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Letter

Tracing Hydrophobic Pollutants in the Deep Sea: A Case Study on Sowerby's Beaked Whales

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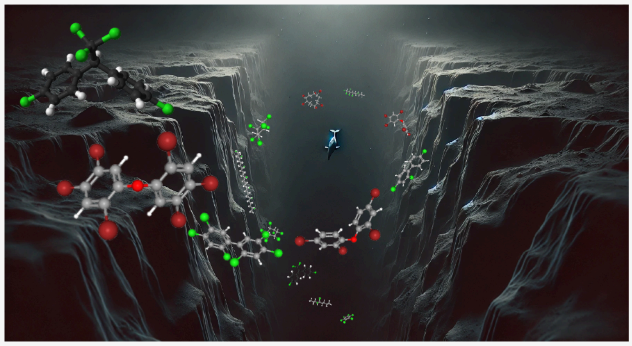

ABSTRACT: Near-total darkness and water depths below 200 m define the deep sea, Earth's largest yet most poorly studied ecosystem. Sowerby's beaked whales (*Mesoplodon bidens*), elusive deep-sea foragers, offer a unique opportunity to assess the impacts of anthropogenic pollutants in this remote environment. This study examined a range of legacy and emerging hydrophobic pollutants, including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), and polychlorinated alkanes (PCAs), across tissues from five stranded whales foraging in Swedish waters. Despite global efforts to reduce pollution, significant pollutant levels in these whales underscore the persistence of legacy contaminants and the widespread use of PCAs. Most pollutants were concentrated in lipid-rich blubber, while PCAs exhibited particularly high levels in whole blood (941−13100 ng/g lipid), indicating tissue-specific accumulation. Blubber pollutant levels were similar to those of harbor porpoises from the same waters, with p,p′-DDE (1020−2280 ng/g lipid) and PCBs (1230−1930 ng/g lipid) exceeding or nearing effect thresholds. Blood concentrations of legacy pollutants were approximately an order of magnitude higher than those in humans from the region, while PCA levels were comparable to those of humans. These findings highlight the urgent need to investigate deep-sea exposure pathways and develop effective management strategies.

KEYWORDS: *Deep Sea, Marine Mammal, Sowerby's Beaked Whale, Tissue Distribution, Persistent Organic Pollutants, Emerging Pollutants, Chlorinated Paraffins*

■ INTRODUCTION

Humans and animals coexist within a shared biosphere, which underscores the interdependence of all species and the ecosystems they inhabit, particularly under the growing impact of diverse stressors. Among the threats pushing the limits of planetary boundaries, chemical pollution and emerging contaminants have been identified as exceeding these boundaries.[1] They represent one of the world's largest environmental risk factors for disease and premature death[2] and pose a growing catastrophic risk to humanity.[3] Yet, research attention on the chemical pollution impact on animals[4] lags behind that on humans,[5] leaving critical gaps in our understanding of their broader ecological and health implications.[6]

Among Earth's diverse ecosystems, the deep sea represents the largest and one of the least studied.[7] Defined as environments beyond continental shelf depths (≈200 m), the deep sea is characterized by extreme conditions, including near-total darkness, average temperatures below 4 °C, and hydrostatic pressures averaging 400 atm, where the absence of sunlight precludes net photosynthetic primary production.[8] Vertical distribution studies suggest that deep waters act as reservoirs for pollutants, such as methyl mercury[9] and perfluoroalkyl acids with higher organic carbon−water partition coefficients[10] (K_{OC}). Additionally, significant enrichment of high K_{OC} pollutants, such as persistent organic pollutants (POPs)[11] and the emerging POPs, polychlorinated alkanes (PCAs, also known as chlorinated paraffins),[12] has been confirmed in the deep sea, with evidence of their accumulation in amphipods at levels exceeding those documented in nearby regions of heavy industrialization.[12,13] These findings indicate a significant impact of high K_{OC}

