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Nontargeted Biomonitoring of Halogenated Organic Compounds in Two Ecotypes of Bottlenose Dolphins (*Tursiops truncatus*) from the Southern California Bight

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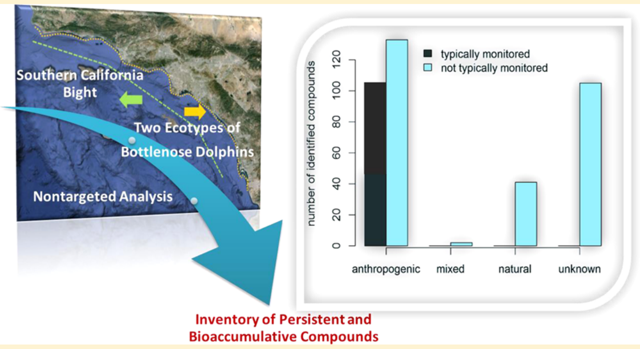
Supporting Information

ABSTRACT: Targeted environmental monitoring reveals contamination by known chemicals, but may exclude potentially pervasive but unknown compounds. Marine mammals are sentinels of persistent and bioaccumulative contaminants due to their longevity and high trophic position. Using nontargeted analysis, we constructed a mass spectral library of 327 persistent and bioaccumulative compounds identified in blubber from two ecotypes of common bottlenose dolphins (*Tursiops truncatus*) sampled in the Southern California Bight. This library of halogenated organic compounds (HOCs) consisted of 180 anthropogenic contaminants, 41 natural products, 4 with mixed sources, 8 with unknown sources, and 94 with partial structural characterization and unknown sources. The abundance of compounds whose structures could not be fully elucidated highlights the prevalence of undiscovered HOCs accumulating in marine food webs. Eighty-six percent of the identified compounds are not currently monitored, including 133 known anthropogenic chemicals. Compounds related to dichlorodiphenyltrichloroethane (DDT) were the most abundant. Natural products were, in some cases, detected at abundances similar to anthropogenic compounds. The profile of naturally occurring HOCs differed between ecotypes, suggesting more abundant offshore sources of these compounds. This nontargeted analytical framework provided a comprehensive list of HOCs that may be characteristic of the region, and its application within monitoring surveys may suggest new chemicals for evaluation.



INTRODUCTION

Currently regulated and monitored environmental contaminants represent a fraction of the total chemical exposure of organisms. Our limited knowledge of the environmental occurrence of unmonitored compounds inhibits a comprehensive understanding of exposure and potential impacts to human and wildlife health. Halogenated organic compounds (HOCs) are of particular concern due to their history of release into the environment, persistence and bioaccumulation, and potential for toxicity. Identifying emerging HOCs of concern, tracking degradation products of anthropogenic legacy pollutants, and understanding the transport and fate mechanisms of biogenic analogues have thus become priority topics in contaminant monitoring.

Targeted analysis, in which only selected chemicals are measured, has traditionally been used to understand the contaminant load of an environmental sample or body burden of an organism. While this approach is irreplaceable in its time- and cost-effectiveness, and quantitative accuracy, it inevitably

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misses many potentially abundant and toxicologically relevant compounds, including unmonitored anthropogenic parent compounds, degradation products, and potentially toxic and bioaccumulative biogenic compounds.[1] For many legacy HOCs, the original parent compound no longer plays the dominant role in the environment. Over time, metabolism and abiotic transformations result in a myriad of degradation products, which may have bioactivities equal to or greater than the parent. For example, it is estimated that 10 to 20% of pesticides are detected as stable transformation products.[2] Naturally produced HOCs can be similar to anthropogenic HOCs in both chemical structure and environmental behavior. Thousands of halogenated natural products are synthesized in the marine environment by sponges, algae, and marine bacteria.[3,4] Some naturally produced HOCs exhibit bioaccumulative properties, environmental concentrations, and toxic potencies that are similar to and/or exceed those of their anthropogenic HOCs.[5−7]

Marine mammals serve as sentinels for characterizing the impacts of persistent anthropogenic HOCs in oceanic and coastal environments.[8] The occurrence of anthropogenic HOCs has been associated with negative health effects in marine mammals, including disruption of endocrine function, impairment of reproductive success, and contribution to cancer.[9−11] Dolphins are apex predators that occupy many marine ecosystems, feed on a variety of aquatic prey, and share a number of physiological similarities with humans, making them representative sentinels for assessing the quality of marine environments[8,12] and characterizing the contribution of HOCs of anthropogenic versus natural origin.

Recently, a nontargeted analytical approach using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) was developed to analyze the suite of HOCs in the blubber of a common dolphin (*Delphinus delphis*).[1] To manage the volume of data resulting from this work (270 individual HOCs were identified, 62% of which were not typically monitored), custom software was developed to create a shared mass spectral library of all identified compounds.[1] In this study, we applied the nontargeted approach to blubber samples from eight male common bottlenose dolphins (*Tursiops truncatus*) stranded between 1995 and 2010 in the Southern California Bight, a section of the eastern North Pacific Ocean centered around the metropolitan Los Angeles region (California, U.S.A.) and home to the heavily dichlorodiphenyltrichloroethane (DDT) contaminated Palos Verdes Superfund site. Each specimen belonged to either a coastal or offshore ecotype.[13] The compounds identified in these samples represent detectable HOCs in the dolphins' habitat. Our aim was to catalog persistent and bioaccumulative HOCs in these sentinel species, identify compounds that are currently unmonitored, and compare the distribution of anthropogenic versus natural HOCs between the two distinct ecotypes of *T. truncatus*.

