Apex marine predators and ocean health: proactive screening of halogenated organic contaminants reveals ecosystem indicator species

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ABSTRACT

Despite decades-long bans on the production and use of certain chemicals, many halogenated organic compounds (HOCs) are persistent and can bioaccumulate in the marine environment with the potential to cause physiological harm to marine fauna. Highly lipid-rich tissue (e.g., marine mammal blubber) functions as a reservoir for HOCs, and selecting ideal indicator species is a priority for retrospective and proactive screening efforts. We selected five marine mammal species as possible indicators for the Southern California Bight (SCB) and applied a non-targeted analytical method paired with an automated data reduction strategy to catalog a broad range of known, known but unexpected, and unknown compounds in their blubber. A total of 194 HOCs were detected across the study species (n = 25 individuals), 81% of which are not routinely monitored, including 30 halogenated natural products and 45 compounds of unknown structure and origin. The cetacean species (long-beaked common dolphin, short-beaked common dolphin, and Risso's dolphin) averaged 128 HOCs, whereas pinnipeds (California sea lion and Pacific harbor seal) averaged 47 HOCs. We suspect this disparity can be attributed to differences in life history, foraging strategies, and/or enzyme-mediated metabolism. Our results support proposing (1) the long- and short-beaked common dolphin as apex marine predator sentinels for future and retrospective biomonitoring of the SCB ecosystem and (2) the use of non-targeted contaminant analyses to identify and prioritize emerging contaminants. The use of a sentinel marine species together with the non-targeted analytical approach will enable a proactive approach to environmental contaminant monitoring.

Keywords: nontargeted mass spectrometry; halogenated organic contaminants; marine mammals; bioaccumulation

Highlights:

- organic contaminants were identified in Southern California marine mammal blubber
- cetaceans accumulated more contaminants than pinnipeds
- common dolphins are proposed as sentinels for emerging contaminants in this region

1. INTRODUCTION

The Southern California Bight (SCB), in the eastern North Pacific Ocean, hosts notable biodiversity in its coastal and pelagic habitats. A multitude of species are exposed to legacy and ongoing contamination by bioaccumulative compounds such as dichlorodiphenyltrichloroethane (DDT) related compounds, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) (Dodder et al., 2012; Maruya and Schiff, 2009). Negative health effects such as endocrine disruption (Brouwer et al., 1989), reproductive impairment (Gilmartin et al., 1976; Roos et al., 2012; Schwacke et al., 2002), and immune system suppression (Hall et al., 1992; Ross, 2002; Schwacke et al., 2012) experienced by marine mammals in other geographic regions, but less studied in the SCB, have been attributed to chronic organic contaminant exposure, potentially increasing susceptibility to infectious disease. HOCs have also been speculated to enhance sensitivity of certain marine mammal species to poisoning by domoic acid (Tiedeken and Ramsdell, 2010), a potent neurotoxin produced by harmful algal blooms that are increasing in magnitude and frequency in the SCB (Gulland and Hall, 2007). Thus, apex predators (i.e., cetaceans and pinnipeds) in this region are integral to the detection of previously unrecognized environmental contaminants in addition to providing exposure data for possible contaminant-related health impacts.

