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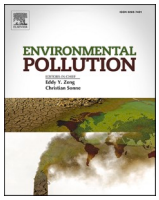

Targeted and nontargeted approaches to uncover complex halogenated pollutants in the historically endangered California brown pelican☆

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ABSTRACT

Historically, California brown pelicans experienced reproductive failure and population decline induced by the pesticide dichlorodiphenyltrichloroethane (DDT). Although the population recovered after the 1972 DDT ban, nontargeted analytical methods have identified over 45 DDT-related compounds (DDT+) and other typically unmonitored halogenated organic compounds (HOCs) in regional upper trophic level wildlife species, presumably originating from the persistent and chemically-complex DDT manufacturing waste deposited in the Southern California Bight (SCB). However, there is limited data on DDT+ in previously endangered bird populations, including the brown pelican. We identified 183 HOCs in seven brown pelican liver samples (131 ± 48, mean ± SD) via nontargeted analysis. Seventeen DDT+ compounds were identified, including four tris(4-chlorophenyl) methane (TCPM)-related compounds, and 17 polychlorinated terphenyls (PCTs), all of which were not assessed in prior pelican research. Quantitative targeted analysis was conducted for three classes: DDT+, polychlorinated biphenyls (PCB), and polybrominated diphenyl ethers (PBDE). Among these three classes, DDT+ was the dominant chemical class ($\sum_{13}$DDT+, 0.56–197 μg/g lw), followed by ($\sum_{14}$PCB, 0.24–96 μg/g lw), and ($\sum_{6}$PBDE, 0.01–5.61 μg/g lw). $\sum_{13}$ DDT+ primarily consisted of *p,p'*-DDE (77 % ± 15 %), *4,4′,4″*-TCPM (12 % ± 10 %), *p,p'*-DDMU (6 % ± 3 %), and *4,4′,4″*-TCPMOH (5 % ± 6 %). These results demonstrate the continuous exposure of known historical HOCs and a chemically diverse set of under-monitored HOCs, including DDT+. Additionally, levels of detection are consistent with recent wildlife studies in the region, suggesting pervasive contamination of a unique profile of HOCs throughout the SCB food web.

1. Introduction

California brown pelicans (*Pelecanus occidentalis californicus*), henceforth referred to as pelicans, are a historically endangered species that experienced drastic population decline due to eggshell thinning caused by the pesticide dichlorodiphenyltrichloroethane (DDT) (Anderson et al., 1975; Jehl, 1973). In 1969, three years before DDT was banned, brown pelicans were successfully rearing young at a rate of 0.004 fledglings per nest, much lower than the 1.2–1.5 fledglings per nest needed to maintain the population (Anderson et al., 1975). The California coastal ecosystem was particularly affected by DDT because the world's largest producer of DDT, Montrose Chemical Corporation, was located in the Los Angeles region (Almada et al., 2023). Their manufacturing waste was disposed via sewage outfall to the Palos Verdes Shelf (PVS), which was designated a U.S. EPA Superfund Site in 1997. Additionally, DDT acid waste was bulk dumped by ship into the offshore deep ocean of San Pedro Basin, located between the Los Angeles coast and Catalina Island, CA (Almada et al., 2023; Wu et al., 2025; Schmidt et al., 2024; Chartrand et al., 2022; Kivenson et al., 2019). The southern California coast was also contaminated with other chemicals from sewage discharge, stormwater runoff, and other types of waste disposal spread across 14 offshore dumpsites, including widely used halogenated organic compounds (HOCs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Dodder et al., 2012; Maruya et al., 2009; Bond et al., 1973).

