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

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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.

Capsule

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

Keywords: Dolphin; brain; PCB; OH-PCB; PBDE

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., 2009).

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 locomotor activity and effects on the neural part of the auditory system (rather than the cochlea). Pre- or postnatal 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., 2008) and OH-PBDEs (Meerts et al., 2000; Ucán-Marin et al., 2008), 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 grey 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^{o}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 x 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 7mL Teflon vials and stored at -80^{o}C.

The brain was removed, weighed, placed inside a Teflon bag, and archived whole at -80^{o}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 ^{o}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., 2008). 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., 2008; 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, PCB101/90, 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 to 0.10 ng/g wet weight (wt.) for OCs; 0.03 to 0.23 ng/g wet wt. for PCBs; 0.01 to 0.80 ng/g wet wt. for OH-PCBs; 0.17 ng/g wet wt. for $MeSO_2$-PCBs/-DDE; 0.04 to 0.19 ng/g wet wt. for PBDEs and other BFRs; and 0.009 ng/g wet wt. for OH-PBDEs.

2.5. Data Analysis

The concentrations of OCs, PCBs, OH-PCBs, $MeSO_2$-PCBs/-DDE, PBDEs and other BFRs, and OH-PBDEs were reported on a wet wt. basis. A concentration mean and standard deviation for a specific compound was determined only if more than 60% of the samples had levels above the MLOQ. To determine the means and standard deviations for a specific analyte, the samples with analyte concentrations below the MLOQ were assigned a random value between zero and the compound-specific MLOQ. In the cases where the number of individuals were n=2, the individual concentration for each animal are given (Table 2). Summary data (mean, standard deviation, and range) comparing the levels of OHCs in CSF and GM were based on Atlantic white-sided dolphin females,

CCSN05-037-La, CCSN05-039-La, and CCSN05-040-La. Because there are well-known differences in the disposition of lipophilic contaminants between female and male cetaceans, the summary data calculated from these three females should not be viewed as representative of the population as a whole. Statistical analysis of the CSF data among the dolphin species (Table 2), or the CSF and GM data in Atlantic white-sided dolphin (Table 3), was not possible due to low and unequal sample sizes and low sample numbers, respectively.

3. Results and Discussion

3.1. Organochlorine pesticides

Of the OC pesticides present in CSF from short-beaked common dolphins (n = 2) and Atlantic white-sided dolphins (n = 8), *p,p*'-DDE was present at the highest concentration (Table 2). Hexachlorobenzene, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, and *p,p*'-DDD also were present in CSF but at lower concentrations. In Atlantic white-sided dolphins (n = 3), the levels of all OCs were higher in cerebellum GM compared to CSF (Table 3). For example, the levels of *trans*-nonachlor were, on average, thirty-four times higher in cerebellum GM compared to the concentrations found in CSF; the levels of *p,p*'-DDE were approximately five times higher in the cerebellum GM than in the CSF (Table 3). The differences in OC concentrations between CSF and cerebellum GM are most likely due to the differences in lipid content of these two tissues (Table 3). However, the lipid content in dolphin CSF was not analyzed because of logistical issues. Nonetheless, in humans, the mean lipid content in CSF is 0.0012% (Illingworth and Glover, 1970) and the lipid content in frontal lobe GM is 36-40%

(O'Brien and Sampson, 1965). We expect similar differences in lipid content to occur in dolphin CSF and dolphin cerebellum GM.

Weisbrod et al. (2001) reported chlorinated pesticide concentrations in blubber, skin, kidney, lung, and liver obtained from Atlantic white-sided dolphins that stranded in similar geographic locations to the dolphins in our study. The levels of *p,p*'-DDE were the highest of all OCs, followed by *trans*-nonachlor, which was similar to what we found in dolphin brain tissue. In the study by Weisbrod et al. (2001), the mean concentrations of *p,p*'-DDE (ng/g wet wt.) were 10 985 in blubber ($n = 6$); 2,021 in skin ($n = 6$); 1,243 in kidney ($n = 2$); 303 in lung ($n = 2$); and 228 in liver ($n = 6$). The concentrations of *p,p*'-DDE in blubber, skin, kidney, lung, and liver reported previously by Weisbrod et al. (2001) were orders of magnitude higher (on a wet wt. basis) than the levels found in CSF (2.23 ng/g wet wt.) and cerebellum GM (12.06 ng/g wet wt.) from Atlantic white-sided dolphins in our study (Table 3).