A comprehensive assessment of contaminant load is required to evaluate the toxicological risk associated with contaminant exposure in wildlife, which may vary depending on the complexity of the contaminant mixture (Desforges et al., 2017). Marine mammals serve as effective indicators of marine pollution due to their high trophic position, large stores of lipid-rich blubber, and relatively long lifespans (Bossart, 2011; Ross, 2000). These characteristics can lead to relatively high concentrations and diverse classes of bioaccumulative HOCs from both anthropogenic and natural sources. Routine targeted screening for tissue HOCs consists of defined lists of compounds that may only account for a proportion of the contaminant load. As a result, many uncharacterized compounds with potential to cause physiological harm may be missed by routine research and monitoring (Shaul et al., 2015). A non-targeted analytical method employing comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC/TOF-MS) is capable of expanding the analysis to include thousands of both known and previously unidentified chemical constituents through enhanced chromatographic resolution and narrower chromatographic peak widths leading to enhanced sensitivity compared to single dimension GC systems (Hoh et al., 2012). The analytical method was combined with searches against reference mass spectra, *de novo* interpretation, and matches to authentic standards. The certainty was categorized based on the method of identification (e.g., matches to authentic standards had the highest confidence) (Hoh et al., 2012). Recent non-targeted studies of dolphins and seabirds have revealed that a substantial proportion of compounds are not routinely monitored (Alonso et al., 2017; Hoh et al., 2012; Millow et al., 2015; Shaul et al., 2015). Prior analyses of blubber samples from eight Pacific common bottlenose dolphins (*Tursiops truncatus*) identified 280 unmonitored and/or unknown compounds (86%) among 327 total HOCs (Shaul et al., 2015). The bottlenose dolphin study established the utility of this analytical method for detecting an expanded range of HOCs in wildlife tissues and identifying priority compounds for further investigation.

In this study, our primary objective was to evaluate apex marine sentinels for monitoring bioaccumulative HOCs using non-targeted analysis. We created an inventory of anthropogenic and naturally occurring bioaccumulative HOCs for each of five marine mammal species as candidate sentinels for the SCB: eastern North Pacific long-beaked common dolphins (*Delphinus* *bairdii*), short-beaked common dolphins (*Delphinus delphis*), Risso's dolphins (*Grampus griseus*), California sea lions (*Zalophus californianus*) and Pacific harbor seals (*Phoca vitulina*). A secondary technical objective of this study was the development of software to automatically identify HOCs from their fragmentation mass spectra, enabling data reduction and sample size expansion (Code 1 and 2, Supplemental Information). This was necessary in order to evaluate chemical accumulation trends across species. Additional objectives were to identify unexpected compounds that may pose an elevated risk based on abundance and frequency of detection, and prioritize unknowns for potential source and structure determination.

2. MATERIALS AND METHODS

Study species were selected based on established habitat range within the SCB, known availability of archived specimens, and documented susceptibility or suspected resistance to domoic acid toxicosis (Danil et al., 2010; Goldstein et al., 2008). Blubber samples were collected between 1990 and 2014, from individuals incidentally killed during fishing activities in the SCB or those that stranded dead. Five blubber samples from each of the five species were analyzed. Samples were restricted to males with priority given to mature (using length as a proxy) by-caught individuals (Table S2) to reduce the variability caused by 1) including females known to offload contaminants, and 2) age and health status (stranded individuals are generally sick). Blubber samples were archived at -20 °C. Approximately 20 g of frozen, full-depth blubber was sub-sampled and processed following protocols outlined by Shaul et al. (2015). Final extracts were analyzed on a Pegasus 4D GC×GC/TOF-MS equipped with an Agilent 6890 gas chromatograph using instrumental parameters optimized for marine mammal blubber by Hoh et al. (2012).

Data were processed using the LECO ChromaTOF mass spectrometer data system (version 4.51.6.0 optimized for Pegasus) and an automated data handling procedure. Details are described in SI-1 and Figure S4. Briefly, custom data reduction software was developed based on the algorithm described by Pena-Abaurrea et al., 2014, which examined mass spectra for ion intensity ratios characteristic of halogenation. Additional rules and a cross-checking procedure were applied to reduce the false positive rate. If the same mass spectrum was present in > 2 samples, the cross-checking procedure required a manual search for the compound in the remaining 23 samples.

