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Identification of DDT+ in Deep Ocean Sediment and Biota in the Southern California Bight

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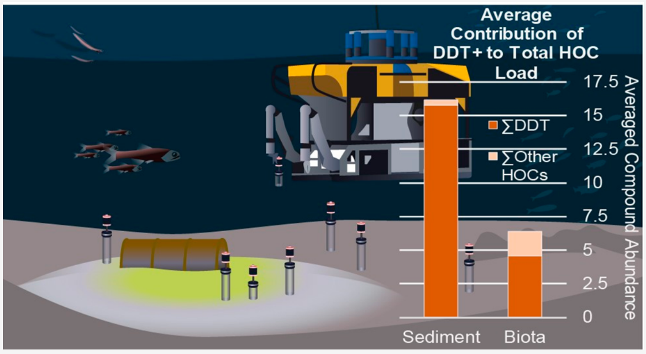

ABSTRACT: The recent rediscovery of offshore DDT waste dumping in the Southern California Bight (SCB) has led to questions about the extent and type of pollution in deep ocean environments. We used a nontargeted analysis to identify halogenated organic compounds (HOCs), including DDT+, in sediment in the San Pedro Basin. Additionally, we examined the chemical profiles of deep ocean biota inhabiting the SCB to assess the bioavailability of DDT+ and HOCs to the deep ocean food web. We detected 49 HOCs across all samples, including 15 DDT+ compounds in the sediment and 10 DDT+ compounds in the biota. Compounds included tris(4-chlorophenyl)methane (TCPM) and its isomers and three unknown DDT-related compounds previously identified in marine mammals. No clear trends were identified regarding DDT+ distribution in sediments. High DDT+ body burdens were found in biota irrespective of collection location, indicating widespread DDT+ contamination in the deep ocean of the SCB. TCPMs were detected in all biota samples except a single surface species, indicating that deep ocean sediment may be a source of DDT+ to the marine food web. This study demonstrates that the analysis of the larger suite of DDT+ is critical to trace deep ocean pollution of DDT in the SCB.

KEYWORDS: *Nontargeted analysis, halogenated organic compounds, contamination, bioaccumulation, TCPM*

■ INTRODUCTION

The Southern California Bight (SCB) has some of the highest recorded concentrations of DDT in the world due to the discharge of DDT manufacturing waste from 1947 to 1982 by the Montrose Chemical Corporation.[1,2] The discharged waste, composed of industrial acid waste with trace amounts of DDT, contaminated the Palos Verdes Shelf (PVS), and the area was designated as an EPA Superfund Site in 1996. Thus, most DDT research in the SCB has focused on the PVS.[3] However, a 1985 report authored by Chartrand et al. provided evidence of a second DDT waste source: the offshore dumping of acid waste associated with the manufacturing of DDT.[2,4]

Offshore dumping of various chemical waste products was a legal practice in the 1900s, and there are 14 known deep ocean disposal sites off the coast of southern California.[2,4,5] One disposal site, Dumpsite 2, is in the San Pedro Channel between Long Beach, CA, and Santa Catalina Island, CA. The 1985 report provides evidence that DDT waste was disposed at Dumpsite 2, but it was often illegally short-dumped before vessels reached the designated dumpsite.[4,6] Venkatesan et al. further reported in 1996 that the ratio of DDT congeners found at the offshore sites did not match those in either the wastewater discharged onto the PVS or the DDT technical mixture.[7] These early findings were corroborated more recently when waste barrels were imaged by underwater vehicles at locations matching the offshore dumpsites identified by Chartrand et al. in 1985.[4,6,8] However, concurrent investigations by the EPA suggested that DDT manufacturing waste may have been bulk-dumped (i.e., not containerized) near the dumpsites rather than disposed in barrels.[9−11] Together, these studies point to a secondary offshore DDT waste source that has been largely unaccounted for in regional environmental surveys, even when examining biota collected in deep waters.[11]

Evidence of potentially significant offshore DDT waste dumping increases the uncertainty in past estimates of the total magnitude of DDT pollution in the SCB. The DDT pollution on the PVS is well-characterized, but there is a need to further investigate the role of offshore deep dumpsites as a source of DDT to the SCB food web.[2,3,6] Additionally, most DDT surveys examine four to eight typical compounds (DDX), such