■ METHODS

A list of defined acronyms and a description of the reference standards are given in the Supporting Information (SI).

Study Area. The Southern California Bight is one of the most populated areas in the U.S., home to approximately 17 million people along a 300-km stretch of the eastern Pacific coastline.[14] It is situated within the California Current Large Marine Ecosystem, which is a productive eastern boundary current upwelling system that supports a complex and influential food web and supplies seafood to a global market.[15] The Southern California Bight has a long and well-documented history of receiving wastewater and stormwater discharge containing DDT, polychlorinated biphenyls (PCBs), and polybrominated diphenylethers (PBDEs).[16,17]

Sample Information (*Tursiops truncatus*). Regional blubber samples were obtained from common bottlenose dolphins found dead on southern California beaches. All dolphins died of natural causes. Sexually mature males from two *T. truncatus* ecotypes (4 individuals from a coastal ecotype and 4 individuals from an offshore ecotype) were selected (see SI Table S5).

Bottlenose dolphins occur as two distinct ecotypes along the California coast: a coastal ecotype that is typically found within 1 km of the shore[18−20] and an offshore ecotype distributed in deeper offshore waters, typically greater than a few kilometers from shore.[20,21] Current stock differentiation for these two ecotypes is supported by morphological and genetic evidence. Differences in tooth size, parasite load, diet, skull morphology, and potentially body size between the coastal and offshore ecotypes of the eastern North Pacific Ocean have been recorded.[22] Perrin et al.[23] also reported significant cranial osteological differences between the two forms, with the differences found mainly in features associated with feeding. Finally, Lowther-Thieleking et al.[13] found significant genetic differentiation between the two ecotypes.

Sample Preparation and Analysis. A detailed description of the sample preparation and instrumental analysis can be found in the SI Methods. Figure 1 summarizes the sample preparation and compound identification procedures. SI Figure S1 gives a representative chromatogram of a dolphin blubber extract.

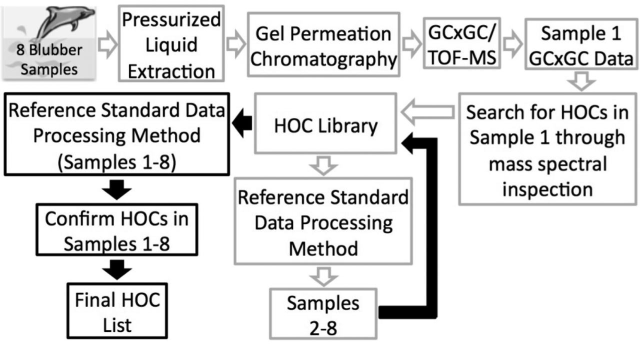

Figure 1. Flowchart of sample preparation and compound identification scheme.

Compound Identification. All isolated chromatographic peaks were examined for identification of their corresponding mass spectra. The procedures are summarized in Figure 1, and a detailed description is given in the SI Methods. Data analysis was conducted with LECO ChromaTOF software version 4.43.3. PCB congeners and DDT and dichlorodiphenyldichloroethylene (DDE) peaks were excluded from identification due to overloading. In the first sample run, 326 peaks were identified as potential HOCs of interest at a signal-to-noise ratio (S/N) of 10 or higher, out of 17 038 total peaks. These HOC peaks were then used to create a reference data processing method that searched the remaining seven samples for the 326 HOCs found in sample 1 ($S/N \geq 50$), with matching based on retention time, and spectral similarity. This

Table 1. Compound Classes Identified in Blubber Samples of Adult Male *Tursiops truncatus* ($n = 8$) Stranded along the Southern California Bight, The Number of Congeners or Isomers within Each Class, The Range of Halogenation, Their Source, And the Number of Compounds within Each Class That Are Not Typically Monitored in Environmental Samples[a]