On average, 479 halogenated mass spectra per sample were selected by the algorithm (out of an average of 9210 chromatographic features per sample), rate of 80% to 85% compared to a fully manual process (Table S3). Core components of the R script are provided in SI-1, and the full R script is provided at https://github.com/OrgMassSpec/IdentifyHalogenatedSpectra. Chromatographic peaks and the corresponding mass spectra selected by the algorithm were manually reviewed, and confirmed halogenated compounds were structurally identified through searches against existing mass spectral libraries generated from prior analysis of marine mammals using the same method (Mackintosh et al., 2016; Shaul et al., 2015) , searches against the NIST 2014 Electron Impact Mass Spectral Library, matches to authentic reference standards, or manual interpretation following the earlier methods. The mass spectra of unknown compounds are provided in SI-2. All identified compounds were also identified in the aforementioned prior studies and are provided in their publications (Mackintosh et al., 2016; Shaul et al., 2015).

3. RESULTS AND DISCUSSION

3.1 Species Contaminant Profile Comparisons. Cetacean blubber contained markedly more compounds than pinniped blubber, with the long-beaked common dolphin, short-beaked common dolphin, and Risso's dolphin averaging 133, 128, and 124 HOCs, respectively. These averages were two- to three-fold higher than average number of HOCs detected in the California sea lion (53 HOCs) and harbor seal (40 HOCs; Table 1).

As a structural class, PCBs had the largest number of individual compounds, and were between 28% to 32% of the total number of identified compounds in cetaceans, and 43% to 45% in pinnipeds (Table 1). For the purpose of identifying which species had the highest number of individual compounds, PCBs are included in the discussion below. However, parent PCB compounds are not included in contaminant profile comparisons because they are well-characterized in the literature and frequently monitored. We included PCB metabolites (methylsulfonyl-PCBs) in profile comparisons because they are typically unmonitored. A total of 194 different HOCs, excluding PCBs, from various anthropogenic, natural, mixed, and unknown sources were identified across all 25 samples, 157 (or 81% of total) of which are not included in routine environmental monitoring surveys.

Hierarchical clustering of samples based on similarity in anthropogenic HOC prevalence identified two primary, separate clusters comprising cetacean and pinniped samples, revealing that cetacean contaminant profiles were more diverse compared to pinnipeds (Figure S1). The heat map shows this difference is not limited to a few compounds, but is consistent across the majority of individual contaminants. The most frequently detected compounds included *p,p'*-DDE, trans nonachor, tris(4-chlorophenyl)methane (4,4',4''-TCPM), BDE-47, BDE-100, and BDE-99, all of which were detected in all 25 samples. Two potential confounding factors influencing contaminant accumulation were animal length (as a proxy for age) and collection year (Table S2). Contaminant profiles within species were not associated with either of these two factors (data not shown).

The HOCs detected in this study were organized into 35 classes based on similarity of molecular structure (Table 1; structural class descriptions in Table S1). DDT-related compounds constituted the most abundant structural class in both of the cetacean and pinniped groups (Figure 1), as well as for each species (Figure S2), which is consistent with the established contaminant signature found in marine mammals from this region (Blasius and Goodmanlowe, 2008; Shaul et al., 2015). The TCPM structural class consisted of eight isomers and was the second-most abundant. It was detected at levels comparable to the chlordane-related and PBDE classes, both of which are legacy HOCs of concern in the SCB (Dodder et al., 2012; Maruya and Schiff, 2009). Following its discovery in Puget Sound and Baltic Sea seals around 1990 (Walker et al., 1989; Zook et al., 1992), 4,4’,4’’-TCPM was determined to be an impurity from the technical preparation of pesticides such as DDT (Buser, 1995) and dicofol (de Boer, 1997). TCPM and its derivative, tris(4-chlorophenyl)methanol (TCPMOH), were measured in marine mammals from North America and Asia in the early 2000s, and were determined to have high biomagnification potential (Kajiwara, K. Kannan, M. Muraoka, M., 2001; Kannan et al., 2004; Minh et al., 2000; Watanabe et al., 2000). Although previously found to be one to three orders of magnitude greater in sea lions off the Northern and Central California coast compared to species from other locations (Kajiwara, K. Kannan, M. Muraoka, M., 2001; Kannan et al., 2004), TCPM and TCPMOH appear to have lost recognition as a pervasive contaminant in the North Pacific over the past decade. The parent compound, 4,4’,4”-TCPM, was detected in every blubber sample in this study. Notably, five additional TCPM isomers were present in nearly all dolphin samples (Figure 2) and constitute a relevant portion of the accumulated structural class.

