Arch Environ Contam Toxicol
DOI 10.1007/s00244-017-0404-y

Persistent Organic Pollutant and Hormone Levels in Harbor Porpoise with B Cell Lymphoma

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Received: 3 January 2017 / Accepted: 12 April 2017

Abstract B-cell lymphoma, a common morphologic variant of non-Hodgkin lymphoma, has been associated with persistent pollutants in humans, but this association is not well-characterized in top-level predators sharing marine resources with humans. We characterized and compared blubber contaminants and hormones of a pregnant harbor porpoise (*Phocoena phocoena*) with B-cell lymphoma, with those in two presumed healthy fishery bycaught porpoises with no lymphoma: a pregnant adult and female juvenile. Common historic use compounds, including polychlorinated biphenyls, polybrominated diphenyl ethers, and pesticides, were evaluated in blubber samples from three porpoises. In addition, blubber cortisol and progesterone levels (ng/g) were determined in all three animals. Total pollutant concentrations were highest in the juvenile porpoise, followed by the lymphoma porpoise and the nonlymphoma adult. Blubber cortisol concentrations were 191% greater in the pregnant with lymphoma porpoise compared with the pregnant no lymphoma porpoise, and 89% greater in the juvenile female compared with the pregnant no lymphoma porpoise. Although both adults were pregnant, progesterone levels were substantially greater (90%) in the healthy compared with the lymphoma adult. Health monitoring of top-level marine predators, such as porpoise, provides a sentinel measure of contaminants that serve as indicators of potential environmental exposure to humans.

Lymphomas are malignant tumors of the lymphoid system reported in humans (Word and Matasar 2012) and higher vertebrate animals (Jaber et al. 2005; Ylitalo et al. 2005). Two major types currently are recognized in humans: non-Hodgkins (NHL) and Hodgkins (Word and Matasar 2012). B-cell lymphoma (BCL) is a common morphologic variant of NHL (80–85% of all cases). B-cell lymphoma is documented in humans (Swerdlow et al. 2008) and other mammals, including horses (Durham et al. 2012) and

Electronic supplementary material The online version of this article (doi:10.1007/s00244-017-0404-y) contains supplementary material, which is available to authorized users.

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Published online: 26 April 2017

orangutans (Ikpatt et al. 2014). However, BCL is relatively uncommon in cetaceans (Newman and Smith 2006) and has not been described in porpoises.

The incidence or diagnosis of NHLs, such as BCLs, has been increasing in humans during the latter twentieth century (Devesa and Fears 1992; Hartge and Devesa 1992). Coincident with this time period was increased use of and environmental contamination by polychlorinated biphenyls (PCBs), before their manufacturing was banned in the United States in 1979 (US Environmental Protection Agency 2016). This convergence prompted several epidemiological studies to determine that the link between PCBs and NHL resulted from several important factors: temporal correspondence between exposure to PCBs and increasing incidence of NHL; toxicological and epidemiological evidence for the immunotoxicity and carcinogenicity of PCBs; and the structural similarity between PCBs and dioxins, which are known human carcinogens that also are associated with NHL (Kramer et al. 2012). Polybrominated diphenyl ethers (PBDEs), organobromine compounds first put into commercial production as flame retardants in the mid 1960s, have raised environmental and health concerns due to their widespread use and increased production since the 1990s (Vonderheide et al. 2008). The use and persistence of PCBs and PBDEs also represents a significant toxicological concern for higher trophic level organisms, such as marine mammals (Rotander et al. 2012). PCBs are associated with deleterious health effects in marine mammals, including increased susceptibility to infectious diseases, immunotoxicity, neoplasia, and endocrine disruption (Ross et al. 1996; Ylitalo et al. 2005; Hall et al. 2006; Hellou et al. 2013).