class	no. compounds	no. bromines	no. chlorines	source	no. not typically monitored
chlordane-related	23	0,UD	6,7,8,9,10,UD	anthropogenic	18
chlorinated benzenes	4	0	3,4,5,6	anthropogenic	4
chlorinated styrenes	2	0	3,7	anthropogenic	2
chlorophosphate	1	0	3	anthropogenic	1
DDT-related	29	0,UD	2,3,4,6,7,9,UD	anthropogenic	23
dichlorobenzophenone	1	0	2	anthropogenic	1
hexachlorocyclohexane-related (HCH)	4	0	4,6	anthropogenic	2
heptachlor-related	4	0	7,8	anthropogenic	3
methylenebistrichloroanisole	1	0	6	anthropogenic	1
methylsulfonyl polychlorinated biphenyls (methylsulfonyl-PCBs)	6	0	5,6	anthropogenic	6
mirex-related	5	0,UD	10,11,12,UD	anthropogenic	4
polybrominated biphenyls (PBBs)	13	4,5,6	0	anthropogenic	12
polybrominated diphenyl ethers (PBDEs)	17	2,3,4,5,6	0	anthropogenic	5
polychlorinated terphenyls (PCTs)	37	0	4,5,6,7,8	anthropogenic	37
pyrrolidinecarbonyl chloride	1	0	1	anthropogenic	1
toxaphenes	19	0	5,6,7,8,9	anthropogenic	NA
tris(4-chlorophenyl)methane (TCPM)	12	0	2,3,4	anthropogenic	12
tris(4-chlorophenyl)methanol (TCPMOH)	1	0	5	anthropogenic	1
brominated anisole	1	3	0	mixed	1
chlorinated polycyclic aromatic hydrocarbon (PAH)	1	0	2	mixed	1
polybrominated dibenzofuran (PBDF)	2	2	0	mixed	2
brominated indoles	2	1,2	0	natural	2
dimethyl bipyrroles (DMBPs)	20	0,2,3,4,5,6	0,1,2,3,4,6	natural	20
methoxy brominated diphenyl ethers (MeO-BDEs)	5	3,4,5	0	natural	5
methoxy brominated/chlorinated diphenyl ether (MeO-B/CDE)	1	3	1	natural	1
methoxy polybrominated biphenyl (MeO-PBB)	1	4	0	natural	1
methyl bipyrroles (MBPs)	8	0,4	0,6,7	natural	8
polybrominated hexahydroxanthenes (PBHDs)	4	3,4	0	natural	4
brominated/chlorinated diphenyl ethers (B/CDEs)	3	3	1	unknown	3
bromomethylbiphenyl	1	1	0	unknown	1
chlorobenzaldehyde	1	0	1	unknown	1
methoxy chlorinated diphenyl ethers (MeO-CDEs)	2	0	8	unknown	2
trichloro benzonitrile	1	0	3	unknown	1
unknown-1	6	0	3,5,6	unknown	6
unknown-2	11	0,UD	3,5,UD	unknown	11
unknown-3	3	3,4	1	unknown	3
unknown-4	17	0	4,6,7,8,9	unknown	17
unknown-5	2	UD	UD	unknown	2
unknown-6	3	UD	UD	unknown	3
unknown-7	2	UD	UD	unknown	2
unknown-8	2	UD	UD	unknown	2
unknowns	48	0,UD	7,UD	unknown	48

[a] Acronyms are listed in the SI. "UD" indicates an undetermined number of halogens. Classes Unknowns-1 to Unknowns-8 contain compounds with suspected identities or similar characteristics, as discussed in the Results section. The class Unknowns contains all other unidentified compounds.

resulted in a total of 395 unique HOCs from the eight dolphin samples. Hits to these 395 peaks were then manually confirmed. The final library resulted from further curation and scrutiny of those peaks and ultimately consisted of 327 unique compounds. A procedural blank was analyzed along with the blubber samples and was processed with the final reference data processing method to ensure that no library compounds were present. Confidence in the compound identifications followed the previously defined categories in Hoh et al.,[1] and descriptions of each level of identification are available in the SI Methods.

Normalized Compound Abundances. For each compound, an abundant fragment ion with minimum interference was selected as the quantification ion. Relative responses of the quantification ions for each compound class were calculated relative to internal standards. Identified compounds were assigned an internal standard as follows. First, ^{13}C-PCB-169 was assigned to compounds eluting between 0 to 1560 s (first dimensional GC: ^{1}D) and 0 to 3.5 s (second dimensional GC: ^{2}D) and BDE-77 to compounds eluting between 1560.1 to 2600 s (^{1}D) and 0 to 3.5 s (^{2}D). Next, compounds were grouped by compound class, and if a class had been assigned to

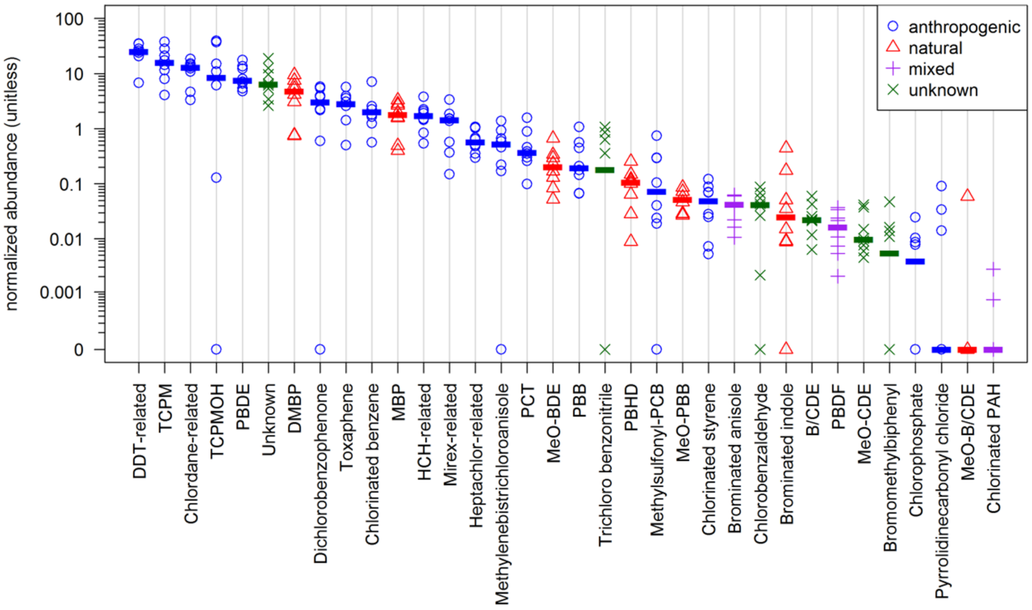

Figure 2. Normalized abundance of each compound class. The abundance is the sum total of the normalized peak areas for all compounds in the class. Each point represents a single dolphin blubber sample ($n = 8$). The line is the median abundance for the compound class. The classes are sorted by median abundance. The source of each compound class is indicated in the legend. Nondetects are shown with a value of zero (multiple nondetects overlap).