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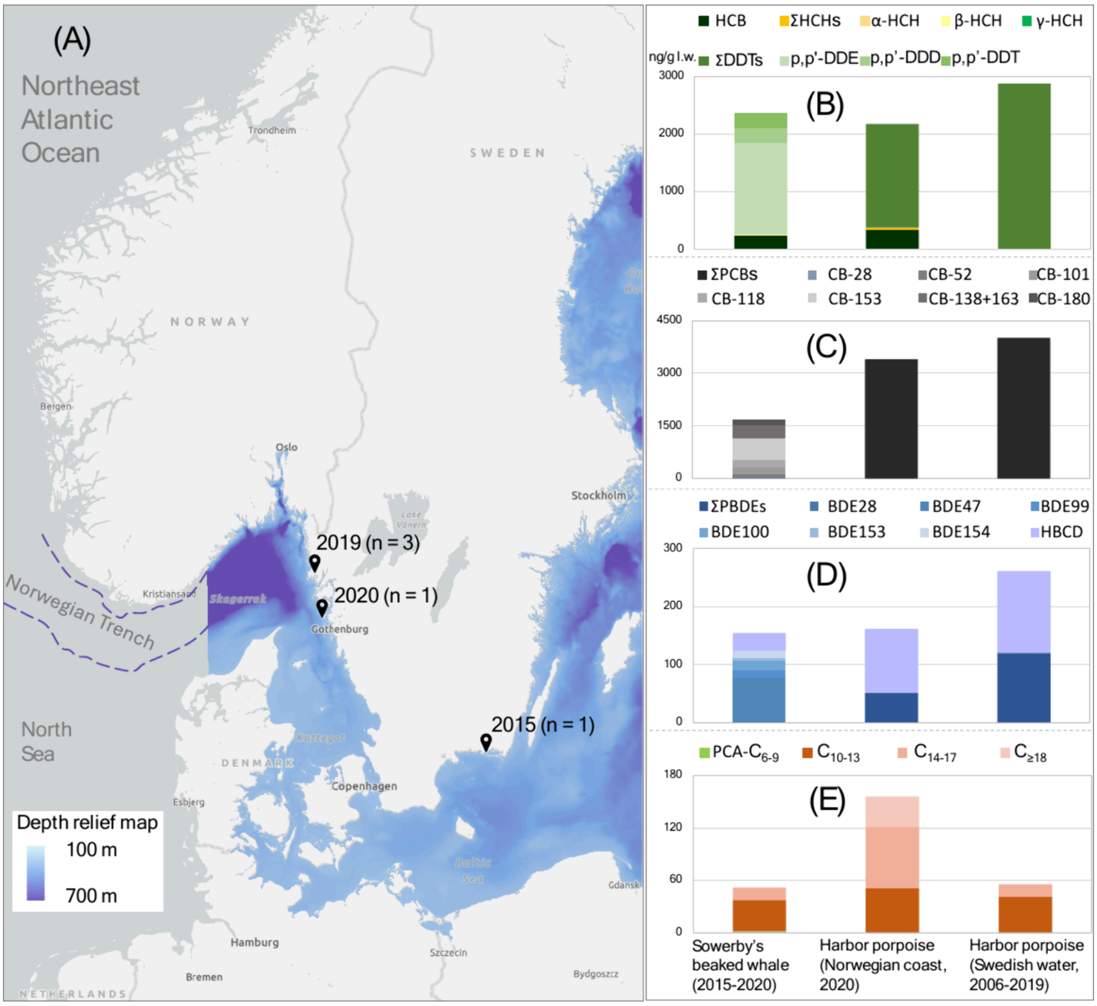

Figure 1. Stranding locations of Sowerby's beaked whales (*Mesoplodon bidens*) overlaid on a depth relief map (A) and blubber concentration comparisons of pollutant groups in harbor porpoises (*Phocoena phocoena*) foraging in Norwegian[26] and Swedish waters:[27,28] (B) OCPs, (C) PCBs, (D) PBDEs, and (E) PCAs. ∑HCH, ∑DDTs, ∑PCBs, and ∑PBDEs were used for harbor porpoise results, as not all the reports provided congener-specific concentrations. Concentrations represent median values. For detailed median values and concentration ranges, see Tables S3 and S4. The depth relief map was modified from HELCOM Map and Data Service.[29]

pollutants on deep-sea habitats. However, approaches to investigate this phenomenon remain limited.