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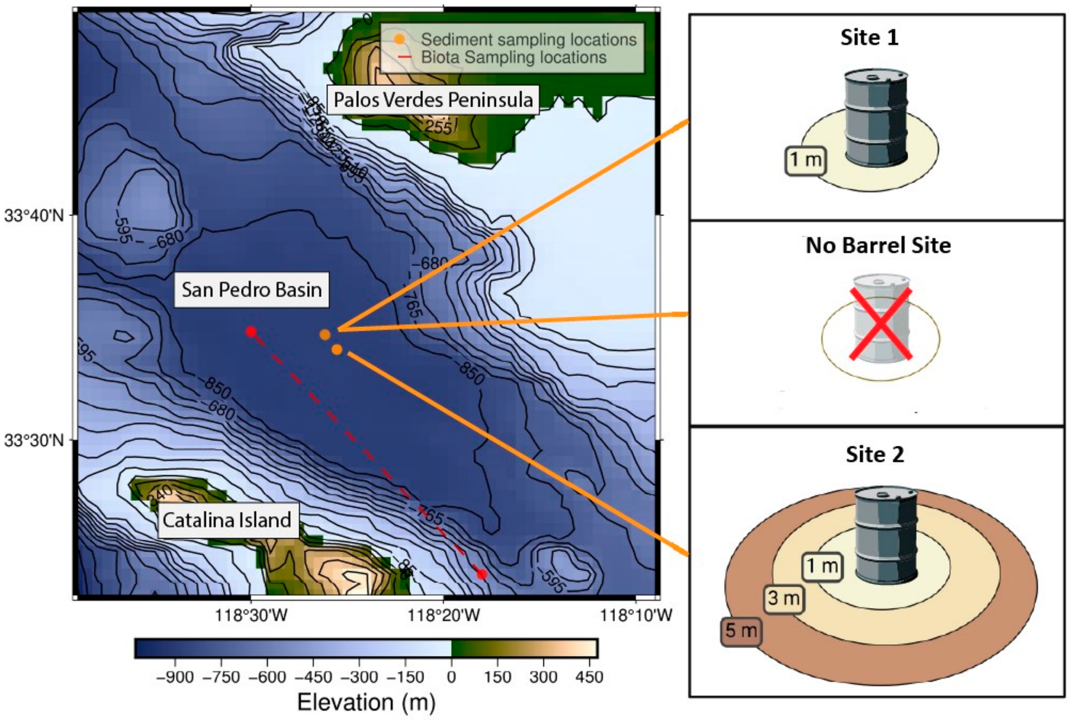

Figure 1. Map of sampling locations for the sediment cores taken from Dumpsite 2, approximately 10 km north of Catalina Island, CA, USA. Site 1 and the No Barrel Site appear to overlap on the scale of the map but are 90 m apart. The red dotted line shows the cruise track for the MOCNESS trawl that collected biota from the water column above Dumpsite 2.

as *p,p′*- and *o,p′*-DDT, DDE, and DDD. However, recent work indicates that marine mammals inhabiting the SCB are exposed to more than 45 DDT-related contaminants.[1] This larger suite of DDT-related chemicals is known as DDT+.[2] DDT+ includes not only DDX but also further degradation products, relatively unknown compounds such as tris(4-chlorophenyl)-methane (TCPM), tris(4-chlorophenyl)methanol (TCPMOH), and their isomers and congeners as impurities of DDT technical product, as well as additional DDT-related compounds.[1,2,13] To our knowledge, only Kivenson et al. in 2019 performed a nontargeted analysis to identify DDT+ and other contaminants present in Dumpsite 2 sediments at different locations than in the present study.[6] Their results showed high variability in sediment DDT+ concentrations across two sediment samples (2−4 and 4−6 cm sediment depth), indicating that dumping was nonuniform. Given the small sample size as well the variability, further investigation of DDT+ profiles in deep ocean sediments are warranted. Additionally, while deep ocean sediments have sparse contaminant data, there are no reports on DDT+ in deep sea biota collected from this location.[12]

Our study aims to investigate the halogenated organic compound (HOC) profile of the deep ocean disposal site (Dumpsite 2) using a nontargeted analysis based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) and custom mass spectral libraries developed from SCB marine mammal surveys.[1,13−20] Here we (1) determine HOC profiles for sediments collected at Dumpsite 2, with a focus on assessing the occurrence of the 45 DDT+ compounds previously identified in regional bottlenose dolphins (*Tursiops truncatus*),[1] and (2) assess the potential for DDT+ bioaccumulation in the deep ocean food web by determining the chemical profiles in one invertebrate and three fish species collected from throughout the water column. The results provide foundational knowledge regarding the type and relative abundance of DDT+ waste in Dumpsite 2 sediments and are the first investigation of DDT+ compounds in deep ocean biota.

