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Organohalogen contaminants and metabolites in cerebrospinal fluid and cerebellum gray matter in short-beaked common dolphins and Atlantic white-sided dolphins from the western North Atlantic

Eric W. Montie [a,*,1], Christopher M. Reddy [a], Wouter A. Gebbink [b], Katie E. Touhey [c], Mark E. Hahn [a], Robert J. Letcher [b]

[a] Departments of Biology (EWM and MEH) and Marine Chemistry and Geochemistry (CMR), Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA 02543, USA
[b] Wildlife and Landscape Science Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, Ontario K1A OH3, Canada
[c] Cape Cod Stranding Network, Buzzards Bay, MA 02542, USA

Organohalogens and/or metabolites in cerebrospinal fluid and cerebellum gray matter in short-beaked common dolphins, Atlantic white-sided dolphins, and gray seal.

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ABSTRACT

Concentrations of several congeners and classes of organohalogen contaminants (OHCs) and/or their metabolites, namely organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), hydroxylated-PCBs (OH-PCBs), methylsulfonyl-PCBs ($MeSO_2$-PCBs), polybrominated diphenyl ether (PBDE) flame retardants, and OH-PBDEs, were measured in cerebrospinal fluid (CSF) of short-beaked common dolphins ($n = 2$), Atlantic white-sided dolphins ($n = 8$), and gray seal ($n = 1$) from the western North Atlantic. In three Atlantic white-sided dolphins, cerebellum gray matter (GM) was also analyzed. The levels of OCs, PCBs, $MeSO_2$-PCBs, PBDEs, and OH-PBDEs in cerebellum GM were higher than the concentrations in CSF. 4-OH-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107) was the only detectable OH-PCB congener present in CSF. The sum (Σ) OH-PCBs/Σ PCB concentration ratio in CSF was approximately two to three orders of magnitude greater than the ratio in cerebellum GM for dolphins.

1. Introduction

Odontocetes (toothed whales, dolphins, and porpoises) bioaccumulate extremely high levels of organohalogen contaminants (OHCs) in their blubber (Hansen et al., 2004; Kannan et al., 1993; Muir et al., 1996; Ross et al., 2000). These OHCs include such legacy chemicals as the organochlorine pesticides (OCs) including dichlorodiphenylethanes (i.e., DDTs), dieldrin, chlordanes, and hexachlorocyclohexanes (HCHs), polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and polychlorinated biphenyls (PCBs); and emerging compounds such as polybrominated diphenyl ethers (PBDEs) (Fair et al., 2007; Johnson-Restrepo et al., 2005; McKinney et al., 2006; Tuerk et al., 2005) and hexabromocyclododecane (HBCD) (Johnson-Restrepo et al., 2008). OHCs such as the PCBs and PBDEs can be biotransformed to hydroxylated products (i.e., OH-PCBs and OH-PBDEs, which are also classified as OHCs). OH-PCBs have been reported in the liver of beluga whales (*Delphinapterus leucas*) (McKinney et al., 2006) and in the plasma of bottlenose dolphins (*Tursiops truncatus*) (Houde et al., 2006). OH-PBDEs have also been found in beluga whale liver (McKinney et al., 2006) and in the blood of Pacific killer whales (*Orcinus orca*) (Bennett et al., in press).

PCBs, OH-PCBs, and PBDEs are considered to be developmental neurotoxicants. Schantz et al. (2003) concluded that there is strong evidence that PCB exposure is associated with negative effects in cognitive development in humans. This association is supported by controlled experiments in rodents. For example, in laboratory rats, developmental exposure to PCBs can cause hearing loss (Goldey et al., 1995; Herr et al., 1996), locomotor deficits (Roegge et al., 2004), and disorders related to learning and memory (Sable et al., 2006). Meerts et al. (2004) showed that prenatal exposure of rat pups to the environmentally relevant PCB metabolite, 4-OH-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107), can cause deficits in

* Corresponding author. Department of Biology, WHOI, MS#32, Redfield Building, Room 338, 45 Water Street, Woods Hole, MA 02543, USA.
E-mail address: emontie@marine.usf.edu (E.W. Montie).
[1] Present address: College of Marine Science, University of South Florida, 140 Seventh Avenue South, KRC 2107, St. Petersburg, FL 33701-5016, USA. Tel.: +1 727 553 1237; fax: +1 727 553 1189.

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locomotor activity and effects on the neural part of the auditory system (rather than the cochlea). Pre- or post-natal exposure of mice or rats to PBDEs can cause changes in spontaneous motor activity and disrupt performance in learning and memory tests (as reviewed by Costa and Giordano, 2007). Thus, it is important to determine the concentrations of PCBs, PBDEs, and their hydroxylated products in odontocete brains as a means to evaluate the risk of these health effects.