Recently, in contrast to sparse historical observational records,[14] there has been a notable increase in strandings and sightings of Sowerby's beaked whales (*Mesoplodon bidens*) in the Skagerrak basin, a deep-water region fed by the southern part of the Norwegian trench and bordered by Sweden, Denmark, and Norway. Examination of five whales stranded between 2015 and 2020 provided valuable insights into their biology, diet, and potential health threats. Analysis of solid remains and environmental DNA in their stomach contents confirmed their reliance on deep-sea ecological niches at depths between 200 and 1000 m, identifying them as deep-sea foragers.[15] This offers a unique opportunity to investigate the bioaccumulation of chemical pollutants within deep-sea habitats.

In this study, we investigated a suite of high K_{OC} pollutants, including various POPs and PCAs, across different tissues of the five stranded Sowerby's beaked whales. We hypothesize that the accumulation of hydrophobic pollutants in these elusive deep-diving cetaceans is likely non-negligible compared to other marine mammals. The inclusion of multiple tissues allows for a more comprehensive assessment, reducing potential underestimation that may occur when biomonitoring relies solely on singular tissues such as blubber. The availability of blood samples enabled cross-domain comparisons with human populations in the surrounding region, shedding light on the severity, prevalence, and broader implications of chemical pollution within this deep-sea ecosystem.

METHODS AND MATERIALS

Sample Collection. Three male and one female dead stranded Sowerby's beaked whales were collected in Swedish waters between 2015 and 2020, together with one male that was shot due to extensive traumatic injuries (Figure 1A). All individuals were subadults, with body lengths ranging from 3.42 to 4.55 m. Laboratory necropsies were conducted on three individuals, while two were sampled in the field, with one of them undergoing necropsy in the field.[15] Existing literature on the life history of Mesoplodon species is scarce. Although previous references indicate that full-grown adults can range from 4.4 to 5.5 m in length,[16] the largest subadult male in our study (4.55 m) did not have erupted teeth, signifying it was not yet sexually mature. Microscopic analyses of its testicular tissue

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were hampered by autolysis but suggested that this male was approaching sexual maturity.[15] While the exact age of maturation is unknown, these subadult whales are likely more than a few years old. Tissue samples were collected from major organs, including blubber, muscle, liver, lung, spleen, whole blood, and brain. Additionally, testicle samples were collected from two of the four males. All samples were preserved frozen (−25 °C) in biobanks for subsequent analysis.

Target Hydrophobic Pollutants. A diverse range of hydrophobic pollutants, with approximate log K_{OC} values ranging from 3 and 9,[17−20] were quantified. These included organochlorine pesticides (OCPs): hexachlorocyclohexane (HCH), and hexachlorobenzene (HCB), and dichlorodiphenyltrichloroethane (DDT) and its metabolites. Additionally, legacy industrial compounds, including six polybrominated diphenyl ether congeners (PBDEs), hexabromocyclododecane (HBCD), and seven polychlorinated biphenyl congeners (PCBs), as well as PCBs' substitutes, PCAs, were analyzed. A list of the congeners and homologues included in the analysis is detailed in the Supporting Information (SI).

Chemical Analysis. Sample extraction and cleanup methods were performed as previously described.[21,22] Briefly, hydrophobic components were solvent-extracted from 5−12 g of wet sample, with lipid weight determined gravimetrically. Internal standards 2,2′,5,6′-tetrachlorobiphenyl (CB-53), Dechlorane 603, and ^{13}C-1,5,5,6,6,10-hexachlorodecane were added to extracts for the analysis of PCBs and OCPs, PBDEs and HBCDs, and PCAs, respectively. For cleanup, a portion of the extract was treated with concentrated sulfuric acid for PCB and OCP analysis, another portion with concentrated sulfuric acid treatment for PBDE and HBCD analysis, and a third portion was processed on a multilayer SPE column for PCA analysis. The injection standards 2,2′,3,3′,4,5,5′,6-octachlorobiphenyl (CB-198) and ^{13}C-1,1,1,3,10,12,12,12-octachlorododecane were added prior to instrumental analysis.