multiple internal standards based on the retention time division, all compounds within the class were assigned the same internal standard (e.g., all 1,1′-dimethyl-2,2′-bipyrroles (DMBPs) containing halogens were assigned the same internal standard even though they could be divided between two internal standards based on retention time). Finally, this quotient was divided by the mass of extracted lipid in each sample to give a normalized abundance for each compound. Synthetic standards for most of the identified compounds were unavailable, therefore calibration curves could not be established and the normalized abundances are considered semiquantitative.

Ecotype Profile Comparison. Ecotype differences were assessed by comparing compound profiles (i.e., the type and abundance of individual HOCs in a sample) using three unsupervised statistical learning methods: hierarchical clustering, *k*-means clustering, and principal components analysis. Prior to analysis, compounds were divided into three groups based on the source (i.e., anthropogenic, natural, or unknown) because we suspected different spatial origins and transport mechanisms for the anthropogenic and natural compounds, and hypothesized the coastal and offshore contaminant profiles would be different. The contaminant profiles from each of the three compound groups were analyzed by the three clustering methods. The compound classes with mixed anthropogenic and natural sources were excluded from the analysis. We concluded there was a difference between the coastal and offshore ecotype contaminant profiles only if all three clustering methods identified two sample clusters corresponding to the two ecotypes.

Mass Spectral Library. Mass spectra of interest and ancillary information (e.g., retention times, abundance, categorization, and fragment ion identifications) were stored in a custom library. The use and structure of the R package that stored the data and generated different formats of the library were previously described.[1] The library is available as a PDF report in the SI, and at http://OrgMassSpec.github.io/ as a text file in the NIST MSP format for import into other software and as the R package SpecLibDolphin2014. The library contains all mass spectra for the set of unique compounds, including unknowns, and ancillary information for the complete set of identifications across the eight samples.

RESULTS

Catalog of Identified HOCs. In total, 1647 HOCs were identified in the eight samples, and 327 of those were unique (i.e., excluding duplicate identifications in multiple samples). DDT, DDE, and PCB chromatographic peaks were saturated and their abundances could not be accurately determined. The occurrence of these compounds in marine environments is well documented; therefore, they were excluded from the remainder of the identification procedure and are not included in the results. An average of 206 (range 182−253) HOCs per sample were identified, indicating the contaminant load was relatively consistent among the samples and suggests the results may be representative of the bottlenose dolphin population in the Southern California Bight.

We identified a wide range of anthropogenic and natural HOCs comprising 34 compound classes based on chemical structure (Table 1). Compounds from the same technical mixture or family of breakdown products (e.g., all DDT-related compounds) were assigned to the same class. Of the 327 unique compounds, 18 classes (180 compounds, 55%) were anthropogenic contaminants, 7 classes (41 compounds, 12%) were of natural origin including several previously undetected dimethyl bipyrrole isomers (DMBP-Br_5, DMBP-Br_4, and DMBP-Br_2Cl_3—confirmed by authentic standards), three classes (4 compounds, 1.2%) were of mixed origin (i.e., having both anthropogenic and natural sources), and five classes (8 compounds, 2.4%) were assigned a structure but had unknown sources. Finally, 94 compounds (29%) were identified as halogenated, but their full structure and the source of the compound were unknown. Eight unknown subclasses consisting of 46 HOCs total contained compounds with either a characteristic fragmentation pattern but a varying number of halogens, or identical mass spectra with different retention times. The remaining 48 unknown compounds were assigned to the general unknown class. Further discussion of the results for each compound class is given in the following sections.

Analytical information on each compound is provided in the SI mass spectral library including the mass spectrum, fragment ion identifications, degree of confidence in the identification, GC×GC retention times, and normalized peak intensities among the eight samples. A table of detection frequencies and normalized abundances for all individual compounds is provided in SI Table S1.

The relative abundance of each compound class was examined (Figure 2). DDT-related compounds were the most abundant (even though the DDE and DDT isomers were excluded from the results). Naturally occurring DMBPs and 1′-methyl-1,2′-bipyrroles (MBPs) were comparable in abundance to anthropogenic classes such as PBDEs and toxaphene. Unknown compounds were also relatively abundant compared to legacy organochlorines. Note the number of compounds belonging to each class varied among the 34 classes (Table 1).

Chlordane-Related Compounds. While technical mixtures of chlordane, a legacy organochlorine insecticide formulation no longer in use, contain over 100 different compounds,[24] five compounds are usually monitored (i.e., α- and γ-chlordane, *cis*- and *trans*-nonachlor, and oxychlordane). We identified 18 additional chlordane-related compounds.