Despite the evidence for neurotoxic effects of PCBs and PBDEs in humans and experimental animals and the propensity of CSF to accumulate hydroxylated-OHC compounds (see below), information about residue patterns and levels of OHCs in the brains of marine mammals is limited (Table S1). To the best of our knowledge, there has been no systematic assessment of OHCs in specific brain structures. With respect to OH-PCB accumulation in the brain, only one study has reported OH-PCBs in the cerebrum of marine mammals (Kunisue et al., 2007). Furthermore, there have been no studies on the analysis of PBDEs and OH-PBDEs in the brains of any cetacean or pinniped species.

We hypothesize that the concentrations and congener profiles of PCBs and PBDEs and their hydroxylated products are different in various brain structures partly because of congener-specific differences in binding affinity for transthyretin (TTR; a thyroid hormone transport protein). In mammals, the three thyroid hormone carrier proteins (albumin, thyroid binding globulin, and TTR) are synthesized by the liver, but only TTR is synthesized in the brain, specifically in the epithelial cells of the choroid plexus (Dickson et al., 1987; Stauder et al., 1986). The choroid plexus is located in the ventricles of the brain and forms the blood–cerebrospinal fluid barrier (part of the blood–brain barrier); it produces most of the cerebrospinal fluid (CSF). TTR that is synthesized in the choroid plexus is secreted into the CSF and transports T_4 from blood into the CSF (Richardson, 2007). Since TTR is synthesized in the choroid plexus, is secreted into the CSF, and selectively binds to some OH-PCBs (Purkey et al., 2004; Ucán-Marin et al., 2009) and OH-PBDEs (Meerts et al., 2000; Ucán-Marín et al., 2009), we hypothesized that odontocete CSF retains OH-PCB and OH-PBDE congeners that have been shown to have a high affinity for TTR in other species.

The objectives of this study were i) to investigate environmentally relevant and persistent, brominated and chlorinated contaminants and metabolites (collectively designated as OHCs) in CSF from opportunistically sampled short-beaked common dolphins (*Delphinus delphis*) ($n = 2$), Atlantic white-sided dolphins (*Lagenorhynchus acutus*) ($n = 8$), and gray seal (*Halichoerus grypus*) ($n = 1$) from the western North Atlantic; and ii) to compare OHC levels in CSF versus cerebellum gray matter in Atlantic white-sided dolphins. Cerebellum gray matter was selected because it is a brain structure that contains Purkinje cells, which are sensitive to dendritic stunting by OH-PCBs *in vitro* (Kimura-Kuroda et al., 2005). This study did not investigate levels of thyroid hormones or binding of OHCs to TTR, but instead focused on measurements of OHCs in CSF and GM of stranded marine mammals as the initial step in assessing the potential for developmental neurotoxicity resulting from OHC exposure in marine mammals.

2. Materials and methods

2.1. Specimens

The gray seal, short-beaked common dolphin, and Atlantic white-sided dolphin specimens used in this study stranded live on the beaches of Cape Cod, Massachusetts, between 2004 and 2005 (Table 1). Magnetic resonance imaging (MRI) was performed on all specimens to study the neuroanatomy of these marine mammals and to develop an approach to investigate how marine biotoxins and anthropogenic pollutants affect the central nervous system (Montie, 2006). Directly related to the present study, Montie et al. (2007) utilized the magnetic resonance images of the Atlantic white-sided dolphin specimens to present an anatomically labeled, MRI-based atlas of the brain and to quantitatively describe the volumetric changes of brain structures during neurodevelopment (Montie et al., 2008). In the present study, the procedures involved in stranding response followed the methods described in Montie et al. (2007, 2008).

2.2. Sample collection

The carcasses were transported to the necropsy facility at the Woods Hole Oceanographic Institution (WHOI), where morphometric measurements were recorded. Carcasses were then prepared for MRI as previously described (Montie et al., 2007, 2008). After medical imaging, the specimen was transported back to WHOI and stored at 4 °C overnight. A complete necropsy was performed the next day and cause of death was determined (Table 1).

CSF was collected by first removing the blubber, nuchal fat, and semispinalis muscle from the dorsal, neck region. The tissue was dissected and removed down to the dura. A 20G × 1 needle equipped with a 10 cc syringe was inserted at the junction where the occipital condyle fuses with the first cervical vertebrae, into the fourth ventricle at the posterior aspect of the medulla and pons. Two to seven mL of CSF were collected in 7 mL Teflon vials and stored at −80 °C.