Published evidence from human studies suggests the more highly chlorinated PCB and PBDE congeners may contribute to the risk of NHL (Hardell et al. 1996, 1998a; De Roos et al. 2005; Bräuner et al. 2012). In marine mammals, similar potential associations are reported in belugas (*Delphinapterus leucas*) (De Guise et al. 1994) and California sea lions (*Zalophus californianus*) (Ylitalo et al. 2005). Although PCBs are not considered directly genotoxic, they may encourage lymphomagenesis through immune dysregulation and immunotoxicity, resulting in genetic mutations (Freeman and Kohles 2012; Kramer et al. 2012). In a rodent study, individual PCB congeners caused DNA adduct formation and cellular mutations. The PCBs were hypothesized to be metabolized into genotoxic metabolites in vitro and in vivo, acting as initiating agents, and inducing cancer (Ludewig et al. 2008). Those PCB congeners most associated with increased NHL risk include coplanar PCBs 156, 180, and 194 (De Roos et al. 2005) and 118, 138, 153, and 170 (Engels et al. 2005; Grulich et al. 2007). Studies suggest PBDE-47 in adipose tissues is associated with increased risk of NHL in humans (Hardell et al. 1998a, b).

An association between pesticide exposure and incidence of lymphoma has been observed, most notably in dogs exposed to lawn pesticide treatments and humans involved in agricultural occupations (Hayes et al. 1991; Zahm and Blair 1992). Within Puget Sound, recent levels of DDTs in marine fish and sediments are generally lower than concentrations of PCBs (Brown et al. 1998; West et al. 2008), with ΣDDT levels measured in whole bodies of outmigrating juvenile Puget Sound Chinook salmon (*Oncorhynchus tshawytscha*) more than two times lower than ΣPCB concentrations (Johnson et al. 2007).

The inland transboundary waters of Washington State have historically received large amounts of endogenous and exogenous pollutants from agricultural, industrial, and household sources (Waldichuk 1983; Johnson et al. 2010). These included wastewater and agricultural runoff, wood and paper pulp mills, and industrial activities, such as manufacturing and gravel mining. Numerous sites within Puget Sound, Washington were heavily contaminated by PCBs from industrial activities (West et al. 2008; O'Neill and West 2009). In addition to these localized sources of pollutants, more global-type sources transported these chemicals to the Salish Sea through atmospheric transport (Noël et al. 2009) and via food webs from prey fish species, such as Pacific herring (*Clupea pallasii*) (West et al. 2008) and salmon (*Oncorhynchus* spp.) (O'Neill and West 2009).

Harbor porpoise (*Phocoena phocoena*) are among the top predators of the coastal trophic system in Washington's inland waters, exposing them to contaminant accumulation from the local food web. Moreover, this species and other regional marine mammals have accumulated persistent organic pollutants (POPs), such as PCBs and PBDEs (Calambokidis and Barlow 1991; Ross et al. 2013). Prey species, such as herring, may play an important role in transferring POPs to predators at higher trophic levels due to the former's relatively high abundance, geographic distribution and lipophilic content (West et al. 2008). Given their coastal inland water home range and prey preferences, harbor porpoise within the Salish Sea are a good model to investigate pollutants and potential links to disease. Therefore, the purpose of this initial study was to characterize and compare the blubber POPs, along with stress and sex hormone concentrations, of an adult pregnant harbor porpoise with B-cell lymphoma, to those in presumed healthy fishery by-caught porpoises with no lymphoma: an adult, pregnant harbor porpoise and a young female yearling, as well as discuss the potential association between POPs and lymphoma.