The dichlorodiphenylethanes (e.g., *p,p*'-DDE, *p,p*'-DDD), the chlorinated cyclodienes present in chlordane (e.g., *cis*-nonachlor, *trans*-nonachlor, *cis*-chlordane, and *trans*-chlordane), and the chlorinated benzenes (e.g., hexachlorobenzene) found in CSF and cerebellum GM have been shown to be neurotoxic in humans and wildlife species (reviewed by Ecobichon (1996)). Environmental exposure of *p,p*'-DDE has been associated with alterations in the brains of American robins (*Turdus migratorius*), including a decrease in the size of two song control nuclei – the robust nucleus of the arcopallium (RA) and the HVC – and a reduction in the volume of the nucleus intercollicularis (IC_o), a brain structure critical for normal sexual behavior (Iwaniuk et al., 2006). Both RA and HVC contain a high prevalence of androgen receptors (Gahr, 2001;

Metzdorf et al., 1999), and testosterone has been shown to increase the size of these brain structures (Alvarez-Borda and Nottebohm, 2002; Ball et al., 2002). Thus, the ability of *p,p*'-DDE to act as potent androgen receptor antagonist (Kelce et al., 1995) may explain the effects of *p,p*'-DDE on brain structure size (Iwaniuk et al., 2006). Chemicals found in the pesticide chlordane (e.g., *cis*-nonachlor, *trans*-nonachlor, *cis*-chlordane, and *trans*-chlordane) are neurotoxicants that block the λ-aminobutyric acid (GABA) receptor found in the brain. The blocking of this ion channel impedes the uptake of chloride ions by neurons and causes a state of uncontrolled excitation, which may explain why chlordane has been shown to negatively affect balance and reaction times in exposed humans (Kilburn and Thornton, 1995).

3.2. PCB and PCB metabolites

In the short-beaked common dolphins (n = 2), the pattern of PCBs present in CSF was dominated by seven congeners: CB-105, -118, -138, -149, -153, -180, and -187, which together comprised 62 – 71% of the ΣPCBs (Table 2). In the male (*n* = 2) Atlantic white-sided dolphins, CSF was dominated by PCB congeners CB-99, -138, -149, -153, -180, -187, which comprised 64 – 67% of the ΣPCBs (Table 2). A similar PCB congener pattern was observed in the CSF of the female (*n* = 6) Atlantic white-sided dolphins; CB-99, -101/90, -138, -149, -153, -180, and -187 comprised approximately 65% of the ΣPCBs (Table 2). In the CSF of all dolphins, CB-153 was the most abundant PCB congener. In Atlantic white-sided dolphins (n = 3) in which both CSF and cerebellum GM were analyzed, the levels of all PCB congeners were higher in cerebellum GM compared to the concentrations in CSF (Fig. 2A). The ΣPCB concentrations were on

average thirty-eight times higher in cerebellum GM than in CSF, which may be explained at least in part by the higher lipid content of cerebellum GM compared to CSF.

The PCB congener pattern in cerebellum GM was similar to the pattern observed in CSF for these Atlantic white-sided dolphins (CCSN05-037-La, CCSN05-039-La, and CCSN05-040-La) (Fig. 2B). CB-153 was the most abundant PCB congener in cerebellum GM and CSF (22.4 ± 0.6% and 22.5 ± 2.2% of total, respectively) followed by CB-138 (13.8 ± 0.9% and 12.2 ± 1.3%, respectively), and CB-149 (7.0 ± 0.2% and 8.2 ± 0.6%, respectively) (Fig. 2B). Weisbrod et al. (2001) also reported concentrations of CB-153 in Atlantic white-sided dolphins to be the highest of all PCB congeners. However, the levels measured in cerebellum GM in our study (40.81 ± 18.75 ng/g wet wt.) (Table 3) were higher than the concentrations reported in liver (9.4 ± 75.9 ng/g wet wt.) by Weisbrod et al. (2001), even when lipid normalized.