DDT-Related Compounds, Tris(4-chlorophenyl)methane (TCPM), and Tris(4-chlorophenyl)methanol (TCPMOH). DDT is a legacy pesticide consisting of several isomers with chlorines in the ortho, meta, and para positions. We identified DDT breakdown products, including two commonly monitored isomers *o,p′*-DDD, *p,p′*-DDD, and four isomers of DDMU. An additional 13 compounds that are likely further degradation products of DDE, DDD, and DDMU[25] were identified (library pages 29−38, 41, 42, 44). We also identified eight compounds (library pages 48−54, 57) with mass spectra containing similar fragmentation to DDE, but with a larger number of chlorines (six to eight).

A total of 12 TCPM congeners were identified: one isomer of TCPM with two chlorines, eight isomers of TCPM with three chlorines, and three isomers of TCPM with four chlorines. One isomer of the presumed primary breakdown product of TCPM, the hydroxylated TCPM (TCPMOH), was identified. Previously, just two isomers of TCPM, each with three chlorines, were identified in a blubber sample of the Atlantic common dolphin, *Delphinus delphis*.[1] The environmental occurrence of TCPM has not been well studied and has several possible industrial sources, including its existence as a minor constituent in technical DDT.[26] However, the number and chlorination range of TCPM we observed has not been previously reported.

Polychlorinated Terphenyls (PCTs). PCTs are organochlorine compounds with similar past uses and production time frame as PCBs and were used as flame-retardants,[27] but for which there is comparatively little environmental data. PCTs consist of three phenyl rings joined together linearly by C−C bonds, with elemental formulas of $C_{18}H_xCl_{14\text{-}x}$. We detected a total of 37 congeners, including nine tetra-, six penta-, eight hexa-, nine hepta-, and five octa-chlorinated congeners. On the basis of their similarity to PCBs, PCTs may be expected to persist and biomagnify in the food chain.[27] Rosenfelder and Vetter (2012) detected 63 tetra- to nonachlorinated terphenyls in harbor porpoise blubber from the North Sea.[28] To our knowledge, these are the first data on their occurrence in Southern California marine mammals.

Dimethyl and Methyl Bipyrroles (DMBPs and MBPs). These nitrogen-containing heterocyclic compounds have no known man-made sources.[29,30] Twenty DMBPs were identified, with halogenation varying between six chlorines, four to six bromines, and mixtures of bromines and chlorines. The most commonly detected isomer of this group to date,[31−33] DMBP-Br_4Cl_2, was also the most abundant congener in our samples, and detected in all eight samples. Several novel isomers that have not been previously reported were also detected: DMBP-Br_4, DMBP-Br_5, and DMBP-Br_2Cl_3. In addition, eight MBPs with varying degrees of halogenation were detected. MBP-Cl_7, also referred to as Q1,[34] was detected in all eight samples and was the most abundant MBP. Three hexachlorinated isomers were the next most abundant MBPs, followed by four tetrabrominated MBPs.

Methoxy Polybrominated/Chlorinated Diphenyl Ethers (MeO-BDEs, -CDEs). MeO-BDEs have been previously isolated from marine algae, sponges, and bacteria.[3,35,36] We identified five MeO-BDEs: 2′-MeO-BDE-68, 6-MeO-BDE-47, 6′-MeO-BDE-99, a tribrominated MeO-BDE, and a tetrabrominated MeO-BDE. The source of methoxy chlorinated diphenyl ethers is unclear, but the hydroxy analogues have been identified as impurities in technical pentachlorophenol.[37] No natural sources are known. Additionally, one mixed halogenated MeO-B/CDE was identified and is a suspected natural product.[38]

Mixed Halogenated Diphenyl Ethers (B/CDEs). Three B/CDE isomers with three bromines and one chlorine were presumptively identified based on the presence of $[M]^+$ and $[M\text{-}Br_2]^+$ ions. There are very limited data on the prevalence of mixed B/CDEs in the environment and their potential sources. Nonabromochlorodiphenyl ethers have been detected in processed sewage sludge from Mid-Atlantic wastewater treatment plant drying lagoons[39] and air and soil samples from e-waste recycling plants in South China.[40] A laboratory experiment showed that monochlorinated polybrominated diphenyl ethers have been formed from the thermal treatment of BDE 47 ($Br_4 \rightarrow Br_3Cl$) and BDE 153 ($Br_6 \rightarrow Br_5Cl$).[41]

Miscellaneous Compounds. PBDEs, polybrominated biphenyls, and trichloroethyl phosphate (the only compound in the chlorophosphate class) are flame-retardants. Manufacture of the former two brominated flame-retardants has been banned in the U.S. since 2013[42] and 1976,[43] respectively, but the latter chlorinated flame retardant remains in use. Methylsulfonyl-PCBs are PCB metabolites.[44] The legacy pesticides toxaphene, mirex, chlorinated benzenes, and hexachlorocyclohexane were also detected in all samples. 4,4′-Dichlorobenzophenone is a degradation product of the pesticide dicofol[45] and DDT,[46] but has not been identified to date in marine mammals to our knowledge. Bioaccumulative compounds with only known natural sources include: mono- and dibromoindole,[47] dimethoxy brominated biphenyl,[30] and polybrominated hexahydroxanthenes.[48] 2,4,6-Tribromoanisole and the dichlorinated polycyclic aromatic hydrocarbon may have both natural and anthropogenic sources.[49] Methylene bis(trichloro)anisole, detected in all eight samples and not previously reported in the environment, is the methylated version of hexachlorophene, which was used as a disinfectant and banned in 1972.[50] Polybrominated dibenzofurans can be formed during the processing of PBDE-containing plastics or the incineration of waste containing brominated flame-retardants,[51] as well as from