PCBs and OCPs were analyzed using dual-column GC-ECD.[22] PBDEs and HBCDs were analyzed using GC-ECNI-MS.[23] PCA analysis, designated as C_nCl_m ($n = 6 - 40$, $m \geq 2$), was performed using chloride-enhanced UPLC-APCI-Orbitrap-MS (Q Exactive, Thermo Fisher Scientific). Quantification of very-short-chain (C_{6-9}), short-chain (C_{10-13}), medium-chain (C_{14-17}), and long-chain PCAs ($C_{\geq 18}$) was based on homologue profile reconstruction[24] and reported in the format recommended by Fernandes et al.[25] Detailed procedures and reference standards are provided in the SI.

QA/QC. At the time of analysis, the laboratory at Stockholm University was nationally accredited for the analysis of PCBs and OCPs and regularly participated in interlaboratory comparison exercises for PCBs, OCPs, PBDEs and HBCD through the QUASIMEME program to ensure analytical reliability and consistency. Recoveries for internal standards CB-53 and Dechlorane were 71 ± 11% and 92 ± 15%, respectively, while recoveries for PCAs were 83 ± 7%. Lipid-normalized concentrations are reported and expressed in units of ng/g lipid weight (l.w.). A procedural blank was included in each batch of sample preparation. The limits of quantification (LOQs) were calculated for each group of pollutants and varied across individual samples based on the analyzed lipid weight. For details refer to the SI.

Statistical Analysis. Pearson correlation tests were performed using PAST Version 5.0.[30] Pollutants with a detection frequency above 80% across all analyzed samples were examined. A significance threshold of $p = 0.01$ was applied. For pollutant concentrations below the LOQ, values were imputed as $LOQ/\sqrt{2}$.[31]

RESULTS AND DISCUSSION

Blubber Biomonitoring and Pollutant Profiles. All investigated hydrophobic pollutants were found to accumulate and distribute across the tissues of all five Sowerby's beaked whales (Table S2). These findings provide empirical evidence of deep-sea exposure to both legacy and emerging pollutants. In blubber, the tissue most commonly used for biomonitoring,[32] Sowerby's beaked whales exhibited comparable levels of $\sum$OCPs, $\sum_6$PBDEs, and $\sum$PCAs to those in harbor porpoises, epipelagic feeders foraging in Scandinavian waters in recent years, but approximately half the levels of $\sum_7$PCBs (Figures 1B−1E). These differences in pollutant profiles may reflect dietary influences that are largely habitat-related. While there is overlap in prey species consumed by Sowerby's beaked whales[15] and harbor porpoises[33] examined from Swedish waters, inshore species such as gobiids and Ammodytidae are more prevalent in porpoise diets.

The predominant compounds detected in Sowerby's beaked whales align with established bioaccumulation patterns in marine mammals: DDE as the major metabolite of DDTs, β-HCH for HCHs, BDE-47 for PBDEs, CB-153 for PCBs, and PCAs-C_{10-13} as the primary PCA mixture (Figure S1). HCB levels were consistent with global trends, reflecting its widespread distribution through atmospheric transport.

Pollutant levels can potentially increase with age, as observed in harbor porpoises, where juveniles had lower POP levels than adults.[27,28] This age-related trend may influence cross-species comparisons. The harbor porpoise data used in this study represent the median values from mixed juveniles and adults (1−16 years old) in Sweden[27,28] and mixed adults or individuals of unknown age in Norway covering both genders.[26] While the Sowerby's beaked whales may partially overlap with harbor porpoises in the second quartile of their age range, limited life history data make it difficult to precisely assess the extent of this overlap or its influence on pollutant levels in Sowerby's beaked whales.