PCBs are clearly transported into the cerebellum of white-sided dolphins despite the presence of the blood brain barrier. Such exposure to PCBs may represent a neurotoxicological risk. Developmental exposure to Aroclor 1254 (a technical-grade mixture of 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). The mechanisms of PCB neurotoxicity most likely involve multiple modes of action, which largely depend upon the congener structure. The mechanisms include effects on thyroid hormone dependent neurodevelopment (Zoeller et al., 2002); binding to and activation of the aryl hydrocarbon receptor (AhR) by planar PCBs by a mechanism similar to that of dioxin (Collins et al., 2008; Powers et al., 2005); impairment of the function of glutamate-nitric oxide-cGMP pathway (Piedrafita et al.,

2008); effects on brain cholinergic muscarinic receptors (Coccini et al., 2006); and damage to dopaminergic neurons (Seegal et al., 2002). In our study, CB-153 (a PCB congener with a non-planar structure) was the most abundant PCB congener in CSF and cerebellum GM. In rats, exposure to CB-153 during development causes reduction in learning ability, behavioral alterations, and reduced long-term potentiation (Holene et al., 1998; Hussain et al., 2000; Schantz et al., 1995).

In the short-beaked common dolphins (n = 2) and Atlantic white-sided dolphins (n = 8), the only detectable OH-PCB congener present in CSF was 4-OH-CB107/4'-OH-CB108 (Table 2). In gray seal CSF (n = 1), 4-OH-HpCS, 4-OH-CB107/4'-OH-CB108, 4-OH-CB146, 3'-OH-CB138, 4-OH-CB163, 4-OH-CB178, and 4'-OH-CB202 were detected. Of these OH-PCB congeners, the concentration of 4-OH-CB107/4'-OH-CB108 was the highest, similar to what was observed in the dolphins (Table 2). Interestingly, the concentration of 4-OH-CB107/4'-OH-CB108 in gray seal CSF (1,636.40 ng/g wet wt) was eighty-two times higher than the highest concentration found in the dolphin CSF sample set (19.72 ng/g wet wt in CCSN05-040-La) (Table 2).

The MLOQs for OH-PCBs in cerebellum GM were much lower than those in CSF (0.01 compared to 0.80, respectively), which increased the detectability of OH-PCBs in cerebellum GM. In Atlantic white-sided dolphins, detectable concentrations of the following OH-PCB congeners were found in cerebellum GM: 4-OH-CB107/4'-OH-CB108, 3'-OH-CB182, 3'-OH-CB183, and 3'-OH-CB180 (Table 3). However, in the Atlantic white-sided dolphin CCSN05-040-La that contained detectable concentrations of 4-OH-CB107/4'-OH-CB108 in both CSF and cerebellum GM, the concentration in CSF

was seventy times greater than that measured in cerebellum GM (19.72 compared to 0.28 ng/g wet wt.) (Table 3).

To our knowledge, there is only one other study that measured the concentrations of OH-PCBs in CSF of any species. In that effort, Takasuga et al. (2004) showed that the levels of OH-PCBs in human CSF were higher than those of PCBs, an opposite pattern to what was observed in serum. In the only other study on OH-PCB residue levels in cetacean brains, Kunisue et al. (2007) reported concentrations of ΣOH-PCBs in the cerebrum of melon-headed whales, striped dolphins, and finless porpoises that ranged from 0.020 to 0.290, 0.020 to 0.330, and 0.170 to 0.240 ng/g wet wt, respectively. These concentrations are approximately 60 to 1000-fold lower than the levels of ΣOH-PCBs in CSF of Atlantic white-sided and common dolphins in the present study.