Materials and Methods

Harbor Porpoise Samples

Detailed pathologic examinations were conducted, and causes of death determined, for the case porpoise with lymphoma (abbreviated as adult lymphoma positive, AL+) and two comparison presumed healthy, fishery by-caught animals: an adult pregnant female with no lymphoma (adult lymphoma negative, AL−) and a female yearling with no lymphoma (YL−), all from Washington State's central inland waters and collected under stranding response permits. Full-thickness blubber samples, extending down to the underlying muscle, for the AL+ were collected from a 167-cm, female, adult harbor porpoise at a standardized site (approximately midway on the dorsum between the blowhole and just anterior to the insertion of the dorsal fin), placed in clean aluminum foil, and frozen at −20 °C. This porpoise was found fresh dead, in poor nutritional condition (dorsal blubber thickness [DBT] = 1.1 cm), on 1 November 2013 on Whidbey Island and was subsequently diagnosed with B-cell lymphoma in the lungs, mediastinal lymph nodes, and spleen. She was pregnant with a 5.5-cm long, early gestational fetus in the left uterine horn. Standard blubber samples also were collected from two apparently healthy fishery by-caught porpoises (excellent nutritional condition) and frozen at −20 °C. The AL− was an approximately 165-cm, adult lactating harbor porpoise (DBT = 1.6 cm) with a 7-cm fetus found 31 October 2012 in Kingston, and YL− was a 110.5-cm female yearling (DBT = 2.1 cm) with blunt force trauma and hemorrhage of the skull base soft tissues and first two cervical vertebrae from fishing gear entanglement, collected 21 September 2014 from Whidbey Island.

Contaminant Analyses

The initial analyte list screened by gas chromatography–mass spectrometry (GC–MS) consisted of 65 compounds (see Online Resource 1). The final target analytes, detected in at least one of the study animals, are reported in ng/g and include six PBDE congeners (47, 49, 66, 99, 100, 153), 16 PCB congeners (101, 105, 110, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, 187, 189), and 15 organochlorine pesticides (alpha-, beta-, and gamma-hexachlorocyclohexane, heptachlor, heptachlor-epoxide, mirex, hexachlorobenzene, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, endrine ketone, *o*, *p*′-DDD, *o*, *p*′-DDE, *o*, *p*′-DDT, *p*, *p*′-DDD, *p*, *p*′-DDE, and *p*, *p*′-DDT).

Blubber aliquots were extracted using accelerated solvent extraction (ASE 350, Thermo, Sunnyvale, CA) as previously described (Robinson et al. 2013a). Briefly, 100-mL cells were loaded with 5 g of silica gel and 55 g of acidic silica gel. The cells were conditioned using a 1:1 v/v mixture of dichloromethane and hexane. The full-thickness blubber punches (0.50–1 g) were homogenized in sodium sulfate using a mortar and pestle and loaded into the cell. Samples were spiked with isotopically labeled surrogate standards and left on the bench for 1 h for quasi-equilibration. Samples were then extracted using hexane. Once extracted, samples were blown down to ∼200 µL and spiked with isotopically labeled internal standards. Samples were analyzed using the instrumentation described below.

DDT, DDE, and DDD related compounds were detected and quantified using a Varian CP-3900 GC system coupled to a Saturn 2100T ion trap mass spectrometer with electron impact ionization using a previously described method (Robinson et al. 2013b). The remainder of the target analytes was detected and quantified using an Agilent 7890 GC coupled to an Agilent 5974 MS in electron capture negative ionization mode as previously described in Trumble et al. (2012). Combined, the average surrogate recovery was 55% for GC–MS analysis.

Hormone Analyses

Blubber extractions followed the methods reported in Kellar et al. (2006). Briefly, approximately 0.1 g of blubber was suspended in 3 mL of phosphate buffered saline and homogenized in a Bullet Blender (Next Advance) using 0.5-mm glass beads. An aliquot of 600 µL of the homogenate was combined with 2 mL of 4:1 ethanol:acetone. This solution was vortexed for 60 s using a multitube vortex mixer (VWR International, VX 2500) and centrifuged (3000 rcf, 15 min). The supernatant was collected and evaporated under nitrogen, and the residue was resuspended in 1000 µL of acetonitrile. This solution was vortexed, and 1000 µL of hexane was added. The acetonitrile portion was aspirated into a new tube and evaporated under a gentle stream of nitrogen for 2 h or until all remaining liquid was gone. The residue was centrifuged, topped with 0.5 mL of assay buffer, and stored at −20 °C until analyzed.