To our knowledge, there has been only one previous study on pollutants in Sowerby's beaked whales, which analyzed $\sum_6$PBDEs in blubber tissue from individuals stranded in the UK between 1992 and 2002.[34] The $\sum_6$PBDE levels observed in the present study (89.1−165 ng/g l.w.) were generally less than half of those reported in the UK study (median: ∼ 250 ng/g l.w., Table S4).

Blubber concentrations of $\sum$OCPs and $\sum_7$PCBs were approximately one to 2 orders of magnitude higher than those of $\sum_6$PBDEs and $\sum$PCAs, with HBCD exhibiting the lowest median and mean values. However, across all other tissues, $\sum$OCPs, $\sum_7$PCBs, and $\sum$PCAs exhibited similar levels, while $\sum_6$PBDEs were consistently 1 order of magnitude lower than these groups (Table S3). The longest chain PCAs detected were up to C_{33} in blood, spleen, and muscle; C_{32} in kidney; C_{29} in blubber, testicle, and lung, and C_{25} in brain (Figure S2). These findings suggest significant tissue-specific differences in the accumulation patterns of these pollutants.

Beyond Blubber: Tissue-Specific Accumulation. The wet weight-based concentrations of most investigated hydrophobic pollutants were significantly correlated with the lipid content of the tissues ($p < 0.01$, Table S5), consistent with previous findings that these compounds predominantly

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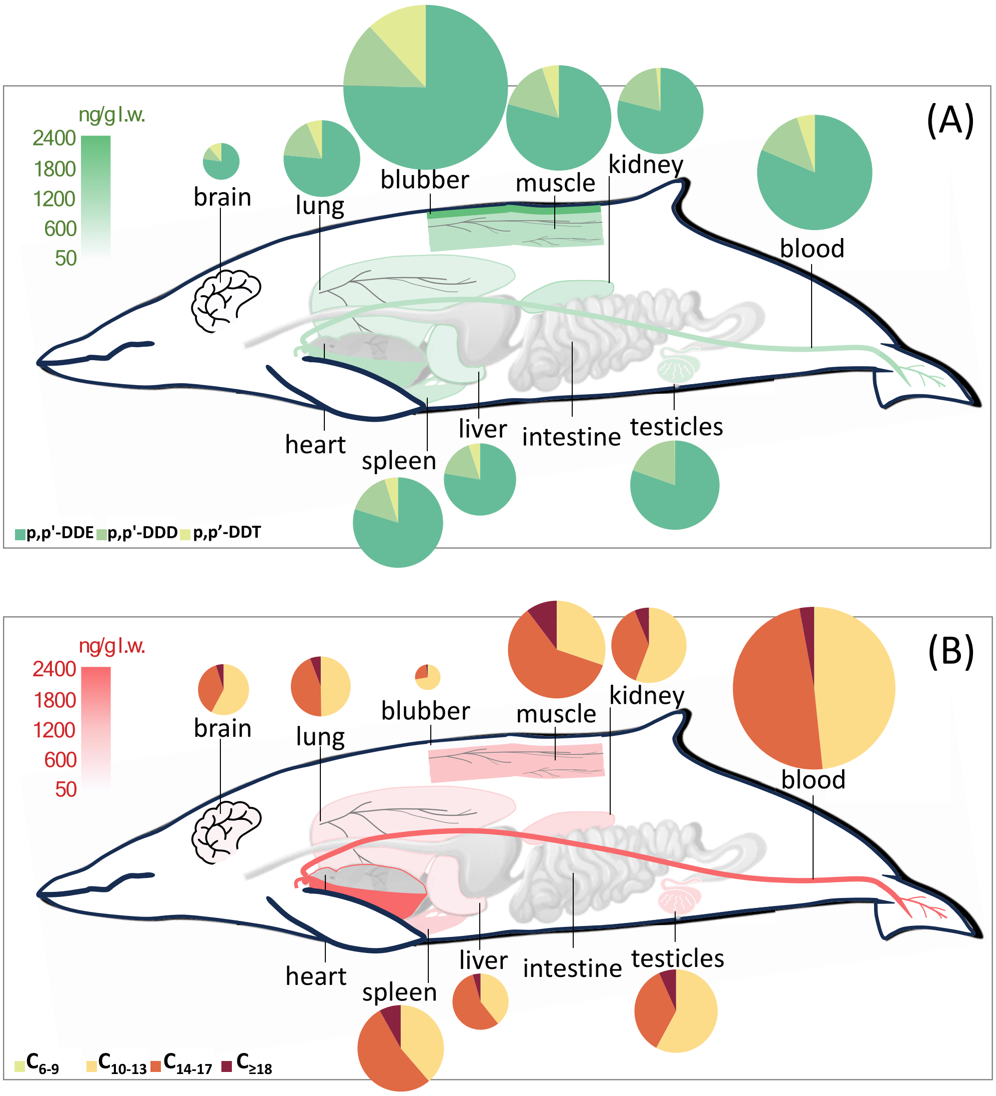