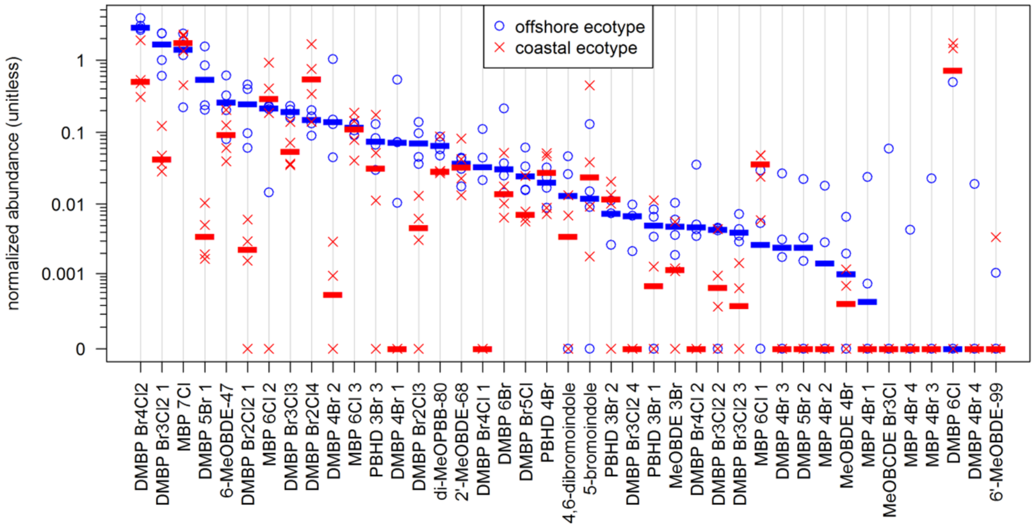

Figure 3. Comparison of HOCs of natural origin in coastal ($n = 4$) and offshore ($n = 4$) *T. truncatus* ecotypes. Each point represents the normalized abundance in a single dolphin blubber sample. The line is the median abundance for the compound. Compounds are sorted by the median abundance in the offshore ecotype. The median abundance of the coastal ecotype (red) is plotted over the offshore median abundance (blue) when both are zero (nondetect).

potentially natural hydroxy-PBDEs via photolysis.[52] Brominated dibenzofurans have been found in environmental samples such as sediments, soils,[51] and seafood,[53] but have not been detected in marine mammals to date. We also detected pyrrolidinecarbonyl chloride in three samples. There is very little information on this compound aside from its description as a synthetic precursor to a carbamate fungicide.[54] Finally, three compounds of unknown origin were detected: trichloro benzonitrile, chlorobenzaldehyde, and bromo methyl biphenyl.

Unknowns. The 94 HOCs in the unknown classes have fragmentation ion cluster(s) with characteristic halogenation patterns of chlorine and/or bromine, but could not be confidently assigned to a compound in the NIST library or identified de novo. Forty-six of the unknown HOCs were assigned to classes Unknown-1 through Unknown-8 based on mass spectral similarities within the class. Preliminary identities are described as follows. Unknown-1 may be chlordane or toxaphene related based on ions at m/z 305/303, 267, 238, and 159/161. Unknown-2 includes compounds identified by the NIST library as chloropropylate along with four compounds hypothesized to be TCPMOH based on spectral similarity. However, due to the absence of molecular ions or larger fragments in their mass spectra, we assigned these compounds to an unknown category. Unknown-3 compounds have previously been identified in Atlantic dolphin blubber,[1] fish oil supplements,[55] passive samplers deployed along the Great Barrier Reef,[56] and a Mediterranean sponge.[48] Unknown-4 compounds may be either polychlorinated diphenyl ethers (PCDEs) or hydroxy PCBs (OH-PCBs). Both of these classes share the molecular formula $C_{12}H_xCl_{10-x}O$ and have similar fragmentation patterns. The isomers identified in our samples possessed the following M+ ions: m/z 306 (Cl_5), 374 (Cl_6), 408 (Cl_7), 442 (Cl_8), 476 (Cl_9).

Ecotype HOC Profile Comparison. Natural product profiles of the coastal and offshore ecotypes were different, as summarized in Figure 3. This result was consistently observed under three unsupervised learning methods: hierarchical clustering, K-means clustering, and principal components analysis. Twelve natural products were detected in the offshore ecotype that were not detected in the coastal ecotype: seven DMBPs, four MBPs, and one methoxy brominated diphenyl ether (MeO-BDE). Several DMBPs were detected in both ecotypes, but had evident differences in abundance with higher levels in the offshore ecotype. Thus, ecotype differences were driven primarily by DMBPs, with the offshore ecotype displaying a greater diversity and abundance of DMBP isomers. Anthropogenic and unknown HOCs had no apparent differences among ecotypes. A description of the clustering methods is provided in the SI Methods, with results for each method as applied to the anthropogenic, natural, and unknown compounds shown in SI Figures S2–S7 and Tables S2–S4.