■ METHODS AND MATERIALS

Sediment Sampling and Collection. Sediment cores were taken from three sites in the proximity of dumped barrels within and near Dumpsite 2, namely, Site 1, Site 2, and a No Barrel Site (Figure 1), using the Schmidt Ocean Institute's Remotely Operated Vehicle (ROV) *SuBastian*, operated from the R/V *Falkor*, during expedition FK210726 in August 2021 (https://www.youtube.com/watch?v=p05c4DjX8AU). Sites 1 and 2 were near visually identified barrels (within ∼5 m). At Site 1, a sediment core was taken 1 m from the barrel (SCB-264). At Site 2, sediment cores were taken 1 m from the barrel (SCB-292), 3 m from the barrel (SCB-290), and 5 m from the barrel (SCB-288). The No Barrel Site (SCB-268) was approximately 90 m from any barrels observed at Sites 1 and 2. In the laboratory, each sediment core was divided into two depths (1−4 cm and 5−6 cm from the surface) that were analyzed separately to determine historical trends (see Table 2). The total carbon (TC) analyzed for HOCs was not significantly different across the sites.

Biota Sampling Locations and Collection. Micronekton (biota) were collected from the water column above the San Pedro Basin (around Dumpsite 2) and the Santa Cruz Basin (offsite control) during two separate cruises (see Table 3). At Dumpsite 2, biota were collected aboard R/V *Roger Revelle* in August 2021 during an afternoon (15:30) to dusk (∼20:30) tow of a 1 m^2 Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) equipped with 202 μm mesh nets.[21] Vertically stratified towing was done in the northwest direction over Dumpsite 2 at a speed of 1 knot. Towing began at 33.4° N, 118.3° W and ended at 33.58° N, 118.5° W (Figure 1).

At the Santa Cruz Basin, biota samples were collected aboard R/V *Roger Revelle* in June 2021 using a 10 m^2 MOCNESS. The MOCNESS was equipped with five individual ~5 mm mesh nets towed between 1 and 2 knots through the basin (33°44′ N, 119°35′ W) in the northwest direction. Samples were collected with individual nets at discrete depth intervals of 990−1480 m and 990−1725 m.

Once the nets were successfully recovered onboard, samples were either coarsely, visually sorted by micronekton type and net number and frozen immediately at −80 °C or rapidly transferred to chilled filtered seawater and stored in walk-in refrigerators until sorting. Biota specimens were identified to species using published taxonomic keys either at sea (Santa Cruz Basin) or when samples returned frozen to Scripps Institution of Oceanography, University of California, San Diego, CA (Dumpsite 2). To obtain sufficient wet biomass for HOC analysis from the lowest level of the food web sampled, one sample of mixed species of zooplankton (swimming animals <2 cm in size) was analyzed (ID 11).

HOC Analysis. Sediment and biota samples were shipped frozen to SGS AXYS Analytical Services Ltd. (Sidney, BC, Canada) and extracted using SGS AXYS Method MLA-028 REV 06 VER 15, which meets the performance and quality control requirements of EPA Method 1699 for the analysis of organochlorine pesticides. Samples were homogenized wet prior to analysis, spiked with $^{13}C_{12}$-labeled *p,p′*- and *o,p′*-DDE, DDD, and DDT, and Soxhlet-extracted with dichloromethane. Sediment extracts were cleaned by fractionation based on polarity using a Florisil column. Biota extracts were additionally cleaned using a Biobead column. Purified extracts were transferred to San Diego State University (San Diego, CA, USA) and brought to 400 μL with isooctane. Analysis was performed using a Pegasus 4D GC×GC/TOF-MS (LECO, St. Joseph, MI) with electron ionization following previously described conditions.[14,18] Raw data were processed using LECO ChromaTOF software (v4.72). HOC analysis followed previously described methods.[16,20] Compounds were classified and named according to their mass spectra and retention times using custom HOC mass spectral libraries, which were generated using the same instrumental method and developed for southern California wildlife (http://orgmassspec.github.io/libraries.html).[1,13,15,16,20,22] A normalized relative abundance was used to compare contaminants between and within samples. Relative abundance was calculated by dividing the peak area of the compound by the peak area of the internal standard ($^{13}C_{12}$-*o,p′*-DDE) and the sample mass. Sediment samples are reported in dry weight (g), while the biota samples are reported in wet weight (g). This provides a unitless abundance value that can be used as a proxy for the compound concentration. To compare the total relative abundance between samples, we used the percent change, defined as ($[RA_1 - RA_2]/RA_2) \times 100$), where RA_i is the relative abundance of sample *i*.