The brain was removed, weighed, placed inside a Teflon bag, and archived whole at −80 °C. The brain was then removed from the freezer and allowed to partially thaw (Fig. 1A) and sliced into 1 cm sections, rostral to caudal, in the coronal plane. Sections were kept frozen by placing them on a Teflon sheet, which was placed onto a metal sheet on top of a hollow tray filled with dry ice (Fig. 1B). The knife was rinsed with acetone, then hexane in between slicing sections. Cerebellum gray matter was dissected from the cerebellum (Fig. 1C), collected in Teflon bags, and archived at −20 °C prior to extraction.

2.3. Extraction and quantification of organohalogen contaminants

The extraction and clean up of CSF and cerebellum gray matter for OC, PCB, PBDE, $MeSO_2$- and OH-PCB, 4-OH-heptachlorstyrene (4-OH-HpCS), and OH-PBDE compounds were based on methods described in detail elsewhere for blood, liver, and brain with some modifications (Chu et al., 2003; Gebbink et al., 2008a,b; McKinney et al., 2006; Muir et al., 2006; Sandala et al., 2004 (and references therein)). Other BFRs including pentabromotoluene (PBT), hexabromobenzene (HBB), 2,2′,4,4′,5-pentabromobiphenyl (BB-101) and total-(α)-hexabromocyclododecane (HBCD) were also measured according to recently published procedures (Gauthier et al., 2009). Briefly, approximately 2.0 g of CSF was spiked with internal standards [six ^{13}C-labeled PCBs (CB-28, -52, -118, -153, -180, and -194), two PBDEs (BDE-30 and -71), 3-$MeSO_2$-2-CH_3-2′,3′,4′,5,5′-pentachlorbiphenyl, four ^{13}C-labeled OH-PCBs (4′-OH-CB120, 4′-OH-CB159, 4′-OH-CB172,4′-OH-CB187), and 2′-OH-BDE28] and extracted via liquid:liquid partitioning. The extraction and clean up of cerebellum gray matter for OCs, PCBs, PBDEs, and $MeSO_2$- and OH-containing compounds were based on procedures described elsewhere for brain with some modifications (Gebbink et al., 2008b). Approximately 2 g of cerebellum gray matter was homogenized and extracted. The quantification of OCs, PCBs, OH-PCBs, $MeSO_2$-PCBs, OH-PBDEs, and the BFRs including PBDEs, PBT, HBB, BB-101 and HBCD using gas chromatography-–mass selective detection (GC–MSD) have been recently described in detail elsewhere (Gauthier et al., 1998, 2009; Gebbink et al., 2008a,b).

2.4. Quality control

The analytes were identified by GC–MS comparison to that of authentic reference standards. The mean recoveries were based on the internal standards (see above) that were added at the beginning of the extraction process. Recoveries of all OHCs were on average 97% ± 21% in the CSF and 50% ± 16% in the cerebellum gray matter. The OHC recoveries in the gray matter samples were lower than the recoveries in CSF because of the challenges of extraction and isolation of OHCs from phospholipids in brain tissue. PCBs and OCs were calculated using an external standard approach, and concentrations of PCBs and OCs were corrected for recovery efficiencies if less than 80%. The concentrations of all other OHCs were determined using an internal standard approach and thus inherently corrected for recovery as well as any other analytical variation. Blank samples (one with each batch of five samples) were analyzed to monitor for background contamination. Traces of pentachlorobenzene, *trans*-chlordane, PCB 101/90, pentachlorophenol, and BDE-99 were found in the CSF blanks; while traces of only pentachlorophenol were found in the cerebellum GM blanks. Of the background OHCs detected, only the concentrations of PCB 101/90 and BDE-99 were background subtracted (per block of five CSF samples) as these concentrations were ~1% of the levels found in the samples. The concentrations of pentachlorobenzene (in CSF), *trans*-chlordane (in CSF), and pentachlorophenol (in CSF and cerebellum GM) were not reported because the background concentrations in the blanks were larger than the concentrations in the samples. The method limits of quantification (MLOQ), based on a conservative signal to noise (S/N) ratio of 10, were as follows for CSF and cerebellum GM samples, respectively: 0.04–0.10 ng/g wet weight (wt.) for OCs; 0.03–0.23 ng/g wet wt. for PCBs; 0.01–0.80 ng/g wet wt. for OH-PCBs; 0.17 ng/g wet wt. for $MeSO_2$-PCBs/-DDE;