Figure 2. Tissue-specific accumulation of DDT with its metabolites (A) and PCAs (B) in Sowerby's beaked whales. Concentrations are lipid-normalized median values (Table S3).

accumulate in lipid-rich tissues, such as blubber and liver.[35] This reflects the role of lipids as solvent compartments for these pollutants.[36] The only exception was long-chain PCAs (PCA-$C_{\geq 18}$), which showed no correlation ($r^2 < 0.1$). This lack of correlation may be attributed to the decreasing trend (represented by the slopes in Table S5) with increasing pollutant hydrophobicity, as observed for PCB and PBDE congeners in this study.

To better understand tissue-specific chemical partitioning in Sowerby's beaked whales, lipid-normalized concentrations were compared. The partitioning patterns of legacy pollutants, using DDTs as an example (Figure 2A), were consistent with findings from previous tissue comparison studies in other marine mammals.[37] Pollutant levels in brain tissue were generally the lowest for most compounds, with the exception of α-HCH, which exhibited the highest concentration in the brain (Table S3). This aligns with prior research suggesting that enantioselective transport across the blood-brain barrier facilitates its enrichment in this tissue.[38]

Surprisingly, $\sum$PCA concentrations in blood (median 2170 ng/g l.w.) were exceptionally high, twice the levels of DDE (976 ng/g l.w.) and $\sum_7$PCBs (860 ng/g l.w.). $\sum$PCA levels in blubber (52.6 ng/g l.w.) were only 2.5% of those in whole blood, ranking them among the pollutants with lowest concentrations in blubber, whereas in other tissues, $\sum$PCAs were consistently among the highest, such as in the brain tissue (Figure 2B, Table S3). High PCA concentrations are less likely driven by the low lipid content in blood, as lipid normalization effectively reduced variance in legacy POP concentrations across tissues. This suggests that tissue distribution, rather than lipid availability, plays a more significant role in determining PCA levels in blood.

These findings not only reflect the environmental presence of PCAs as one of the most widely used[39] industrial chemicals globally[40] but also suggest that potential contaminant-mediated effects may be tissue- or blood-specific.[41] Traditional biomonitoring approaches often focus on blubber as the target tissue for hydrophobic pollutants, including PCAs. However, this approach may substantially underestimate PCA accumulation within ecosystems, highlighting the critical need for broader, tissue-specific analyses to more accurately assess the environmental and ecological impacts of emerging pollutants.

Whales Outpace Humans in Chemical Burden. Having the whole blood data enabled comparison of chemical

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concentrations in these deep-sea foraging mammals with those in human serum/plasma from the same region (Table S6). Most pollutants, especially legacy POPs (PCBs, OCPs, and PBDEs), were about 1 order of magnitude higher in the whales than in humans (Figures 3A−3C). Even compared with a PCB-contaminated human population in Denmark that experienced significant near-field exposure,[42] the median whale blood levels (860 ng/g lipid) were nearly twice as high as the median human level (481 ng/g lipid, Table S6).