Cortisol levels (ng/g) in each blubber aliquot were determined using a Correlate-EIA cortisol kit (ENZO ADI-900-071), whereas progesterone was determined using a Correlate-EIA progesterone kit (ENZO ADI-901-011). Samples were individually vortexed prior to hormone quantification, and cortisol and progesterone assays were run according to manufacturer instructions. The amount of antibody-bound labeled cortisol or progesterone in the samples was assessed against standard calibration curves. To control measurement error contributed by all extraction

and quantification steps, each sample was extracted and measured at least twice and reported as the average concentrations. Extraction efficiency was determined for each separate assay kit by spiking selected subsamples with dilutions of progesterone. The extraction efficiency was calculated as the amount of quantified progesterone of the spiked samples minus the amount in nonspiked samples, divided by the amount of progesterone added before extraction. Hormone kits and QA/QC protocols (triplicate/parallelism) were in accordance with the National Committee for Clinical Laboratory Standards Evaluation Protocols.

Viral Screening

Because infectious agents may be associated with development of lymphomas (Bossart et al. 1997), such as the Epstein-Barr virus, a herpesvirus associated with BCL in humans (Saha and Robertson 2011), tumor sections from AL+ mediastinal lymph nodes were submitted to the Zoological Medicine and Wildlife Disease Laboratory (University of Florida, College of Veterinary Medicine, Gainesville, FL) for morbillivirus and herpesvirus screening by reverse transcriptase and standard polymerase chain reaction (PCR), respectively, based on established methods (VanDevanter et al. 1996; Tong et al. 2008).

Results

The lymphoma was classified as a multicentric B-cell lymphoma with plasmocytoid differentiation. Neoplastic cells effaced and infiltrated into the mediastinal lymph nodes and parts of the lung and spleen. Diagnosis of B-cell lineage was confirmed by positive immunohistochemistry using an anti-CD79a antibody marker. Further characterization of the tumor type is ongoing. Of the 76 target pollutants, 38 were detected in at least one of the three porpoises, with 34 recovered from both the AL+ and AL− and 35 from the YL− (Table 1). Total pollutant concentrations (in ng/g lipid) were slightly higher in the YL− porpoise (8300) than the AL+ (7900). Total blubber PBDE (Fig. 1) and ΣPCB (Fig. 2) concentrations were highest in the YL− with 1700 and 4700, respectively, compared with AL+ and AL−, whereas total pesticide concentration (Fig. 3) was greatest in the AL+ (4500). Additionally, of the 34 analytes recovered from the AL+ and AL−, 25 (74%) were higher in the AL+. With the greatest total number of contaminants detected in the YL−, individual analyte concentrations were correspondingly greatest in this animal, compared among all three porpoises. DDTs were detected in the AL+ and YL−, but not in the AL−; however, quantification was limited due to unknown chemical interference. PBDEs 28 and 33 were only detected in the YL− (12.7) as was PCB congener 169 (27.3). ΣDDE levels in the AL+ porpoise was 3700 but could not be precisely quantified due to the value being beyond the linearity of the calibration curve.

Blubber cortisol levels (ng/g lipid) in the lymphoma porpoise followed a pattern of AL+ > YL− > AL−. AL+ was 230,000, which was 177 and 191% higher than the comparison-control animals, YL− (14,000) and AL− (5500), respectively (Fig. 4). The juvenile cortisol level was 89% greater than that in the pregnant porpoise with no lymphoma. Blubber progesterone levels (AL− > AL+ > YL−) were more than doubled in AL− (110,000) compared with the AL+ porpoise (42,000), whereas blubber progesterone in the YL− was 110. Viral screening (PCR) for herpesvirus and morbillivirus was negative.

Discussion

Lymphomas are one of the most common neoplasms reported in humans and many animal species. Compared with other species, neoplasms of all types are far less common in marine mammals, with the exception of California sea lions (Venn-Watson et al. 2012) and dolphins and whales (Bossart et al. 1997; Jaber et al. 2005). Howard et al. (1983) described several cancerous tumor types in nine different cetacean species. However, this study presents the first reported B-cell lymphoma in a harbor porpoise.