DISCUSSION

Catalog of Identified HOCs. A significant fraction (280 of 327, 86%) of the identified HOCs is not typically monitored in environmental surveys. The mixed, natural, and unknown compound classes (147 compounds in total) are comprised entirely of unmonitored compounds. Among the anthropogenic compounds, 133 of 180 (74%) are not typically monitored and thus have limited occurrence data, and 17 of the 18 anthropogenic compound classes were either all or partially composed of chemicals that are not typically monitored (Table 1). The exception was toxaphene, a highly complex mixture that has historically been analyzed as an unresolved group.[57] Here, we have referred to the complete toxaphene class as typically monitored. Our count of anthropogenic contaminants did not include PCBs and DDT/DDE, and therefore underestimates the monitored HOC burden. However, including an estimated 55 PCB congeners[1] and the routinely monitored four DDT/DDE isomers, there remained a greater number of unmonitored anthropogenic compounds ($n = 133$) compared to monitored anthropogenic compounds ($n = 106$). Generalizing these observations in bottlenose dolphins, contaminant monitoring of other biota in the Southern California Bight may omit a significant fraction of bioaccumulative contaminants. It is likely that an underestimation of the HOC body burden in apex predators extends beyond the current study region.

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Monitoring only the major components of technical mixtures may exclude minor components and breakdown products from observation. For example, the chlordane-related compound class contained unmonitored compounds with abundances comparable to typically monitored technical mixture components (see SI Figure S8). Five compounds, *cis*- and *trans*-nonachlor, α- and γ-chlordane, and oxychlordane, are typically monitored. However, the first four only comprise approximately 45% of the technical mixtures.[24] Furthermore, the abundance profile of chlordane-related compounds observed in the dolphin blubber differs from the technical mixture profile, suggesting varying extents of biomagnification or persistence of the individual compounds. Unmonitored chlordane compounds were detected in all eight samples, indicating they are prevalent in the regional environment. If these unmonitored products begin to dominate the HOC load, the typically monitored components of the technical mixture may no longer provide an accurate assessment of occurrence.

In addition to abundance, frequency of detection is a criterion to prioritize chemicals for monitoring and/or biological impact assessment. The frequency of detection varied among HOCs, from between one to eight samples (Figure 4). However, a larger number of unmonitored HOCs had high detection frequencies compared to monitored HOCs. For example, 54 unmonitored HOCs were detected in all eight samples, compared to 17 typically monitored HOCs. This suggests typically monitored HOCs are not the only widely distributed contaminants within the Southern California Bight.

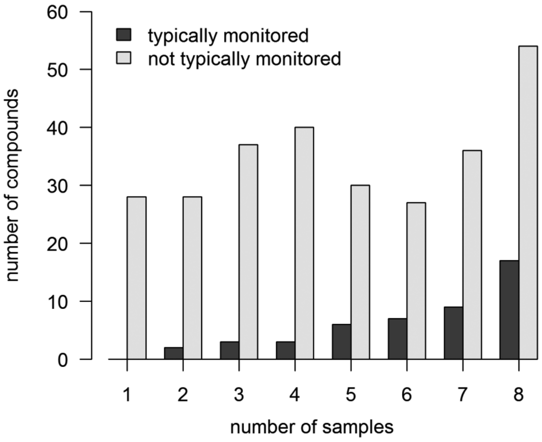

Figure 4. Histogram of the number of typically and not typically monitored compounds identified in the given number of samples.

Contaminant Profiles of Coastal and Offshore Ecotypes. We hypothesized that the coastal ecotype may contain a higher prevalence of anthropogenic compounds due to a closer proximity to urban sources. The data did not support this hypothesis, i.e., we did not observe a significant difference in the anthropogenic contaminant profiles. If the primary route of exposure to the anthropogenic HOCs is through diet, then any difference observed between ecotypes would be expected to be reflected in contaminant levels of their respective prey. Overall, there is little information on the feeding habits of the two *T. truncatus* ecotypes analyzed in the present study, although results from Perrin et al.[23] and Walker[22] indicate the two ecotypes largely feed on different prey. One explanation for our results is that prey for both the offshore and coastal ecotypes obtain their contaminant burdens from the same sources in the Southern California Bight.[58] For example, some anthropogenic sediment contaminants in the Southern California Bight are transported farther than 1 km from the shoreline,[59] potentially resulting in similar contaminant profiles in coastal and offshore food webs to which the two dolphin ecotypes are exposed.

In contrast, the observed natural product profile was different between the two ecotypes. DMBPs, followed by MBPs, were more prevalent in the offshore ecotype. This indicates that sources of these compound classes may be more abundant in offshore habitats of the Southern California Bight. Rocky reef habitats are more prevalent around the offshore Channel Islands (e.g., Santa Catalina, San Clemente, Santa Cruz, and Santa Rosa) than along the mainland coast.[60] Such hard bottom habitats (e.g., coral reef and/or macroalgae dominated systems) are known to be particularly rich in halogenated natural products.[61] Similar to our coastal/offshore comparison, Baird's beaked whale samples from offshore Pacific Ocean waters contained 60-fold higher DMBP levels than the same sample type from the Sea of Japan, despite having relatively similar anthropogenic PCB levels.[31] The modes by which DMBPs and MBPs enter the marine food web have not yet been identified, but MBPs have been shown to biomagnify with increasing trophic level.[62] There is evidence for biosynthesis of DMBPs by marine bacteria,[3] but further research is required to identify the sources and distribution in the Southern California Bight.