RESULTS AND DISCUSSION

HOCs in Sediment and Biota. In total, we detected 49 unique HOCs across all sediment and biota samples (Table 1 and Supporting Information (SI) Table 1). A greater diversity of HOCs was identified in the biota than in the sediment. Biota contained 42 HOCs (86% of the total detected HOCs), and the sediments contained 28 HOCs (57%), with 22 HOCs common among the two groups. Given that HOCs bioaccumulate, the detectability of the compounds in biota may be greater than in sediment.[23] The average relative abundance and standard deviation for each compound are given in SI Tables 2 and 3.

Table 1. Numbers of HOCs Detected in Sediment and Biota Samples by Compound Class[a]

compound class	source	no. in sediment	no. in biota	total no. detected
chlordane-related	anthropogenic	1	2	2
chlorinated compound	anthropogenic	1	3	4
DDT-related	anthropogenic	12	8	12
DMBP	natural	0	4	4
MBP	natural	0	1	1
Mirex	anthropogenic	0	1	1
PCB	anthropogenic	10	18	18
TCPM-related	anthropogenic	3	2	3
unknown	unknown	1	3	4
total		**28**	**42**	**49**

[a] Source indicates anthropogenic, natural, or unknown origins. Definitions of abbreviated compound class names can be found in the SI.

The 49 HOCs were classified into nine groups based on their chemical structure.[1,13,16,20] The structural class with the highest number of compounds was polychlorinated biphenyls (PCBs) ($n = 18$), followed by DDT+ (including DDT-related and TCPM-related chemicals) ($n = 15$). Five compounds did not match with the custom mass spectral libraries[1,13,15,16,20,22] or with the 2017 NIST Electron Ionization (EI) library. One compound had a fragmentation pattern similar to that of DDT and DDD, with fragment ions at $m/z = 235$, 199, and 165 but an earlier retention time. The other four compounds had unknown fragmentation patterns and were thus labeled as “unknown”. The mass spectra for these compounds are given in the SI.

Sediment HOC Profiles in Dumpsite 2. There were 28 HOCs detected across the sediment samples ($n = 10$) at Dumpsite 2, including 15 DDT+ compounds from both the DDT-related and TCPM-related classes (Figure 2). Kivenson et al.[6] detected 19 organochlorines, including 14 DDT+ compounds, in two sediment samples analyzed by GC×GC/TOF-MS from Dumpsite 2. Our current work detected four DDT-related compounds not identified by Kivenson et al. (DDNU and three additional unknown DDT-related compounds). Conversely, Kivenson et al. detected bis(4-chlorophenyl)methane (DDM) and dichlorobenzophenone (DBP), which were not detected in our sediments. In both studies, *p,p′*-DDE was the most abundant compound in most samples. We noted exceptions in the 5−6 cm fraction at the 1 m location at Sites 1 and 2 (ID 22 and ID 10) and in the 1−4 cm fraction at the No Barrel Site (ID 27). In these three samples, *o,p′*-DDD, DDT-related 12,[13] and *p,p′*-DDD were the most abundant, respectively.