Many variables may affect measured pollutant loads in blubber and include season sampled, condition of the blubber sampled, and the animal's age, health, and nutritive condition, gender, reproductive status, prior maternal offloading, and diet (Jepson et al. 2005, 2016). In general, contaminant levels were highest in the YL−, likely reflecting receipt of maternal transfer of pollutants during gestation and lactation (Desforges et al. 2012). Between the two pregnant adults, contaminants were consistently higher in the AL+. However, the concentrations noted in the lymphoma case may have been partly due to mobilization of pollutants from the blubber, as often is observed as an animal loses its nutritional condition due to chronic illness or poor diet (Jepson et al. 2005). Differences between the two individuals may be reflective of age or number of pregnancies and therefore additional maternal offload. Transfer rates to offspring tend to decrease with mother's age; therefore, contaminant levels are higher in primiparous compared with multiparous females, because they have not yet offloaded their contaminants to their offspring (Borrell et al. 1995). Transfer of contaminants to offspring follows birth order, diminishing with the dam's age, with the highest load transferred to the first-born compared with

Table 1 Persistent pollutants in the blubber of harbor porpoises in the Salish Sea, Washington State

Analyte	Contaminant concentrations (ng/g lipid) in the blubber of:		
	AL+	AL−	YL−
PBDEs			
PBDE 28 + 33	ND	ND	12.7
PBDE 47	525.5	299.8	1177.3
PBDE 49	97.8[a]	37.7[a]	105.8
PBDE 66	9.8	2.4[a]	20.8[a]
PBDE 99	150.1	130.0	110.3
PBDE 100	143.0	93.8	150.1[a]
PBDE 153	40.4	32.9[a]	2.4[b]
PBDE 154	58.3	67.9	33.3
Total PBDEs	1024.9	708.9	1702.3
PCBs			
PCB 101	188.7	106.9	385.9
PCB 105	44.8	25.4	90.8
PCB 110	69.1	38.7	103.0
PCB 114	2.4	3.1	ND
PCB 118	140.8	77.0	377.6
PCB 123	4.1[a]	5.7	ND
PCB 126	5.6[a]	6.1	9.5
PCB 138	692.2	424.7	1520.1
PCB 153	623.4	393.4	1429.9
PCB 156	4.8[a]	2.9[a]	8.3
PCB 157	3.2[a]	2.7	8.4
PCB 167	11.3	ND	24.4
PCB 169	ND	ND	27.3[a]
PCB 180	267.8	202.4	362.1
PCB 187	306.8	225.4	375.3
PCB 189	4.8	3.6	4.1
Total PCBs	2369.8	1518.0	4726.7
Pesticides			
Alpha-HCH	31.2	37.4	50.2
Beta/Gamma-HCH	46.8	39.9	59.7
Heptachlor	ND	2.9	1.0[a]
Heptachlor-epoxide	ND	5.3	8.1
Mirex	14.1	12.0	ND
HCB	20.2	15.7	86.7
Trans-chlordane	6.1	6.9	8.2
Cis-chlordane	82.0	62.7	181.3
Trans-nonachlor	67.3	48.8	155.5
Cis-nonachlor	40.6	27.4	85.4
Endrine ketone	6.8	5.5	9.8
ΣDDTs	96.7	ND	55.6
ΣDDEs	3697.0	469.7	1085.5
ΣDDDs	402.7	54.2	137.1
Total pesticides	4511.5	812.3	1924.1
Total pollutants	7906.2	3039.2	8353.1

Each value represents the mean of three replicate extractions unless otherwise noted

AL+, pregnant adult harbor porpoise with lymphoma; AL−, pregnant adult harbor porpoise with no lymphoma; YL−, female yearling porpoise with no lymphoma; *HCH* hexachlorocyclohexane; *HCB* hexachlorobenzene; *ND* not detected