The ΣOH-PCB/ΣPCB concentration ratios in CSF were approximately two orders of magnitude higher than the ratio in cerebellum GM (Fig. 3). In Kunisue et al. (2007), the ΣOH-PCB/ΣPCB ratio for cerebrum in melon-headed whales, striped dolphins, and finless porpoises were approximately three orders of magnitude lower than the ratios in CSF and approximately one order of magnitude lower than the ratios in cerebellum GM in dolphins from our study (Fig. 3). In addition, the ΣOH-PCB/ΣPCB concentration ratio for the pons and medulla oblongata (brainstem) reported for polar bears from East Greenland (collected in 2002) was one order of magnitude lower than the ratios for dolphin CSF in our study (Gebbink et al., 2008a,b).

When comparing the ΣOH-PCB/ΣPCB concentration ratio among different brain tissues in this study and in previous studies with other wildlife species, there is an

indication that CSF retains higher concentrations of OH-PCBs compared to other brain structures (Fig. 3). This observation suggests that the determination of OH-PCBs in one brain compartment is not representative of the brain as a whole, and that CSF-specific localization of OH-PCBs may be occurring. This observation may possibly be explained by an association of OH-PCBs with TTR. TTR is synthesized in the choroid plexus of the brain, is secreted into the CSF, and has been shown to selectively bind some OH-PCBs in humans and birds (Purkey et al., 2004, Ucan-Marin et al., 2009). Furthermore, 4-OH-CB107 (the only OH-PCB congener detected in cetacean CSF in our study) has been shown to have excellent binding selectivity for TTR in human plasma (Purkey et al., 2004). The presence and properties of TTR in cetaceans is not well understood. However, TTR has been identified in the liver of the Atlantic white-sided dolphin; the partial amino acid sequence showed 84%, 80%, 74%, and 75% identity to TTRs from pig (*S. scrofa*), human (*H. sapiens*), mouse (*M. musculus*), and rat (*R. norvegicus*), respectively (Montie, 2006). Whether 4-OH-CB107 or other OH-PCB congeners selectively bind to Atlantic white-sided dolphin TTR is unknown. However, if this hypothesis is true, it may explain why the ΣOH-PCB/ΣPCB concentration ratio is higher in CSF compared to ratios in other brain structures.

In our study, the concentration of 4-OH-CB107/4'-OH-CB108 in the gray seal CSF sample was approximately two orders of magnitude higher than the levels present in dolphin CSF (Table 2). Furthermore, the ΣOH-PCB/ΣPCB ratio in the gray seal CSF sample was 5279, approximately 5000 times higher than the ΣOH-PCB/ΣPCB ratio in dolphin CSF. This difference could be explained by possible differences in biotransformation rates and/or metabolite retention between pinnipeds and cetaceans.

Cytochrome P450 (CYP) monooxygenases mediate the catalytic conversion of PCBs to OH-PCBs. In humans, fifty-nine CYP genes have been identified and approximately half of these belong to CYP subfamilies that code for enzymes that mediate the metabolism of xenobiotics. CYP1A1-, CYP2A-, CYP2B-, and/or CYP3A-type enzymes, depending on the species, are involved in PCB metabolism (Letcher et al., 2000; Yoshimura et al., 1987). In rats, CYP1A1 can metabolize CB-105 and -118 to form 4-OH-CB107 (Gauger et al., 2007; Sjodin, 1998) but additional CYP enzymes may have these catalytic capabilities. PCB residue patterns in cetaceans suggest that CYP2B-type enzyme activity is low (Duinker et al., 1989; Tanabe et al., 1988; Norstrom et al., 1992). Low rates of metabolism of 2,2',5,5'-tetraCB (CB-52) in pilot and beluga whale and low rates of CYP2B-activities in beluga also support this hypothesis (White et al., 1994, 2000). In grey seals, CYP1-, CYP2-, and CYP3-like enzymes are expressed in liver and extrahepatic tissues (Nyman et al., 2000), and CYP1A- and CYP3A-like enzymes are involved in the metabolism of mono-ortho PCB congeners (such as CB-105 and -118) (Li et al., 2003). It is possible that the metabolism of PCBs to OH-PCBs is greater in pinnipeds than cetaceans, but there are no studies that have directly compared the metabolic capabilities of representative taxa from these two orders. Nonetheless, the very high concentrations of 4-OH-CB107/4'-OH-CB108 in the gray seal CSF sample (1636.40 ng/g wet wt.) warrant further studies of OH-PCBs in pinniped CSF.