However, within the SCB, these isomers have only been observed by prior non-targeted studies of bottlenose dolphins (Mackintosh et al., 2016; Shaul et al., 2015). The consistent ratio of TCPM isomers observed across dolphin samples suggests similar exposure to a consistent mixture of isomers, constituting a persistent and unmonitored chemical mixture that could permeate trophic levels throughout the region.

3.2 Halogenated Natural Products. The 30 identified halogenated natural products (HNPs) belonged to six different structural classes and accounted for 15% of the HOCs. These compounds are produced by algae or sponges and have a biomagnification potential similar to anthropogenic HOCs (Pangallo and Reddy, 2010). Despite their global distribution and profusion in marine food webs and biota, the health implications of exposure to HNPs are largely unknown (Shaul et al., 2015). We observed a more diverse array of HNPs in the dolphin species compared to the pinnipeds, with dimethyl bipyrroles (DMBPs) the most prevalent and abundant class (Table 1, Figure 1). 1,1'-Dimethyl-tetrabromo-dichloro-2,2'-bipyrrole (DMBP Br_4Cl_2) was the most common individual natural product, and was detected in all but one sea lion and four harbor seal samples (Figure S2). This compound was previously detected in California sea lion blubber at concentrations rivaling that of BDE 47 (Stapleton et al., 2006). Heptachlorinated 1-methyl 1',2-bipyrrole (MBP 7Cl) was the second-most common HNP and was found in all cetacean samples and three sea lion samples. This widespread natural product, also referred to as Q1, has been identified in Atlantic marine mammals (Teuten et al., 2006).

HNP profiles have been shown to be unique to different marine species and ecotypes within a geographic region (Dorneles et al., 2010; Hauler et al., 2013; Pangallo and Reddy, 2010; Shaul et al., 2015). The species in this study displayed nearly unique HNP profiles (Figure 3). California sea lions clustered separately from harbor seals, and all common dolphins grouped separately from the Risso's dolphins (with the exception of two short-beaked common dolphins), suggesting that foraging strategies and locations may be discernable using HNP profiles. In contrast, anthropogenic profiles were unique between cetaceans and pinnipeds, but species within these taxonomic groups were not different (Figure S1).

The difference between HNP and anthropogenic contaminant clustering may be due to the origin of these compounds to the local marine environment. Legacy anthropogenic compounds originated from terrestrial sources and now contaminate broad areas of marine sediment in the SCB (Dodder et al., 2012; Maruya and Schiff, 2009). HNPs may have spatially distinct ecological profiles based on algal and sponge community composition, leading to unique bioaccumulation patterns based on the habitats and foraging strategies of the different species. HNPs have important potential applications beyond toxicological assessments, including the differentiation of ecotypes or populations within species (Dorneles et al., 2010; Shaul et al., 2015), evaluating trophic relationships (Pangallo and Reddy, 2010), and could enhance indirect chemical methods for determining diet composition of free-ranging marine mammal species (Bowen and Iverson, 2012).

3.3 Unknown Contaminants. Based on isotopic profiles in their mass spectra, 45 observed compounds were determined to contain halogens, but their complete structural identities could not be determined through mass spectral searches or *de novo* interpretation (mass spectra in SI-2 and accessible online at https://github.com/OrgMassSpec/SpecLibMarineUnknown2018). Ten of the halogenated unknown compounds have not, to our knowledge, been previously identified in marine biota; they are first 10 spectra described in SI-2. The number of unknowns accounted for 24% of the total number of identified HOCs and outnumbered each of the DDT-related (n=24), chlordane-related (n=14) and PBDE (n=16) structural classes. These unidentified compounds could be emerging contaminants from current anthropogenic activities, previously undiscovered legacy pollutants, or halogenated natural products. For example, the unknown-8-2 isomer (SI-2, page 46) was relatively abundant in cetacean blubber and a significant regression was found with ΣDDT-Related (R^2=0.79, p<0.001), ΣChlordane-Related (R^2=0.74, p<0.001), ΣTCPM (R^2=0.82, p<0.001, and the TCPMOH Isomer 2 metabolite (R^2=0.88, p<0.001); Figure 4), indicating the unknown compound may have a similar history of anthropogenic use in this region. Unknown-8-2 did not have a significant regression with ΣPBDE, a compound class with a different history compared to the other anthropogenic contaminants, including later peak use (Dodder et al., 2012). There was a significant, but less robust regression with ΣDMBP (R^2=0.4, p<0.001), indicating unknown-8-2 may not be a natural product.