[a] Two replicate extractions

[b] Single extraction

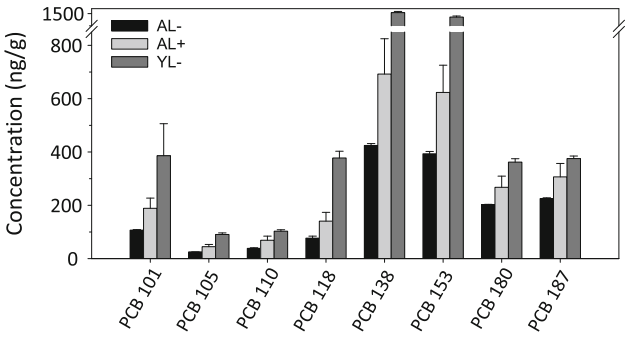

Fig. 1 Mean blubber PCB levels and means (ng/g lipid) in pregnant adult harbor porpoises with (AL+) and without (AL−) lymphoma, and a female yearling with no lymphoma (YL−). Nondetected and analytes making up less than 5% of the total burden were omitted from graphs

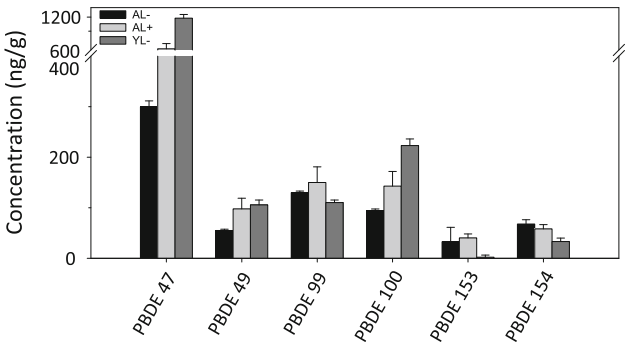

Fig. 2 Mean blubber PBDE levels and means (ng/g lipid) in pregnant adult harbor porpoises with (AL+) and without (AL−) lymphoma, and a female yearling with no lymphoma (YL−). Nondetected and analytes making up less than 5% of the total burden were omitted from graphs

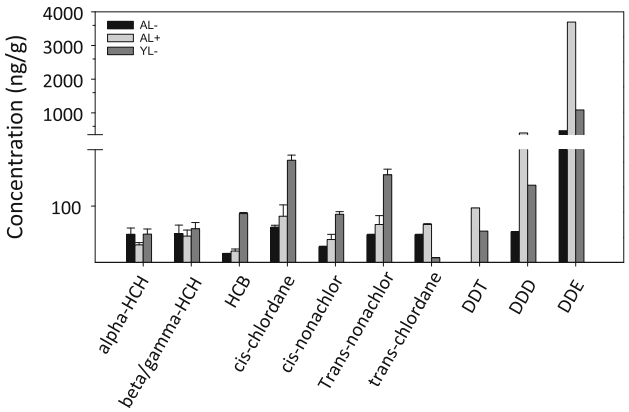

Fig. 3 Mean pesticide levels and means (ng/g lipid) in pregnant adult harbor porpoises with (AL+) and without (AL−) lymphoma and a female yearling with no lymphoma (YL−). Nondetected and analytes making up less than 5% of the total burden were omitted from graphs

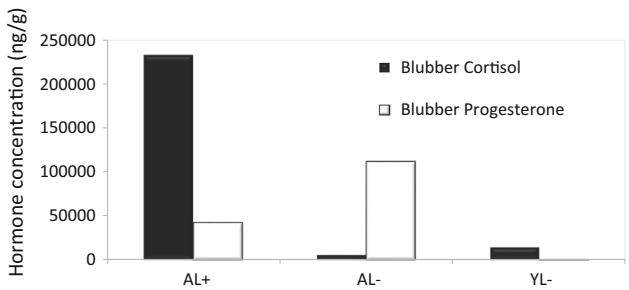

Fig. 4 Blubber cortisol and progesterone level (ng/g lipid) in pregnant adult harbor porpoises with (AL+) and without (AL−) lymphoma and a female yearling with no lymphoma (YL−)

subsequent calves (Ylitalo et al. 2001). The age of the AL+ was estimated to be between 8 and 12 years based on tooth growth layer group counts (Lockyer 1995); however, the age of the AL− was not estimated.

