and this compound also possesses estrogenic potential (Boberg et al., 2010). Other known metabolites of parabens include protocatechuic acid (3,4-dihydroxybenzoic acid [3,4-DHB]), methyl protocatechuate (OH-MeP), and ethyl protocatechuate (OH-EtP) (Wang and Kannan, 2013).

Earlier, we reported the accumulation of parabens and their metabolites in marine mammals for the first time, and the reported concentrations of 4-HB in dolphins and sea otters were some of the highest ever reported in the literature (Xue et al., 2015b). Although 4-HB has natural sources (Peng et al., 2006; Zapata and McMillan, 1979), the significant positive relationship between MeP and 4-HB in marine mammals was

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suggestive of anthropogenic sources of origin for these compounds in the marine environment (Xue et al., 2015b). In this study, we report for the first time the concentrations and accumulation profiles of parabens and their metabolites in liver, kidney, brain, muscle, fat, egg, and plasma of several species of fish and birds, including albatrosses and bald eagles. The samples originated from a wide range of locations, including inland waters, the coastal marine environment, and the Pacific Ocean.

2. Materials and methods

2.1. Standards and reagents

Analytical standards of MeP (~98%), BuP (~98%), and 4-HB (~98%) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). 3,4-DHB (≥97%), OH-MeP (~97%), and OH-EtP (~97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). $^{13}C_6$-4-HB was purchased from Cambridge Isotope Laboratories. Analytical standards of EtP, PrP, BzP, and HeP were purchased from AccuStandard Inc (New Haven, CT, USA). d_4-HeP and d_4-BzP were obtained from C/D/N Isotopes Inc (Pointe-Claire, Quebec, Canada). Mixed solutions of $^{13}C_6$-MeP and $^{13}C_6$-BuP were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions of target analytes and internal standards (ISs) were prepared at 1 mg/mL in methanol and stored at −20 °C until use. The chemical structures of the target analytes are shown in the Supporting information (Table S1). Methanol (HPLC grade), acetone (ACS grade), and acetonitrile (ACS grade) were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA).

2.2. Sample collection

A total of 254 tissue samples from 12 species of fish and seven species of birds as well as black bears were analyzed in this study. Fish species analyzed include smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*), burbot (*Lota lota*), lake trout (*Salvelinus namaycush*), lake sturgeon (*Acipenser fulvescens*), white suckers (*Catostomus commersonii*), channel catfish (*Ictalurus punctatus*), walleye (*Stizostedion vitreum*), longnose suckers (*Catostomus catostomus*), striped mullet (*Mugil cephalus*), red drum (*Sciaenops ocellatus*), and Atlantic sharpnose shark (*Rhizoprionodon terraenovae*). Birds species analyzed were Laysan albatross (*Phoebastria immutabilis*), black-footed albatross (*Phoebastria nigripes*), bald eagle (*Haliaeetus leucocephalus*), herring gull (*Larus argentatus smithsonianus*), double-crested cormorant (*Phalacrocorax auritus*), common loon (*Gavia immer*), and white-tailed sea eagle (*Haliaeetus albicilla*). Fish samples were collected from several locations across the United States, including Great Lakes waters, New York State waters, and Florida coastal waters. The sampling locations of birds were grouped as the Baltic Sea coast, Midway Atoll (central North Pacific Ocean), Great Lakes region, and other U.S. locations. Black bear samples were collected from Michigan.

Fish and bird tissue samples were collected as part of our previous investigations to elucidate bioaccumulation of organic contaminants; the details with regard to sample collection have been described elsewhere (Johnson-Restrepo et al., 2005; Kannan et al., 2000; Kannan et al., 2001a; Kannan et al., 2001b; Kannan et al., 2002, 2005; Sinclair et al., 2006). Smallmouth and largemouth bass were collected between 2001 and 2003 from 19 inland lakes and rivers in New York State. Liver and muscle samples were collected and stored in pre-cleaned glass jars. Fish from the Great Lakes (Lake Superior, Lake Michigan, and Lake Huron) and eight inland Michigan lakes and rivers were collected between 1996 and 1997; homogenized "skin-on" or "skin-off" fillets were analyzed. Liver and muscle tissues of fish from Florida coastal waters, collected in 2004 from the Indian River Lagoon and adjacent offshore coastal waters, also were analyzed. Tissues collected from Atlantic sharpnose sharks included muscle, liver, kidney, brain, and gill. All fish samples were stored at −20 °C until analysis.