Although these pollutants have been banned for decades, and their levels in humans have decreased significantly,[43] their levels in Sowerby's beaked whales, foraging at depth, were still significant. For instance, p,p′-DDE levels in three of the five whales exceeded the effect threshold (1430 ng/g l.w.) associated with decreased lymphocyte proliferation in bottlenose dolphins,[44] with the other two nearing this threshold (1020 and 1320 ng/g l.w. in blubber). Similarly, PCB concentrations (1230−2680 ng/g l.w. in blubber and liver) reached 25−50% of the 5420 ng/g l.w. toxicity threshold established for cetaceans.[45] The highest liver concentrations of p,p′-DDE and $\sum_7$PCBs were found in an emaciated female with significantly reduced muscle mass. This individual also had the highest liver concentrations of $\sum_6$PBDEs (420 ng/g l.w.) that were four times higher than the median and HBCD concentrations (168 ng/g l.w.) that were 40 times higher. Dietary information[15] suggests this whale was at least a few years old (weaned and no longer dependent on its mother) but had not reached sexual maturity. Consequently, she would not have offloaded pollutants via reproduction. This contrasts with the very low PCB concentrations found in a lactating female harbor porpoise in Swedish waters,[28] likely due to pollutant offloading through lactation. Nonetheless, she was cachectic/in a negative energy balance, which may have influenced her pollutant profile.

It is worth noting that comparing whole blood with serum and plasma might underestimate the differences between whales and humans. The presence of red blood cells in whole blood could influence pollutant distribution.[46] Studies in humans have shown that plasma can have 1.5 to 2 times higher POP concentrations on a lipid-weight basis than whole blood,[47] although similar research in whales or other marine mammals is lacking.

Emerging pollutants, such as PCAs, which are still widely used in large volumes globally,[55] showed comparable levels in both near-field humans and far-field whales (Figure 3D). Notably, the most hydrophobic PCA homologues ($C_{\geq 18}$) were predominant in one of the three whole blood samples, exhibiting an unprecedented concentration of 9100 ng/g l.w. − the highest ever recorded in a living organism. The homologue fingerprint revealed a remarkable abundance of very-long-chain PCAs, with a high proportion of $C_{\geq 24}$ homologues (Figure S3). This pattern was also observed in the spleen of the same individual and the muscle of a killer whale stranded in the same water body in 2018.[56] Although the sample size is small, these results suggest a potentially high detection frequency for these compounds in Sowerby's beaked whales. Recent studies have linked PCA-$C_{\geq 18}$ to hepatocyte damage through oxidative stress[57] and inflammatory damage in cardiomyocytes,[58] but toxicity thresholds for risk assessment remain largely undefined for cetacean species.

Continuous efforts have been made to protect the shared biosphere that supports both marine ecosystems and human health, including banning and restricting legacy pollutants