Although not directly comparable to immunotoxic thresholds observed in other studies and marine mammal species, ΣPCB ranges in this study were near or less than those determined to cause dose-related alterations in gene expression, immune, and endocrine function (vitamin A and thyroid hormones) (1300 ng/kg lipid) (Mos et al. 2010) or to alter or reduce natural-killer T-cell function in harbor seals (*Phoca vitulina*) (17,000 ng/kg lipid) (Ross et al. 1996). Lack of inferred or demonstrated data on immune function effects of PCBs and PBDEs in harbor porpoises precludes further comparisons beyond those to other regional porpoise populations. Blubber PCBs in porpoises from Washington, Oregon, and California were 2000–129,000 ng/g lipid (Calambokidis and Barlow 1991), whereas in British Columbia measured 5000–17,000 ng/g lipid (Jarman et al. 1996). These harbor porpoise populations were thus likely to include individuals with PCB levels near the immunotoxic range observed in harbor seals.

Although the US sales and distribution of PCBs ended in the 1970s, PCBs are commonly reported in animal tissues worldwide (Hall et al. 2006; Trumble et al. 2012). The likely dominant pathway of acquiring contaminants in the adult porpoises was through bioaccumulation upon ingestion of mature prey items, such as Pacific herring, known to be significantly contaminated with PCBs (West et al. 2008). Recent work in Salish Sea harbor seals demonstrated a decline in PCBs (81%) and PBDEs (71%) in sampled tissues from 1984 to 2003 (Ross et al. 2013). The decline was attributed to marked reduction of source input of these chemicals and increased regulatory implementations (Ross et al. 2013). Ongoing efforts continue to reduce source inputs of contaminants into the Salish Sea and promote environmental remediation of existing sites of pollution (Washington Department of Fish and Wildlife 2016). Although a definitive causal relationship between contaminants, cortisol, and BCL is yet to be demonstrated, this research adds to the weight of evidence of a possible link; further samples are needed to elucidate these associations.

PBDEs levels were greatest in the YL− porpoise, likely reflecting the animal's young age and, therefore, not yet having offloaded contaminants through transplacental transfer and lactation. Nonetheless, the levels of PBDE-47 recorded in the present study were at least 30 times greater than the mean observed in human patients with NHL (Meironyté Guvenius and Norén 2001). While evidence linking PBDEs with lymphoma is less-defined than that for PCBs, findings to date indicate that these modern additives to plastics may similarly effect human health (and possibly other mammals) as DDT and PCBs, by inducing genetic recombination that are linked to a number of diseases, including cancer (Helleday et al. 1999). DDTs were lower compared with concentrations reported in a bottlenose dolphin (*Tursiops truncatus*) with immunoblastic lymphoma (Jaber et al. 2005), in dolphins in the Indian River Lagoon, and Charleston, South Carolina (Fair et al. 2010), and were below toxicological significant levels (Vos et al. 2002). The high proportion of DDEs, metabolites of DDT, suggests the DDT exposure was not recent.