Some OH-PCBs (e.g., 4-OH-CB106) have been shown to bind to the human thyroid hormone receptor (TR) (You et al., 2006), while other OH-PCB metabolites derived from CB-105 and -118 have been shown to activate the rat TR in transient transfection assays (Gauger et al., 2007). Other studies report that OH-PCBs can inhibit

thyroid function in the brain. For example, Kimura-Kuroda et al. (2005) reported that 4'-OH-CB106 and 4'-OH-CB159 significantly inhibited thyroid hormone (T_3)-dependent extension of Purkinje cell dendrites extracted from mouse cerebellum *in vitro*. Regardless of the exact mechanism, prenatal exposure of rat pups to 4-OH-CB107 has been shown to cause deficits in locomotor activity and effects on the neural part of the auditory system (Meerts et al., 2004).

$MeSO_2$-PCBs were not detected in CSF. However, cerebellum GM in Atlantic white-sided dolphins (n = 3) contained, 3'-$MeSO_2$-CB49, and 4'-$MeSO_2$-CB49. There are no other reported studies of $MeSO_2$-PCBs in brain structures of cetaceans. In the pons and medulla oblongata collected from polar bears, the concentrations of $MeSO_2$-PCBs ranged from 9 to 23 ng/g wet wt (Gebbink et al., 2008a,b). The lack or low levels of $MeSO_2$-PCBs in CSF and cerebellum GM may suggest that the blood brain barrier is effectively excluding such PCB metabolites from the brain, assuming that $MeSO_2$-PCBs are metabolically formed in the dolphin and/or accumulated from the diet.

3.3. Brominated Flame Retardants and OH-PBDEs

In the short-beaked common dolphins (n = 2), of all the PBDE congeners and BFRs monitored, trace levels of BDE-47 and BDE-100 were present in CSF (Table 2). CSF of the Atlantic white-sided dolphins (n = 8) contained BDE-47, BDE-99, BDE-100, and BDE-153/BB-154, with BDE-47 as the major congener. In Atlantic white-sided dolphins in which both CSF and cerebellum GM were analyzed (n = 3), the levels of all identified PBDEs were higher in cerebellum GM compared to the concentrations in CSF (Fig. 4). BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154/BB-153, and BDE-

183 were found in cerebellum GM (Fig. 4). These patterns are likely a function of the higher lipid content of cerebellum GM compared to CSF. As in CSF, in cerebellum GM, BDE-47 was the predominant brominated flame retardant measured.

To our knowledge, this study is the first to report PBDEs in brain tissue collected from cetaceans. Tuerk et al. (2005) reported levels of PBDEs in blubber obtained from Atlantic white-sided dolphins that stranded in similar geographic locations to the dolphins in our study. BDE-47 was the major PBDE congener in the blubber of those dolphins, similar to what we found in brain tissue. The concentrations of PBDEs in blubber were orders of magnitude higher than the levels in CSF and cerebellum GM samples from Atlantic white-sided dolphins. A combination of decreased lipid content and the blood-brain barrier may restrict the accumulation of PBDEs in brain compartments to the degree present in blubber.

The greatest concern regarding exposure to PBDEs is their developmental neurotoxicity (reviewed by Costa and Giordano (2007)). Studies with experimental animals have indicated that pre- and postnatal exposure to PBDEs impairs motor activity and cognitive behavior. Exposure of neonatal rats to BDE-47 (the most prominent PBDE congener in CSF and cerebellum GM) caused poor performance in horizontal locomotor activity (Eriksson et al., 2001). In addition to the effects on the cerebellum, exposure of neonatal rats to some PBDEs (e.g., BDE-153) impairs learning and memory and decreases hippocampal cholinergic receptors (Viberg et al., 2003). Mechanisms of PBDE neurotoxicity are still being elucidated, but include interference with thyroid hormone signaling, disruption of signal transduction pathways, and oxidative stress (reviewed by Costa and Giordano (2007)).