After DDT was banned in the US in 1972, pelican populations began

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to recover. In 2009, they were delisted as an endangered species, but their population health remains threatened by other factors including declining fish stocks, adverse weather, and disease outbreaks (Anderson et al., 2017; Duerr et al., 2023a). Beginning in 2015, applications of nontargeted analysis (NTA) using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC/TOF-MS) demonstrated complex HOC exposure profiles in sediment, mid-water fish, black skimmers, and marine mammals from the Southern California Bight (SCB). Across these samples, 25–81 % of the detected HOCs were not typically monitored (Millow et al., 2015; Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2024; Stack et al., 2022). Further, regional DDT pollution was found to be more complex than previously thought, with over 45 DDT-related compounds identified (Millow et al., 2015; Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2024; Stack et al., 2022; Mackintosh et al., 2016). Collectively, the DDT-related chemicals are referred to as DDT+, which differs from the conventionally monitored DDX (*p,p'*- and *o,p'*-DDT, DDE, and DDD) in that the term DDT+ encompasses both DDX and the expanded group of DDT byproducts, impurities, breakdown products, and additional related compounds (Almada et al., 2023; Kivenson et al., 2019; Stack et al., 2024). The biological effects of the expanded DDT+ group are relatively unknown, but typically unmonitored chemicals like tris (4-chlorophenyl)methane (TCPM) and tris(4-chlorophenyl)methanol (TCPMOH) cause changes in gene expression pathways related to endocrine function and xenobiotic metabolism (Navarrete et al., 2021; Wilson et al., 2023). TCPM-related chemicals are considered impurities in DDT manufacturing (Almada et al., 2023; Buser, 1995), and they were found at concentrations up to 27 μg/g lipid weight (lw) in SCB bottlenose dolphins (Mackintosh et al., 2016). Comparatively, *p,p'*-DDE concentrations ranged from 19 to 496 μg/g lw, suggesting that TCPM-related chemicals can exist at comparable concentrations to *p,p'*-DDE (Mackintosh et al., 2016).

This research on other SCB species raises concern for pelican exposure to DDT+ and other HOCs. While their migratory range spans from central Mexico to Canada, pelicans breed in the SCB on Anacapa and Santa Barbara Islands—near the known dumpsites—as well as in central Baja California (Fig. S1). Pelicans consume similar prey (e.g., anchovies, mackerel) as California sea lions and some cetaceans (Duerr et al., 2023a), suggesting similar exposure to the complex HOCs previously detected in SCB marine mammals. Additionally, as a long-lived piscivorous seabird, pelicans are particularly sensitive to the bioaccumulation of contaminants within their habitat, thus allowing them to serve as early indicators of ocean health. However, despite recommendations for contaminant monitoring and incidences of decreased reproduction, there is little to no data on recent contaminant loads in pelicans (Anderson et al., 2017; Zeeman et al., 2010). For this pilot study, we aim to reexamine pelican contaminant loads using a combination of nontargeted and targeted approaches for persistent and bioaccumulative HOCs in order to 1) identify known and unknown HOCs, including those that are not typically monitored, in pelicans and 2) quantitatively measure the concentrations of DDT+, PCBs, and PBDEs in pelicans to compare with historic values.

2. Materials and methods

2.1. Sample information

Pelican liver samples were provided by International Bird Rescue's (IBR) two wildlife centers that receive wild aquatic birds for rehabilitation in the metropolitan areas of Los Angeles and San Francisco, California. Liver samples were chosen due to their ability to provide comprehensive contaminant analysis across broader demographics (e.g. age and sex), thus allowing insight into long-term and recent exposures within a population (Espín et al., 2016; Castagna et al., 2024). A total of seven liver samples were collected between February and May of 2022, five from IBR Los Angeles, and two from IBR San Francisco (Table 1). All pelicans were dead on or shortly after arrival or were euthanized due to poor prognosis.

2.2. Sample preparation and nontargeted instrumental analysis

Pelican liver samples were extracted following previously described methods (Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2022). Briefly, approximately 8 g of liver were homogenized with diatomaceous earth using a mortar and pestle, spiked with internal standards (50 μL of $^{13}C_{12}$-PCB-169, $^{13}C_{19}$-TCPM, and $^{13}C_{12}$-FBDE-69 at 4 μg/mL), and extracted by pressurized liquid extraction (Dionex ASE 300, ThermoFisher Scientific) with dichloromethane (100 °C, 1500 psi). The resulting extracts were concentrated to 5 mL of a 1:1 ethyl acetate: cyclohexane solution. A 0.4 mL portion was removed and gravimetrically weighed to determine the total lipid content of each sample. The remaining solution (4.6 mL) was injected into a gel permeation chromatography (GPC) system (J2 Scientific, Columbia, MO) following the same methods as Shaul et al. (2015). The eluent fractions were then collected and evaporated to a final extract of 1 mL. The extracts were spiked with a recovery standard ($^{13}C_{12}$-PCB-189, 50 μL at 2 μg/mL) and injected into Pegasus 4D GC×GC/TOF-MS (LECO, St. Joseph, MI). Three procedural blanks were prepared in the same manner.