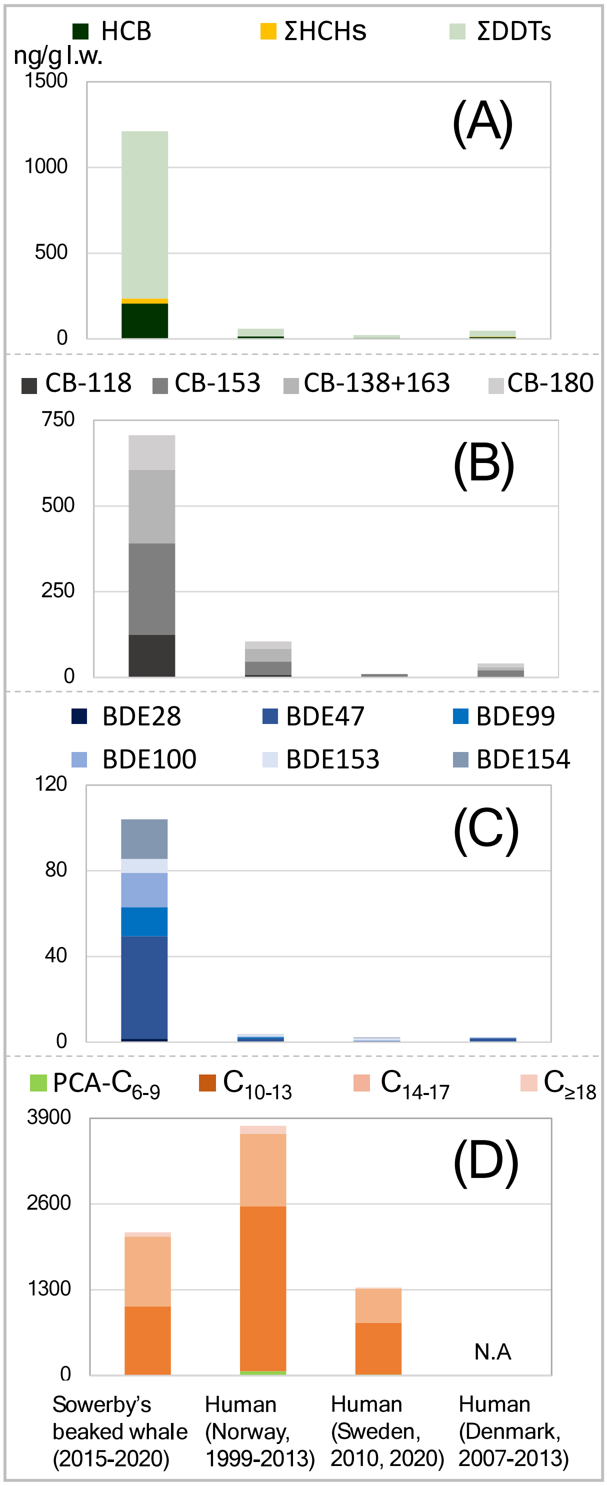

Figure 3. Blood concentration comparisons of pollutant groups between Sowerby's beaked whales and humans living in surrounding countries: (A) OCPs, (B) PCBs, (C) PBDEs, and (D) PCAs. PCAs in the Danish results were not available (N.A.) at the time of data review. Serum/plasma data for humans were obtained from the MoBa[48] and ATEAM cohorts[24,49] (Norway), Riksmaten 2010−11[50] and NorthPop Birth cohorts[22] (Sweden), and Aarhus Birth,[51,52] Odense Child,[53] and Rigshospitalet cohorts[54] (Denmark). Concentrations represent the median values for whales and the mean of the median values across human cohorts. For detailed tabular data, see Table S6.

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under the Stockholm Convention,[44] recommending limited consumption of fish with high pollutant levels,[59,60] and removing emerging pollutants through wastewater treatment.[61] These measures have resulted in reduced near-field and dietary exposures in humans[62] and lower pollutant levels in wastewater outflows to aquatic environments.[63] However, the Sowerby's beaked whales in this study exhibited legacy pollutant levels that were significantly higher than those found in humans, as well as comparable levels of emerging pollutants. Marine mammals, with their long life spans, key positions in the food web, and large lipid reserves, are particularly vulnerable to the health risks of pollutant bioaccumulation.[64] These accumulated pollutants may contribute to biodiversity loss and compromise ecosystem integrity.[65] The Sowerby's beaked whales in this study provide a rare opportunity to assess potential risks of anthropogenic pollutants, but many deep-sea species, including those yet to be discovered,[66] may face similar threats and potentially go extinct before their vulnerabilities are even understood.[67] Further research is urgently needed to identify the pathways leading to such high exposure to hydrophobic pollutants in deep-sea ecosystems and to develop more effective strategies for reducing pollution in these vulnerable environments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.5c00115.

> List of analyzed pollutants, sample preparation details, instrumental analysis, LOQs, raw data for individual samples, median and mean summary, pollutant blubber levels in marine mammals, Pearson correlation analysis, pollutant serum/plasma levels in humans, pollutant profiles, PCA fingerprints across tissues, and PCA-$C_{\geq 18}$ predominated fingerprints (PDF)

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Notes

The authors declare no competing financial interest.

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