3.4 Differences in Cetacean vs. Pinniped Profiles. Pinnipeds appear to accumulate fewer compounds across a smaller number of structural classes, however the compounds that do accumulate in pinniped blubber are found at similar abundance to those in cetaceans (Figure 1). Although California sea lions feed on the same pelagic schooling fish as common dolphins, and even regularly feed in industrialized embayments (Meng et al., 2009), the multitude of HOCs in their contaminant profile is reduced (53 average total HOCs in sea lions versus 133 and 128 average total HOCs in long- and short-beaked common dolphins, respectively; Table 1). Possible explanations for this discrepancy are likely physiological, such as differences in life history strategies and enzyme-mediated metabolism. Pinnipeds undergo periods of fasting during annual breeding and molting seasons (Ling, 1970). These processes are energetically costly, enhancing blubber turnover and mobilizing contaminants to the bloodstream (Peterson et al., 2014). Small cetacean species maintain a homogenous HOC profile across blubber depth indicating more stable concentrations over time (Méndez-Fernandez et al., 2016). Cetaceans also have a lower capacity for metabolizing PCBs compared to other terrestrial and marine mammals, such as seals (Routti et al., 2008; Tanabe et al., 1988), due to lower or absent CYP2B liver enzyme activity (Boon et al., 1997). An enhanced capacity for metabolizing and excreting certain organic contaminants was proposed to explain the much lower levels of TCPM and TCPMOH observed in pinnipeds compared to cetaceans (Minh et al., 2000). Enhanced metabolism has also been proposed as an explanation for the absence of HNPs from pinniped blubber despite presence in prey items (Pangallo and Reddy, 2010) and the lack of methoxy-BDEs detected in California sea lions (Stapleton et al., 2006). The expanded range of HOCs evaluated in this study revealed additional structural classes that appear to align with this phenomenon (Figure 1).

3.5 Best Sentinel Determination. Based upon the multitude and abundance of detected HOCs, cetacean species are more effective regional bioindicators for cataloging emerging and unknown HOCs. The following discussion excludes PCBs because they were not previously included in the associated bottlenose dolphin study (Shaul et al., 2015). Whereas 103 anthropogenic and naturally occurring unmonitored compounds, and 45 unknowns, were detected in the cetacean samples from the current study (94% of all unmonitored compounds from both cetaceans and pinnipeds), only 46 typically unmonitored HOCs from anthropogenic and natural sources, and 12 unknowns, were detected in pinniped blubber. Within the cetaceans, the long-beaked common, short-beaked common, and Risso's dolphin samples averaged 90, 89, and 89 HOCs per sample, respectively. In contrast, an average of 209 HOCs per sample (n=8) was detected in Pacific common bottlenose dolphins in the prior study using the same blubber sampling and instrumental method (Shaul et al., 2015). The bottlenose dolphins were collected from the same region, during approximately the same timeframe (1995-2010). The data processing methods were different. Halogenated compounds in the bottlenose dolphins were identified by a fully manual process. For the current project, as described in detail in SI-1, we used an automated process (with manual cross-checking between samples as a final step). Direct comparison of the two processes determined the automated process detection rate of halogenated compounds was 80% to 85% of the manual process. The difference in detection rates is not large enough to account for the difference in the number of HOCs detected in bottlenose dolphins compared to the other species.