2.3. HOC identification and analysis

Data from the GC×GC/TOF-MS was processed using LECO ChromaTOF software (version 4.72.0.0) using automatic peak finding and mass spectral deconvolution with a signal-to-noise threshold (S/N) of 100. Mass spectral data were then filtered using an automated data reduction software as detailed by Cossaboon et al. (2019). This software used a customized R script to filter through the provided mass spectral data and flag potential HOCs based on ion intensity ratios characteristic

Table 1
Sample information for the California brown pelican liver samples (n = 7) analyzed. Table is ordered by time in care.

Sample ID[a]	Sex	Age (years)	% Lipid	Time in Care	Health Conditions
LA-SY-F-0578	F	2	1.08	0 days[b]	emaciated, hypothermic
LA-SY-F-0606	M	2	1.94	0 days[b]	emaciated, hypothermic, roundworms present
LA-TY-M-0568	M	3	1.59	0 days[b]	emaciated
LA-ATY-F-0564	F	>3	1.22	0 days[b]	emaciated
LA-ATY-F-0054	F	>3	3.71	11 days[a]	weak, ataxic, hypothermic, left wrist abscess, vascular fragility, euthanized due to massive pectoral hemorrhages
SF-ATY-F-1859	F	>3	4.21	10 weeks, 2 days	emaciated and hypothermic on admission, with poor perfusion and toe tip necrosis, euthanized due to persistent foot infection
SF-HY-M-1285	M	<1	3.6	23 weeks, 6 days	multiple body wounds secondary to fishing line entanglement, necrosis, euthanized due to insufficient flight for release

[a] Samples are referred to by location, age and sex, where LA = Los Angeles, SF = San Francisco, ATY = After Third Year (>3 years old), TY = Third Year (3 years old), SY = Second Year (2 years old), HY = Hatch Year (<1 year old), and F = Female and M = Male.
[b] Birds stranded during southern California's Brown Pelican mass stranding event of May–June 2022.

of halogenated compounds. A manual review procedure was applied to all filtered compounds to confirm halogenation in the mass spectra as well as a cross-checking procedure for compounds that were absent in any of the samples. Confirmed HOCs were identified and structurally classified based on retention times and fragment ion similarities with compounds in existing mass spectral libraries from previous studies (Shaul et al., 2015; Cossaboon et al., 2019; Mackintosh et al., 2016; Hoh et al., 2012). Chromatographic peak abundances for each compound were normalized to an internal standard and the lipid content of the corresponding sample as outlined by Shaul et al. (2015). Raw peak areas for individual compounds were first divided by the $^{13}C_{12}$-PCB-169 peak area then by the lipid weight (g) of each sample, resulting in a unitless normalized value. The normalized response of each compound was used in various R scripts to generate heatmaps and boxplots for analysis. Compounds found exclusively in the blanks have been excluded from analysis. The ratio of raw peak areas for the $^{13}C_{12}$-PCB-169 internal standard and the $^{13}C_{12}$-PCB-189 recovery standard were 36 % and 45 % among the samples, indicating consistent recovery through the sample preparation. It should be noted that the ratio does not indicate the internal standard's absolute recovery. Rather this confirms the general adequacy of the methods performed and reliability of the relative comparisons of the normalized responses.