Regional Differences. Fifty-six percent of the compounds identified in the Pacific bottlenose dolphins were also found in a *D. delphis* stranded in 2006 off Cape Cod, MA (U.S.A.) in the western North Atlantic Ocean[1] analyzed using an equivalent analytical method. Twenty-nine DDT-related compounds were detected in the present study compared to eight in the *D. delphis* (excluding DDT and DDE). Twelve isomers of TCPM, which is a minor component of technical DDT (among other potential sources), were observed in the present study compared to two in the *D. delphis*. The magnitude and pervasiveness of DDT contamination in the Southern California Bight[59] can explain the higher number of related compounds in the Pacific *T. truncatus*. Polychlorinated terphenyls (PCTs) were not detected in the Atlantic dolphin, compared to 37 isomers in the *T. truncatus*. PCTs have been detected in other areas of the Atlantic, including a monk seal from North Africa,[28] marine mammal blubber from Africa, the European Arctic, and Iceland,[63] the liver of two different ray species off the coast of Rio de Janeiro, Brazil,[64] and sediments and biota from the James River and Chesapeake Bay (U.S.A.).[65,66] In contrast, two polychlorinated styrenes, byproducts of the synthesis of chlorinated hydrocarbons,[67] were detected in the *T. truncatus*, compared to 16 in the Atlantic *D. delphis*.

Naturally produced pyrroles also exhibited geographic specificity. The Atlantic *D. delphis* contained 28 MBP and 10 DMBP congeners, while the Pacific *T. truncatus* contained eight MBP and 20 DMBP congeners, similar to the inverse relationship of these two classes in the Atlantic and Pacific Oceans observed in other studies.[6,68] Previous research has also shown that MBPs in samples from the Atlantic Ocean are dominated by the more brominated congeners,[62,69−71] while those from the Pacific are dominated by the more chlorinated congeners,[72] as we observed in the Pacific *T. truncatus*.

Implications. When applied to marine mammals (dolphin blubber) as sentinels, this study shows that the nontargeted analysis framework can successfully discover multiple classes of

HOCs and provide a comprehensive list of HOCs that may be characteristic of an expansive region, in this case, the Southern California Bight. The identified HOCs are a subset of an exposure universe of persistent, bioaccumulative, and potentially biomagnifying compounds. Upper trophic level organisms may be exposed to these compounds through food-web transfer, and potential adverse health effects from long-term exposure for both marine mammals and humans (through seafood consumption) are of concern. This nontargeted monitoring framework can play a critical role in environmental health protection and evaluation of contaminants on a regional basis. In addition, the framework is complementary to other approaches that rely on databases of registered chemicals and modeling to predict the occurrence of new persistent contaminants.[73−75] However, the limited number of registered chemicals in the database restricts top-down modeling approaches. Missing compounds may include impurities, byproducts, unpredicted transformation products, region-specific chemicals with small production volumes, and, as the present study has illustrated, naturally occurring chemicals. However, the nontargeted framework presented here is a bottom-up approach that enables the identification of such chemicals. If further application of this framework continues to show relatively high abundances and detection frequencies of chemicals that are not currently monitored, then those compounds could be prioritized for toxicity testing and larger-scale targeted monitoring using quantitative methods.

Mass spectral library searches are limited to the available spectra, and de novo interpretation can be hindered by lack of fragmentation and/or molecular ions. Therefore, the identification of chemicals by this nontargeted approach remains challenging and time-consuming. For this reason, we established a mass spectral library for the HOCs identified in the dolphin blubber, 201 out of 327 of which are not included in the current National Institute of Standards and Technology (NIST) 2011 electron ionization (EI) mass spectral library. Further expansion of mass spectral libraries will improve identification efficiency in future analyses and is necessary for the nontargeted monitoring framework to realize its full potential. Examination of other marine mammals and top predator species in eastern Pacific Ocean food webs is a next step in assessing the comprehensiveness of the framework.

Several classes of natural HOCs were detected at abundances comparable to anthropogenic HOCs, highlighting the importance of examining their potential relevance to ocean and human health. Insofar as dolphins are appropriate sentinels for human exposure, it is hypothesized that humans are exposed to these natural HOCs through seafood consumption. However, many questions on the sources, occurrence, and toxicology of natural HOCs remain unanswered at this time. For toxicology alone, investigations on the interactions both within the diverse classes of natural HOCs observed, as well as between natural and anthropogenic HOCs would further reveal the similarities and differences in potency between these ubiquitous, bioaccumulative substances.

Humans are exposed to complex mixtures of HOCs at relatively low doses throughout our lifetimes,[76] with the primary exposure route often being through diet.[77] This is in stark contrast to the manner in which traditional toxicological studies and risk assessments are carried out, which have primarily focused on single chemicals at higher than environmentally relevant doses.[76,78] Even when all components of a complex mixture of chemicals are present at levels below their respective no adverse effect concentrations, the additive "mixture effect" of such components can result in deleterious outcomes.[79] A critical step in tackling the above-mentioned issues involves gaining a better understanding of what chemicals in the environment make up these complex exposure mixtures.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed descriptions of the analytical methods. In addition, glossary of acronyms, eight supplemental figures and five tables are included. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

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