This indicates the bottlenose is the most contaminated marine mammal among the six species studied in the SCB. However, bottlenose dolphins do not often strand, limiting availability of full-depth blubber samples from this species, and hampering ongoing alternate research that relies on live biopsy samples (Figure S3). Archived specimens are also exceptionally rare for the Risso's dolphin, which typically range offshore. Therefore, based on similar HOC profiles and readily available sample material, it is recommended that long- and short-beaked common dolphins (which contained 80% of the unmonitored compounds found among all species in this study) serve as effective sentinels for fulfilling the two main priorities of the non-targeted work in the SCB: retrospective evaluation of contaminant trends and proactive screening for emerging contaminants of concern.

3.6 Domoic Acid Susceptibility. Species with observed sensitivity to domoic acid toxicosis (i.e., long-beaked common dolphins, short-beaked common dolphin, and California sea lions) did not contain unique contaminant signatures compared to those that have not been documented to experience mass mortality events following blooms (i.e., common bottlenose dolphins, Risso's dolphins, and harbor seals (Gulland and Hall, 2007; Lefebvre et al., 1999). In fact, domoic acid-related stranding events were not observed for the most contaminated species, the Pacific common bottlenose dolphin. This suggests contaminants do not play a profound role in the domoic acid sensitivity exhibited by particular SCB marine mammal populations at the species level. This result reinforces existing speculation that prey preference likely has the greatest impact on the risk of poisoning by domoic acid (Lefebvre et al., 1999). The Northern anchovy has been implicated as the primary vector of exposure and is a preferred prey item for California sea lions, long-, and short-beaked common dolphins (Lowry and Carretta, 1999), all of which experience domoic acid toxicosis (Danil et al., 2010).

3.7 Implications for Contaminant Monitoring. The population of the five coastal counties bordering the SCB is projected to increase to over 20 million people by 2020 (Maruya and Schiff, 2009), thus there is a demand for vigilance in monitoring of both legacy and emerging contaminants in this ecologically and economically valuable coastal zone. Therefore, as new compounds are released into the environment, non-targeted contaminant analysis allows researchers and managers to comprehensively screen for emerging compounds of concern, fast-tracking them for possible source, structure, and toxicity testing. Further evaluation and characterization of the unknown compounds highlighted in this study is imperative for assessing seafood safety and possible distribution of such contaminants on a global scale, particularly when the abundance of these compounds rivals that of many legacy pollutants. Additionally, chemicals identified in this study may serve as unique tracers to gather information about the physiology and ecology of marine mammal species that serve as sentinels of marine environmental quality.

SUPPLEMENTARY DATA

The following is the supplementary data related to this article:

Supporting Information Part 1 (SI-1) and Supporting Information Part 2 (SI-2).

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Author Contributions

N.G.D., E.H., S.J.C., D.W.W., K.D., and K.A.M. designed the research plan, S.J.C., D.W.W., and K.D. contributed sample material, J.M.C performed the sample preparation, J.M.C., N.G.D., and E.H. performed the instrumental and data analysis, N.G.D. wrote the custom software, and J.M.C., N.G.D., E.H., S.J.C., D.W.W., K.D., and K.A.M wrote the paper.

Notes

The authors declare no conflict of interest.