2.4. Quantitation of DDT+, PCB, and PBDE

After the extracts were run on the GC×GC/TOF-MS, additional cleanup was performed prior to targeted analysis on an Agilent 5977A Series Gas Chromatography-Mass Spectrometer (GC-MS) (Agilent Technologies, Santa Clara, CA). To clean the extracts, the ~1 mL extract was loaded onto an Enviro-Clean Silica SPE cartridge (UCT, Bristol, PA, USA). The extract was then eluted with 5 mL hexane, 5 mL 1:1 hexane: dichloromethane, and 5 mL dichloromethane. The hexane and 1:1 hexane:dichloromethane fractions were combined and evaporated to 1 mL before injection onto the GC-MS. The GC-MS was operated in select ion monitoring (SIM) mode using an electron impact (EI) source. Detailed instrument conditions can be found in Table S1 and all quantification and qualifying ions assigned to each target analyte can be found in Table S2. In total, 33 HOCs were selected for quantitation, including 13 DDT+ compounds, 14 PCBs, and 6 PBDEs (Table S3). Analytes were quantified using a nine-point calibration curve with a concentration range of 0.05 ppb–500 ppb. Individual calibration point concentrations in ppb are as follows: 0.05, 0.1, 0.5, 1, 2.5, 10, 50, 100, 500. Carbon-labelled DDT and PCB standards ($^{13}C_{12}$-p,p'-DDT, DDD, DDE; $^{13}C_{12}$-o,p'-DDT, DDD; $^{13}C_{19}$-TCPM, $^{13}C_{19}$-TCPMOH, $^{13}C_{12}$-PCB-169) were added to all calibration standards at a final concentration of 10 ppb. Data was analyzed using Agilent Mass Hunter Quantitative software (Version 7.0.457.0). First, all peaks detected using the SIM mode acquisition method were reviewed to ensure retention time match between the target analyte in the calibration curve and sample. Peak areas were then manually reviewed to ensure proper integration for both the target analyte and corresponding internal standard (Table S2). Partial regression curves were applied to quantify the amount of target analyte present in each sample. Since the internal standards were used for the quantitation, the results are recovery corrected. The overall average recovery rate was 87 %. The blanks contained no detectable concentrations of any analytes, except for p,p'-DDE, which was detected at low levels in all three blanks. The concentration of p,p'-DDE detected in the blanks was subtracted from the concentration detected in the samples.

3. Results and discussion

3.1. HOC profiles in pelicans

In total, 183 distinct HOCs were identified across the seven samples. The number of HOCs per sample was variable, with an average of 131 ± 48 HOCs per sample and range of 47–177. Overall, 39 of the 183 HOCs (21 %) were detected in all seven samples and 43 HOCs (23 %) were found in at least six, suggesting widespread prevalence of these HOCs. Hierarchical clustering of the samples based on individual HOC profiles showed no clear clustering based on age or sex, however, relative HOC abundance was highest in pelicans emaciated at death (Fig. 1). During periods of distress (e.g. starvation, migration, mass strandings), fat stores are depleted, which may result in elevated concentrations as lipid soluble HOCs are redistributed to highly metabolic organs such as the liver (Malcolm et al., 2003; Wienburg and Shore, 2004; Hela et al., 2006). Pelicans have been greatly affected by emaciation, as observed in the mass stranding events of 2009, 2010, and 2022 (Duerr et al., 2023a; Duerr et al., 2023b; Jaques, 2016; Nevins et al., 2011), and may therefore be subject to increased toxicity of these HOCs as they are remobilized to target organs and concentrated relative to their body mass (Bustnes et al., 2010; Bogan and Newton, 1977; Holmstrup et al., 2010; Thompson et al., 1977). However, a larger study is needed to further investigate potential associations between pelican body condition and HOC body burden.

Notably, the two pelicans collected from the San Francisco location had, on average, 3.8 times lower average total abundance than pelicans collected from Los Angeles (n = 5) (Table S5). However, one of these birds was the only first-year bird in the study, and this bird had the lowest average abundance overall, which supports the likelihood of contaminant accumulation over time. Location of capture is not expected to be a significant factor in contaminant load given that this species has a strong north-south annual cycle: breeding occurs in the southern end of its range on the Pacific coast of Mexico, Baja California, and the California Channel Islands while non-breeding dispersal ranges as far north as British Columbia, Canada (Stinson, 2022). While interpretations of the differences noted between the two sample locations are limited by this study's sample size (n = 7), spatial trends should be further investigated to determine the unique chemical exposures experienced by northern and southern region seasonally resident pelicans.