OH-PBDEs were detected in CSF and cerebellum GM (Tables 2 and 3). In CSF, trace levels of 6'-OH-BDE49 were detected in gray seal (n = 1), short-beaked common dolphins (n = 2), and Atlantic white-sided dolphins (n = 8); in addition, 6-OH-BDE47 was present in dolphin CSF (Tables 2 and 3). In Atlantic white-sided dolphins (n = 3), cerebellum GM contained detectable levels of 6'-OH-BDE49 and 4'-OH-BDE49 but not 6-OH-BDE47. Interestingly, in cerebellum GM, the concentration of 4'-OH-BDE49 (which was not detected in CSF) was approximately fifty times greater than that of 6'-OH-BDE49. Gebbink et al. (2008a,b) analyzed for OH-PBDEs in pons-medulla oblongata (collectively termed brainstem) in polar bears but did not find detectable levels of these brominated compounds. In plasma collected from polar bears, Verreault et al. (2005) reported quantifiable levels of 4'-OH-BDE49. It is possible that 4'-OH-BDE49 is a metabolite of the precursor BDE-47, as discussed by Verreault et al. (2005).

4. Conclusions

A number of OHCs identified in CSF and cerebellum in this study of short-beaked common and/or white-sided dolphins, as well as a gray seal from the western North Atlantic, have been demonstrated to be developmental neurotoxicants in controlled experiments with rodents. Thus, these chemicals may affect neurodevelopment in exposed marine mammals. One of the most important points when addressing this risk is that marine mammals are not just exposed to one OHC but rather a "cocktail" of legacy OCs and PCBs, emerging contaminants such PBDEs, their metabolites and/or degradation products, other developmental neurotoxicants (e.g., lead, methylmercury, perchlorate, dioxins, etc.), as well as natural, marine neurotoxins (associated with

stochastic exposure to harmful algal blooms). The significance of these multiple co-exposures is still unclear, but the potential for additive and/or synergistic effects on the central nervous system should be considered. Presently, as a scientific community, we are ill-equipped to address these questions in marine mammals. Future research should focus on developing approaches to better assess the impact of OHCs on the central nervous system in marine mammals (such as those methods described in Montie et al. (2007; 2008)) and then apply these techniques to health assessment and epidemiology studies with cetaceans and pinnipeds.

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Figure Legends

Fig. 1. Collection of cerebellum gray matter samples from Atlantic white-sided dolphins. (A) The brain was removed and archived whole at -80^{o}C. (B) The brain was sliced into 1 cm sections, rostral to caudal, in the coronal plane. (C) Cerebellum gray matter was dissected from the cerebellum and archived at -20 ^{o}C prior to extraction. The region that was collected is marked by a yellow circle.

Fig. 2. PCB congeners in cerebrospinal fluid (CSF, n=3) and cerebellum gray matter (GM, n=3) in Atlantic white-sided dolphins (CCSN05-037-La, CCSN05-039-La, CCSN05-040-La) from the western North Atlantic. (A) Mean concentrations ± standard deviations for each PCB congener (ng/g wet wt). (B) Mean percentages ± standard deviations for each congener calculated from the total PCB mix. Red = CSF; Blue = cerebellum GM.

Fig. 3. A comparison of mean ΣOH-PCB/ΣPCB concentration ratios in different brain structures collected from a gray seal, short-beaked common dolphin, Atlantic white-sided dolphins, and from wildlife reported elsewhere. [1] Kunisue et al. (2007), [2] Gebbink et al. (2008a,b). CSF = cerebrospinal fluid.

Fig. 4. Mean concentrations ± standard deviations of PBDE congeners (ng/g wet wt) in cerebrospinal fluid (CSF) and cerebellum gray matter (GM) of Atlantic white-sided dolphins (CCSN05-037-La, CCSN05-039-La, CCSN05-040-La) from the western North Atlantic.