The ΣDDT+ concentrations at Sites 1 and 2 were not similar. The 1 m locations at Site 2 had 26% and 91% less ΣDDT+ than those at Site 1, depending on sediment depth. At Site 2, the ΣDDT+ abundance was not highest nearest the barrel (Table 2), similar to previous work that reported increasing ΣDDT+ sediment concentrations with increasing distance from barrels.[6] Site 2 samples were collected at

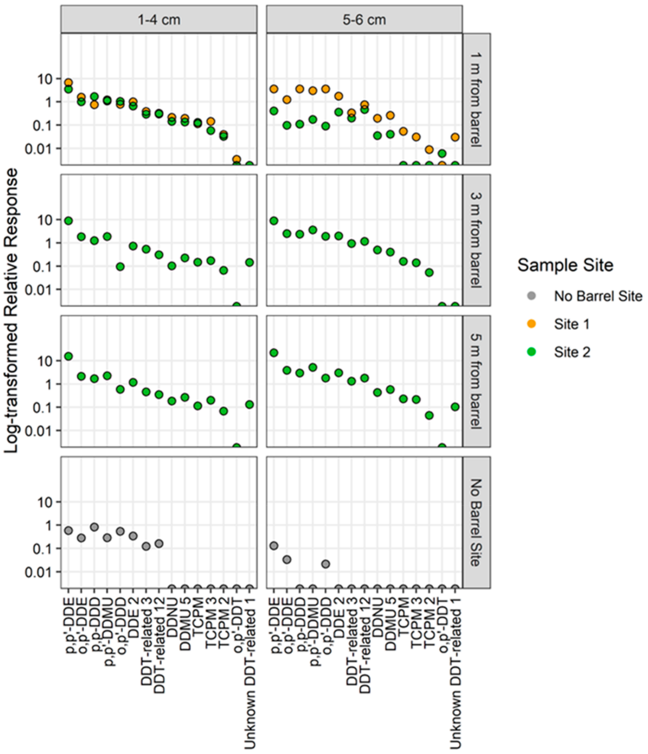

Figure 2. Log-transformed relative abundances of the 15 DDT+ compounds detected in the sediment samples based on sampling location and sediment depth.

multiple distances from the barrel, and we found that ΣDDT+ in the 1–4 cm fraction was 3 times higher at the 5 m location (ID 28) compared to the 1 m location (ID 7) and more than 4 times higher in the 5–6 cm fraction. We found the ΣDDT+ abundance at the No Barrel Site (90 m from any barrel) was 77% and 99% lower compared to Site 1 (1 m) at the respective sediment depths. Further sampling should be done to understand whether these differences are meaningful. Similar to Kivenson et al., who described nonuniform ΣDDT concentrations in Dumpsite 2 sediments, we found that no clear trend emerged from these data with respect to ΣDDT+ distributions relative to barrel locations.

The No Barrel Site exhibited a different HOC profile compared with barrel-adjacent samples. Eleven HOCs were detected at the No Barrel Site, compared to the 29 HOCs at the barrel-adjacent sites (Figure 2). The No Barrel Site lacked nine PCBs and seven DDT+ compounds. The seven absent DDT+ compounds were *o,p′*-DDT, DDNU, DDMU 5 (an isomer of *p,p′*-DDMU, previously described by Shaul et al.[13]), three TCPM isomers, and one unknown DDT-related compound (Figure 2). These findings could indicate a number of scenarios: (1) compounds absent at the No Barrel Site are potential candidates to chemically fingerprint deep ocean dumping; (2) there are specific environmental conditions, microbes, or benthic–pelagic fauna present close to the barrels that are not present at the No Barrel Site, leading to different degradation pathways and thus different accumulation profiles for DDT+; (3) the variation reflects the sloppiness of historical waste dumping; or (4) the seven DDT+ compounds apparently absent at the No Barrel Site are present but below the limit of detection.

Biota HOC Profiles. Across all four biota species, 42 HOCs of both natural and anthropogenic origins were detected, including 10 DDT+ compounds (SI Table 3). The *p,p′* and *o,p′* isomers of DDE and DDD were observed along with other less commonly targeted DDT+ compounds, including *p,p′*-DDMU, an additional DDMU isomer, TCPM, and an additional TCPM isomer. Two other DDT-related compounds previously identified in bottlenose dolphins were detected: DDE 2 (an isomer of DDE) and DDT-related 12.[1,13] Five DDT+ compounds present in the sediment were absent in the biota: *o,p′*-DDT, DDNU, and one unknown DDT-related compound as well as the previously characterized DDT-related 3 and TCPM 2 (an isomer of TCPM).[1,16] These five DDT+ compounds had relatively low abundance in the sediment, contributing ∼5% to the total DDT+ abundance. This indicates that these five compounds are not highly abundant in sediment and therefore may not transfer to biota at detectable levels or that the compounds may be metabolized within the biota.