The 183 HOCs spanned 25 chemical structural classes, based on the classifications in previous work on other avian and marine mammal species (Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2022; Mackintosh et al., 2016; Hoh et al., 2012). Previous SCB pelican research has assessed <10 of these structural classes through targeted approaches (Anderson et al., 1975; Jehl, 1973; Zeeman et al., 2010; Blus et al., 1970), therefore the current study indicates that the contaminant profiles of regional pelicans is more chemically diverse than previously known (Table 2). Comparison with previous nontargeted studies in SCB wildlife depict strong similarities in HOC profiles, with 158 of 183 (86.3 %) pelican HOCs previously described in cetaceans, pinnipeds, and condors (Shaul et al., 2015; Cossaboon et al., 2019; Mackintosh et al., 2016; Hoh et al., 2012). Additionally, an average of 88 % (±11 %) of structural classes in pelicans were also detected in these upper trophic marine mammals, and 22 % (±8.5 %) in deepwater fish and sediment (Table S4). When compared with coastal and inland condors, pelicans share 58 % and 29 % of the same contaminant classes respectively. These similarities in HOCs across marine predators suggest the widespread prevalence of both legacy and unmonitored contaminants in the SCB marine ecosystem. While further comparative analysis is outside the scope of the current study, future research reviewing contaminant profiles across the SCB food web may provide deeper insight on regional patterns of HOC exposure.

3.2. Previously unreported HOCs in pelicans based on the nontargeted analysis

We identified a total of 13 DDT+ compounds, three of which are commonly monitored (*p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDMU). The additional 10 DDT+ compounds that are not typically monitored were up to 20 % of total DDT+ abundance. These compounds were identified and described in previous nontargeted analysis of other southern California

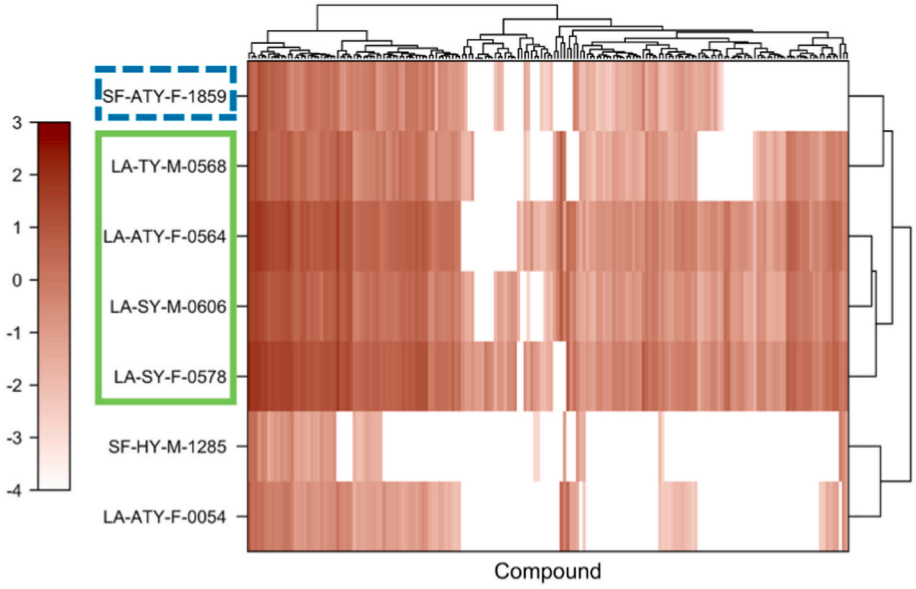

Fig. 1. Hierarchical clustering heatmap of the log_{10}-transformed normalized response of individual compounds identified in California Brown Pelicans. Compound responses were determined by normalizing the peak area abundance to the respective internal standard abundance ($^{13}C_{12}$-PCB-169) and total lipid mass of each sample. Green solid box indicates pelicans emaciated at death; dashed blue box indicates pelican was emaciated upon admission. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Number of detected compounds and average total normalized response (±SD) for each designated structural class in the current study. Prior California brown pelican research that analyzed compounds within the class are cited. Table is ordered by highest average response.