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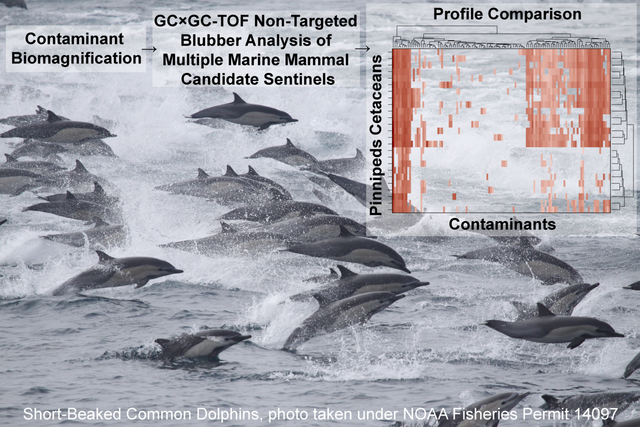
A) Common Compounds in Cetaceans
B) Common Compounds in Pinnipeds
C) Compounds in Cetaceans Only
Relative Response (log_{10})
100
10
1
0.1
0.01
DDT-related
Chlordane-related
TCPM
PBDE
DMBP
TCPMOH
Chlorinated benzene
HCH-related
MBP
Mirex-related
Unknown-8
Unknown
MeO-BDE
PCT
PBB
Unknown-4
PBHD
Heptachlor-related
Unknown-7
Methylsulfonyl-PCB
Pyrrolidinecarbonyl chloride
Unknown-6
Chlorinated styrene
Brominated anisole
B/CDE
Toxaphene
Methylenebistrichloroanisole
Dichlorobenzophenone
MeO-PBB
Unknown-5
MeO-B/CDE
Unknown-3
Dieldrin
MeO-CDE

A) Cetaceans
B) Pinnipeds
Percent of Total Relative Response
100
75
50
25
0
TCPM
TCPM 5
TCPM 3
TCPM 6
TCPM 4
TCPM 1


10
1
0.1
0.01
0.001
0
Relative Response (log_{10})
Risso's Dol. (7)
Risso's Dol. (3)
Short-Beaked Dol. (10)
Short-Beaked Dol. (9)
Risso's Dol. (25)
Risso's Dol. (16)
Risso's Dol. (2)
Long-Beaked Dol. (21)
Long-Beaked Dol. (17)
Long-Beaked Dol. (20)
Long-Beaked Dol. (8)
Short-Beaked Dol. (11)
Short-Beaked Dol. (24)
Short-Beaked Dol. (14)
Long-Beaked Dol. (6)
Sea Lion (18)
Sea Lion (5)
Sea Lion (12)
Sea Lion (19)
Sea Lion (15)
Harbor Seal (22)
Harbor Seal (13)
Harbor Seal (23)
Harbor Seal (1)
Harbor Seal (4)
MBP 7Cl
DMBP Br4Cl2
DMBP 4Br 3
DMBP 5Br 2
DMBP 4Br 1
MeOBDE 4Br
PBHD 3Br 2
DMBP Br2Cl3
MBP 4Br 2
MBP 4Br 3
DMBP 6Cl
MBP 6Cl 3
MBP 6Cl 1
PBHD 4Br
DMBP Br5Cl
DMBP Br2Cl2 1
DMBP 4Br 2
DMBP Br3Cl2 1
di-MeOPBB-80
DMBP 5Br 1
6-MeOBDE-47
2'-MeOBDE-68
DMBP 6Br
MBP 6Cl 2
DMBP Br2Cl4
PBHD 3Br 3
DMBP Br3Cl3
DMBP Br4Cl 1
DMBP Br4Cl 2
MeOBCDE Br3Cl

ΣDDT-Related
R² = 0.79, p < 0.001
ΣChlordane-Related
R² = 0.74, p < 0.001
ΣTCPM
R² = 0.82, p < 0.001
TCPMOH Isomer 2
R² = 0.88, p < 0.001
ΣPBDE
R² = 0.02, p = 0.46
ΣDMBP
R² = 0.40, p < 0.001
ΣMBP
R² = 0.19, p = 0.30
Anthropogenic Contaminant
Natural Product
Structural Class Relative Response
Unknown 8-2 Relative Response