Progesterone, a crucial metabolic intermediate in the production of endogenous steroids, including the sex hormones and corticosteroids, was more than 2.5 times greater in the blubber of the AL− porpoise (111,000 ng/g lipid; AL− > AL+ > YL−) compared with values from the AL+ 42,000 ng/g lipid). Although lymphomas are not generally considered to be under hormonal control, human epidemiologic studies suggest different protective effects are conferred by endogenous and exogenous reproductive hormones depending on the NHL subtype. For example, risk of NHL was reduced by 50% in women exposed to estrogen (Nelson et al. 2001), whereas prolonged exposure to the pregnancy hormone progesterone may inhibit development of B-cell NHLs (Lee et al. 2008; Prescott et al. 2009). Interestingly, the presence of estrogen and progesterone receptors on the cancer cell surface may play a role in the growth and resolution of some BCL tumors. For instance, a 9-year-old mare was diagnosed with subcutaneous nodules and a granulosa-theca cell tumor, typed as a T-cell rich BCL, which was negative for estrogen and positive for progesterone receptors (Henson et al. 1998). Upon removal of the ovarian tumor, the subcutaneous nodules resolved but recurred upon administration of exogenous progestins. While an understanding of what constitutes stress in harbor porpoise is somewhat lacking, there is understanding that stress or stress hormone levels change in response to changes in metabolism and growth, development, immune functions, reproductive capacity, and social status. Many other apparent factors, however, can influence characteristic stress responses in marine mammals, including genetic (e.g., species), developmental (e.g., life history stage), and environmental (e.g., temperature, nutrition, pollutants) factors. While we reveal a potential link between cortisol levels and lymphoma in the harbor porpoise, it is difficult to eliminate other potential stressors.

Recent human research suggests a link between increased cortisol (stress) and BCL/carcinomas (Moreno-Smith et al. 2010). Cortisol is secreted by the hypothalamus–pituitary–adrenal axis in response to an underlying stress and is potentially cytotoxic to lymphoma and leukemia cells (Boumpas et al. 1993), which may suppress the production of cytokines that mediate the inflammatory reaction (Chrousos 1995). Moreover, animal studies have demonstrated that corticosteroids may stimulate virally derived tumor growth (Romero et al. 1992). In addition, to illustrate the complexity of the interaction between cortisol and lymphoma, Godbout and Glaser (2006) suggest that chronic exposure to environmental pollutants may contribute to lymphoma formation, which then may elevate cortisol.

Although challenging to pinpoint the exact etiology or pathogenesis of neoplasm formation in marine mammals, Ylitalo et al. (2005) demonstrated a clear association between blubber PCB concentrations and carcinoma in California sea lions. Indirect evidence supports the hypothesis of immune dysregulation as a means by which PCBs may contribute to NHL (Kramer et al. 2012). Human epidemiological studies and select animal models demonstrate that PCBs are associated with immune dysregulation through modification of innate and adaptive immunity (Kramer et al. 2012). Effects on immune cells and signaling molecules may manifest as changes in lymphocyte subsets and function (Belles-Isles et al. 2002), increased incidence of infections (Hall et al. 2006) and changes in immune organs, such as the thymus (Park et al. 2008). Additionally, a dose-related inverse association between fatty fish consumption and the proportion of cytotoxic T cells has been observed (Hagmar et al. 1995). Factors that elicit immunosuppression, such as contaminants, may create an environment more conducive to malignancy formation through impairing immunosurveillance by natural killer or cytotoxic T cells or disrupting other protective mechanisms (Godbout and Glaser 2006; Kramer et al. 2012). A shift in the proportion of cytokines produced by T cells may permit virus replication, resulting in an increase in frequency of tumor promotion (Glaser et al. 2005).

The results of this preliminary study add to the weight of evidence suggesting a potential role of PCBs, and possibly a regulatory role by hormones, in NHL carcinogenesis in harbor porpoise. Together with increased number of cases or samples, causal links may be formulated for this species. Harbor porpoise are a top predator species that feed on predominantly fatty prey and live in close association with coastal-based pollutants. Therefore, continued monitoring of contaminants in coastal waterways, as well as

investigation of marine species health, will help to reveal the role environmental pollutants and hormones play in NHL carcinogenesis.

Acknowledgements Essential funding for collection of these samples was provided through grants from the John H. Prescott Marine Mammal Rescue Assistance Grant Program. The marine mammal stranding network members of Washington State helped procure porpoise tissues. Aleta Hohn (NOAA Fisheries, Southeast Fisheries Science Center, Beaufort, NC) and Jennifer Olson (The Whale Museum, Friday Harbor, WA) provided age information for the lymphoma-positive case.

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