Structural Class	Abbreviation	No. HOCs in class	Source	Avg. total class Response[a]	Analyzed in prior research (ref.)
Polychlorinated biphenyl[b]	PCB	61	anthropogenic	219.09 ± 240.47	(Anderson et al., 2017; Zeeman et al., 2010; Blus et al., 1970)
Dichlorodiphenyltrichloroethanes	DDT+[c]	13	anthropogenic	129.01 ± 130.75	(Anderson et al., 1975; Jehl, 1973; Zeeman et al., 2010; Blus et al., 1970)
Tris(4-chlorophenyl)methane	TCPM[d]	3	anthropogenic	23.75 ± 27.64	
Tris(4-chlorophenyl)methanol	TCPMOH[d]	1	anthropogenic	18.37 ± 22.29	
Polybrominated diphenyl ether	PBDE	10	anthropogenic	10.85 ± 12.56	Zeeman et al. (2010)
Chlorinated benzene	–	2	anthropogenic	9.21 ± 11.64	
Chlordane-related	–	15	anthropogenic	7.38 ± 8.76	Zeeman et al. (2010)
Toxaphene	–	13	anthropogenic	5.42 ± 7.24	Zeeman et al. (2010)
Hexachlorocyclohexane	HCH-related	1	anthropogenic	4.47 ± 5.73	Zeeman et al. (2010)
Polychlorinated terphenyl	PCT	16	anthropogenic	2.33 ± 2.87	
Polychlorinated diphenyl ether	PCDE	9	anthropogenic	1.86 ± 2.97	
Mirex-related	–	2	anthropogenic	1.85 ± 2.33	Zeeman et al. (2010)
Polybrominated hexahydroxanthene derivative	PBHD	4	natural	1.52 ± 1.76	
Brominated anisole	–	1	natural	1.37 ± 1.39	
Heptachlor-related	–	4	anthropogenic	1.34 ± 1.59	Zeeman et al. (2010)
Brominated indole	–	1	natural	0.31 ± 0.26	
Dieldrin	–	1	anthropogenic	0.28 ± 0.31	(Jehl, 1973; Zeeman et al., 2010; Blus et al., 1970)
1′-methyl-1,2′-bipyrrole	MBP	2	natural	0.26 ± 0.32	
Polybrominated Biphenyl	PBB	4	anthropogenic	0.22 ± 0.27	
Methoxy brominated diphenyl ether	MeO-BDE	1	natural	0.17 ± 0.21	
Methoxy chlorinated diphenyl ether	MeO-CDE	1	anthropogenic	0.10 ± 0.14	
Chlorinated styrene	–	2	anthropogenic	0.07 ± 0.12	
1,1′-dimethyl-2,2′-bipyrroles	DMBP	1	natural	0.06 ± 0.07	
Methoxy polybrominated biphenyl	MeO-PBB	1	natural	0.04 ± 0.04	
Unknown	Unk.	14	unknown	7.99 ± 6.77	

[a] Average Response represents the total response of the structural class averaged across the sample set.
[b] PCBs were identified only to the degree of chlorination.
[c] DDT+ encompasses both traditional DDX compounds and the expanded DDT + compounds.
[d] TCPM and TCPMOH are known impurities of DDT and are considered part of the newly recognized DDT+ class. They are separated here to highlight their high response value.