Structural class	No. HOCs in class	(No. not typically monitored)	Source	Long-beaked common dolphin (*D. capensis*, n=5) Average no. HOCs	Short-beaked common dolphin (*D. delphis,* n=5) Average no. HOCs	Risso's dolphin (*G. griseus*, n=5) Average no. HOCs	California sea lion (*Z. californianus,* n=5) Average no. HOCs	Harbor seal (*P. vitulina*, n=5) Average no. HOCs
B/CDE	1	1	unknown	0	0	0	0	0
Brominated anisole	1	1	mixed	1 (1)	0	0	0	0
Chlordane-related	14	9	anthropogenic	8 (3)	8 (4)	7 (3)	5 (2)	1 (0)
Chlorinated benzene	1	1	anthropogenic	1 (1)	1 (1)	1 (1)	0	1 (1)
Chlorinated styrene	1	1	anthropogenic	1 (1)	0	0	0	0
DDT-related	24	16	anthropogenic	13 (7)	12 (5)	10 (4)	4 (2)	3 (1)
Dichlorobenzophenone	1	1	anthropogenic	0	0	0	0	0
Dieldrin	1	0	anthropogenic	0	1 (0)	0	0	0
DMBP	16	16	natural	8 (8)	12 (12)	13 (13)	2 (2)	0
HCH-related	2	0	anthropogenic	1 (0)	1 (0)	1 (0)	1 (0)	0
Heptachlor-related	3	2	anthropogenic	1 (1)	2 (1)	1 (0)	0	0
MBP	6	6	natural	4 (4)	4 (4)	3 (3)	2 (2)	0
MeO-B/CDE	1	1	natural	0	0	1 (1)	0	0
MeO-BDE	3	3	natural	2 (2)	2 (2)	2 (2)	0	0
MeO-CDE	1	1	unknown	0	0	0	0	0
MeO-PBB	1	1	natural	1 (1)	1 (1)	1 (1)	0	0
Methylenebistrichloroanisole	1	1	anthropogenic	1 (1)	1 (1)	1 (1)	0	0
Methylsulfonyl-PCB	7	7	anthropogenic	2 (2)	1 (1)	1 (1)	1 (1)	3 (3)
Mirex-related	5	4	anthropogenic	3 (2)	3 (2)	2 (1)	1 (0)	1 (0)
PBB	10	9	anthropogenic	4 (3)	4 (3)	4 (3)	1 (0)	2 (2)
PBDE	16	6	anthropogenic	10 (1)	9 (2)	9 (1)	8 (0)	6 (0)
PBHD	3	3	natural	1 (1)	1 (1)	2 (2)	0	0
PCT	6	6	anthropogenic	2 (2)	1 (1)	3 (3)	0	0
Pyrrolidinecarbonyl chloride	1	1	anthropogenic	0	0	0	0	0
TCPM	8	8	anthropogenic	6 (6)	6 (6)	6 (6)	2 (2)	1 (1)
TCPMOH	2	2	anthropogenic	1 (1)	1 (1)	1 (1)	0	0
Toxaphene	13	5	anthropogenic	4 (0)	7 (0)	5 (0)	0	0
Unknown	22	22	unknown	5 (5)	5 (5)	6 (6)	0	0
Unknown-3	2	2	unknown	1 (1)	1 (1)	0	0	0
Unknown-4	12	12	unknown	6 (6)	4 (4)	4 (4)	1 (1)	0
Unknown-5	2	2	unknown	1 (1)	1 (1)	2 (2)	0	0
Unknown-6	3	3	unknown	1 (1)	1 (1)	0	0	0
Unknown-7	2	2	unknown	1 (1)	1 (1)	1 (1)	0	1 (1)
Unknown-8	2	2	unknown	1 (1)	1 (1)	1 (1)	0	0
Average PCBs	-	-	anthropogenic	43	39	35	24	17
Average Total HOCs (excluding PCBs)	194	-	mixed	90	89	89	29	22
(Range)				(77 – 98)	(77 – 102)	(70 – 108)	(14 – 55)	(11 – 32)


Contaminant Biomagnification
GC×GC-TOF Non-Targeted Blubber Analysis of Multiple Marine Mammal Candidate Sentinels
Profile Comparison
Cetaceans
Pinnipeds
Contaminants
Short-Beaked Common Dolphins, photo taken under NOAA Fisheries Permit 14097