While the sample size was too small for statistical analysis, we note that individuals of the same fish species collected from the two sites varied in their DDT+ and HOC loads (Table 3). For both fish species *Cyclothone acclinidens* and *Melanostigma pammelas*, those collected at the control site (Santa Cruz Basin) had 2 to 6 times higher DDT+ abundance compared to the samples collected ∼100 km distant at the examined dumpsite in the San Pedro Basin. This suggests that DDT+ contamination may be widespread beyond Dumpsite 2 and may not be directly correlated to the presence of barrels or

Table 2. Sample Information and ΣDDT+ Relative Abundances for Sediment Samples (*n* = 10), All Taken at a Depth of ∼900 m[a]

sampling site	sampling location	sediment depth (cm)	sample ID	sample mass (g)	no. of HOCs	ΣDDT+ abundance
No Barrel Site	90 m from any barrel	1–4	ID 27	8.39	10	3.01
		5–6	ID 24	5.31	5	0.19
Site 1	1 m from barrel (SCB-264)	1–4	ID 26	5.15	25	13.23
		5–6	ID 22	5.32	22	17.71
Site 2	1 m from barrel (SCB-292)	1–4	ID 7	9.39	23	9.8
		5–6	ID 10	7.36	14	1.53
Site 2	3 m from barrel (SCB-290)	1–4	ID 16	7.03	23	16.24
		5–6	ID 2	6.23	23	23.63
Site 2	5 m from barrel (SCB-288)	1–4	ID 28	5.69	25	24.99
		5–6	ID 6	3.45	25	42.01

[a] Sample mass reported as dry weight. ΣDDT+ abundance was calculated by summing the relative abundances for all of the DDT-related and TCPM-related compounds in the sample.

Table 3. Sample Information and Compound Abundances for the Biota Samples (n = 7)[a]

						total relative abundance	
sampling location	taxonomic id	sampling depth (m)	sample ID	no. of individuals per composite	sample mass (g)	ΣDDT+	ΣHOCs
San Pedro Basin	*L. stilbius*	546−0	ID 13	2	3.15	0.265	1.211
	C. acclinidens	546−657	ID 25	61	5.01	1.334	3.518
	C. acclinidens	657−784	ID 19	146	10.1	2.073	3.978
	M. pammelas	657−784	ID 15	6	5.03	5.813	0.684
Santa Cruz Basin	bulk zooplankton	990−1480	ID 11	not quantified	10	3.955	1.939
	C. acclinidens	990−1480	ID 12	68	10.1	6.836	1.025
	M. pammelas	990−1725	ID 23	11	10	11.651	0.561

[a] Each biota sample was a composite composed of a varying number of individuals. The number of bulk zooplankton was not determined due to their small size. Sample mass is reported as wet weight. ΣDDT+ includes all DDT- and TCPM-related compounds, and ΣHOCs includes all other non-DDT+-related compounds.

proximity to the dumpsite. Notably, two TCPM-related compounds (TCPM and TCPM 3) were detected in all biota samples except *Leuroglossus stilbius* (ID 13), which contained neither TCPM isomer. This may be, for the first time, evidence of TCPM entering the food web from deep sediments. A better understanding of species-specific variations and vertical and spatial gradients in biota is needed before stronger conclusions can be drawn.

Overall, we found a diverse set of HOCs, including DDT+ compounds, in bottom sediments and biota from deep ocean sites off the coast of southern California. Our findings demonstrate the importance of examining the larger suite of DDT+, as opposed to the four to eight compounds that are typically monitored, because the unmonitored compounds can significantly contribute to the contaminant body burden across a range of marine taxa.[1] The majority of the DDT+ compounds (87%, n = 13) detected in the sediment and biota were previously detected in SCB birds and marine mammals.[1,13,15,16,20] This discovery is critical and suggests that DDT+ from deep ocean sediment enters the water column and subsequently the marine food web. DDT pollution in SCB should be recognized as an ongoing environmental concern requiring further research.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.4c00115.

> Glossary, unknown compound mass spectra, and SI Tables 1−3 documenting all detected halogenated organic compounds with their names, retention times, quantitative ions, and relative abundances (PDF)

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Notes

The authors declare no competing financial interest.

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