wildlife (Table S6) (Millow et al., 2015; Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2022; Mackintosh et al., 2016). Additionally, we identified three isomers of tris(4-chlorophenyl)methane (TCPM) and one tris(4-chlorophenyl)methanol (TCPMOH) compound. TCPM and TCPMOH are likely impurities of DDT and therefore considered part of DDT+ (Almada et al., 2023; Mackintosh et al., 2016; Buser, 1995). TCPM and TCPMOH are associated with developmental toxicity and affect a variety of biological pathways (Wilson et al., 2023; Navarrete

et al., 2021). This study presents the first detection of TCPM and TCPMOH in pelicans, but they have been previously detected in sediment, marine mammals, human adipose tissue, and breast milk, demonstrating their environmental ubiquity (Mackintosh et al., 2016; Walker et al., 1989; Lebeuf et al., 2001; Falandysz et al., 1998; Jarman et al., 1992; Minh et al., 2000a; Minh et al., 2000b; Rahman et al., 1993; Schwarzbauer et al., 1999; Watanabe et al., 1999). TCPM and TCPMOH were the third and fourth most abundant structural classes across the samples (Fig. 2), indicating their persistence, bioaccumulative propensity, and potential to exert ecotoxicological effects.

We detected 16 polychlorinated terphenyl (PCT) isomers, making it the second largest structural class in terms of unique compounds. Existing data on PCTs in California wildlife has been limited to a few marine mammals, and this is believed to be the first report of PCTs in pelicans. PCTs are industrial chemicals structurally similar to PCBs and generally used in the same applications (i.e., electronic equipment, lubricants, sealants, etc.), with some Aroclor mixtures composed of up to 40 % PCTs and 60 % PCBs (De Boer et al., 2000). Additionally, PCTs are environmentally ubiquitous and found to strongly correlate with widespread PCB contamination ($R^2 > 0.8$) but were not as closely regulated or monitored (De Boer et al., 2000; Jensen and Jørgensen, 1983; Wester et al., 1996). Existing data likely underestimates the magnitude of PCT contamination, which is currently estimated to be ~10 % of total PCB concentrations (Bustnes et al., 2010). This underestimation may be due to analytical challenges posed by the complex nature of PCTs: a theorized 8000+ congeners, possible degradation during sample extraction, and significant co-elution with PCBs in single-column GC-analysis (De Boer et al., 2000; Wester et al., 1996; Rosenfelder and Vetter, 2014). Updated techniques involving GPC clean-up and GC×GC techniques, such as those used in this study, may yield improved separation of PCT congeners (De Boer et al., 2000; Wester et al., 1996). This is further supported by recent NTA work, which detected up to 37 PCTs in southern California bottlenose dolphins and 77 PCTs in raptors from Japan, highlighting the benefit of NTA monitoring for comprehensive contaminant analysis (Shaul et al., 2015; Cossaboon et al., 2019; Tue et al., 2021; Alonso et al., 2017).

We detected 16 PCTs with 4–9 chlorines, whereas many global studies suggest that homologues with 5–7 chlorines are the most commonly detected in marine wildlife (Rosenfelder and Vetter, 2014; Tue et al., 2021; Galceran et al., 1993; Rebryk et al., 2022). In the present study, the number of PCT isomers within each homologue was: 4Cl (n = 3); 5Cl (n = 1), 7Cl (n = 2); 8Cl (n = 9); 9Cl (n = 1) (**SI-2**). The higher chlorination may allow for greater persistence and bioaccumulation, potentially increasing the hazard of those PCTs (International Agency for Research on Cancer, 2016). We found a strong positive correlation between total PCB and PCT abundance in the current sample set ($R^2 = 0.75$, p = 0.011) (Fig. S2).

We detected 14 unknown HOCs, of which five have been previously detected in the SCB wildlife and nine are unique to this study (**SI-2**). Classification as an unknown HOC was determined by patterns of chlorination or bromination in its isotope clusters. Overall, the combined relative abundance of these unknown HOCs was similar to well-known contaminants such as PBDEs (n = 10) and chlordane-related compounds (n = 15), indicating the potential of unknown and/or unmonitored HOCs to contribute to total burden. Additional discussion on the nontargeted analysis of the remaining 22 structural classes identified in this study can be found in the work of Shaul et al. (2015).