Liver, kidney, brain, egg, muscle, and fat tissues of albatrosses were collected from adult Laysan and black-footed albatrosses on Sand Island, Midway Atoll, in the central North Pacific Ocean during 1992 to 1996. Liver, muscle, and kidney tissues of bald eagles were collected from carcasses of bald eagles found dead in the Upper Peninsula of Michigan in 2000. Blood samples of bald eagles were collected from nesting eagles in Michigan, by venipuncture of the brachialis vein using a 10-mL syringe. Eggs of double-crested cormorants and herring gulls were collected from Little Charity (Saginaw Bay) and Scarecrow Islands (Thunder Bay) in Lake Huron and Tahquamenon Island (Whitefish Bay) in Lake Superior in May 1998. Common loon liver and egg samples were collected from North Carolina, Maine, and New Hampshire. Livers of white-tailed sea eagles were collected between 1979 and 1999 from eastern Germany and Polish coastal areas of the Baltic Sea. All bird tissue samples (liver, kidney, brain, muscle, and fat) were either wrapped in solvent-cleaned aluminum foil or placed in pre-cleaned glass jars (egg) and stored at −20 °C until analysis. Bald eagle plasma samples were stored in 15-mL polypropylene tubes at −20 °C. Liver and kidney tissues of black bears from Michigan were analyzed. It is worth noting that the samples analyzed in this study were collected several years ago, for the analysis of legacy contaminants, and therefore the concentrations measured may not reflect the current trends. However, this is the first study to report paraben levels and establishes baseline for future trend analysis. Sampling locations are shown in Fig. S1.

2.3. Sample preparation

The sample preparation procedure for the analysis of parabens and their metabolites has been described elsewhere (Wang et al., 2015a, 2015b; Xue et al., 2015b; Xue and Kannan, 2016). First, the surface of each tissue sample was removed with clean scissors and tweezers (as a preventive measure to eliminate potential extraneous contamination during necropsy and storage). Then, 200 to 300 mg of tissue (inner portion of each tissue sample) were accurately weighed and spiked with 50 ng of IS mixture ($^{13}C_6$-MeP, $^{13}C_6$-BuP, d_4-HeP, d_4-BzP, and $^{13}C_6$-4-HB). After equilibration for 30 min, 5 mL of acetone were added to the sample. The mixture was homogenized in a mortar and transferred to a 15 mL polypropylene (PP) tube by washing with a 2 mL mixture of methanol and acetonitrile (1:1, v/v). The combined extracts were shaken in an oscillator shaker for 60 min and then centrifuged at 5000 ×*g* for 5 min (Eppendorf Centrifuge 5804, Hamburg, Germany). The supernatant was then transferred to a new PP tube, and the mixture was concentrated to near dryness under a gentle nitrogen stream. One milliliter of a mixture of methanol and acetonitrile (1:1, v/v) was added, and an ultra-low temperature (−20 °C) treatment was employed to separate lipids from the organic solvent layer. After storage at −20 °C overnight and immediate centrifugation at 5000 ×*g* for 5 min, the supernatant was transferred into a vial for liquid chromatography tandem mass spectrometry (HPLC-MS/MS) analysis.

For plasma samples, a liquid-liquid extraction (LLE) method was used (Wang et al., 2015b). Briefly, 500 μL of plasma were transferred into a 15-mL PP tube. All procedural blanks and samples were spiked with a known amount of ISs (50 ng $^{13}C_6$-MeP, $^{13}C_6$-BuP, d_4-HeP, d_4-BzP, and $^{13}C_6$-4-HB) prior to extraction. The samples were buffered with 500 μL of 1 M ammonium acetate and 40 μL of β-glucuronidase (4 μL/mL) and incubated at 37 °C for 12 h in an incubator shaker (JEIO TECH SI-300, Seoul, Korea). Samples were then extracted twice with 5 mL each of ethyl acetate (5 × 2 mL). For each successive extraction, the mixture was shaken in an oscillator shaker for 60 min and then centrifuged at 5000 ×*g* for 5 min. The supernatants were combined and then washed with 1 mL of ultra-pure water (Barnstead International, Dubuque, IA, USA). After centrifugation again at 5000 ×*g* for 5 min, the supernatant was transferred into a 15-mL glass tube and concentrated to near-dryness under a gentle nitrogen stream. Finally, 0.5 mL of mixture of methanol/acetonitrile (1:1, v:v) were added and vortex mixed for analysis by HPLC-MS/MS.

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