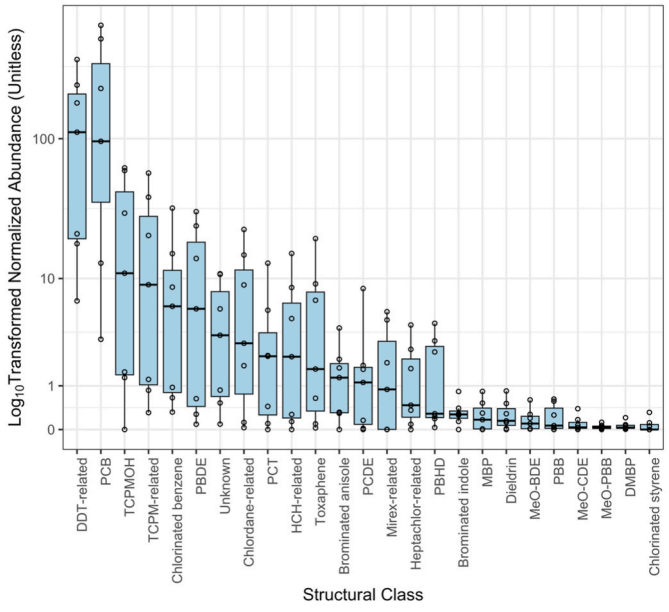

Fig. 2. Summed normalized relative abundance of HOC structural classes for all pelicans (n = 7). Boxplots are ordered by median value.

3.3. Concentrations of DDT+, PCBs, and PBDEs

We quantified three of the most abundant chemical classes based on the nontargeted analysis (DDT+, PCB, and PBDE) and their commercial availability. Overall, we obtained standards for 13 of the recently identified 45 DDT+ compounds, 14 PCBs, and 6 PBDEs (Table S3). Note, the target analytes chosen for quantitation represent a fraction of the total number of compounds identified through nontargeted analysis: 35 % of DDT+, 23 % of PCBs, and 60 % of PBDEs. DDT+ analytes were selected based on availability of authentic standards, while PCB and PBDE quantification was limited to the most abundant congeners within the SCB. Therefore, the reported concentrations underrepresent the total concentrations of all identified compounds in the chemical classes as observed by the nontargeted analysis.

For this discussion, DDT+ includes DDX (*p,p'*- and *o,p'*-DDT, DDE, DDD) and seven additional compounds from the expanded suite of 45+ DDT-related compounds, including TCPM and TCPMOH-related chemicals (Table S3). ∑DDT+ accounted for 24–85 % of the total quantified chemical concentration and ranged from 0.56 μg/g to 197 μg/g lw. ∑PCBs contributed 10–73 % to the body burden and ranged from 0.25 to 81 μg/g lw, while ∑PBDEs made up 0–7 % and ranged from 0.00 to 20 μg/g lw (Table 3). Among the quantified analytes, ∑DDT+ made up the majority of total body burden in all but one sample, a female adult collected from the San Francisco IBR center, in which ∑PCBs made up 73 % (Fig. S3). This variation may be due to geographical differences in contaminant exposure (via regional dietary preference) or potential contaminant off-loading to its offspring. However, due to the limited sample size and sample information, the cause(s) remain unclear.

The ∑DDT+ concentrations were primarily driven by four compounds: *p,p'*-DDE (77 % ± 15 %), 4,4′,4″-TCPM (12 % ± 10 %), *p,p'*-DDMU (6 % ± 3 %), and 4,4′,4″-TCPMOH (5 % ± 6 %). The contributions of *p,p'*-DDE to ∑DDT+ is consistent with previous literature (Mackintosh et al., 2016; Elliott et al., 2023; Sericano et al., 2014; Sharpe and Garcelon, 2010; Herring et al., 2010) in all but two adolescent samples from the Los Angeles center, in which *p,p'*-DDE made up 53–56 % of the total. In those two samples, TCPM and TCPMOH combined contributed 36–41 % of the total concentration. *p,p'*- and *o,p'*-DDD were detected at low levels (n.d. to 0.79 μg/g lw) and contributed less than 1 % of the total concentration. The compounds *p,p'*- and *o,p'*-DDT, as well as *o,p'*-DDE were not detected. Historically, the six DDX compounds (*p,p'*- and *o,p'*-DDT, DDD, and DDE) were considered the major components of DDT pollution and monitored in most DDT studies. Here, we find that three of the four major contributing compounds are not part of DDX. TCPM and TCPMOH are not typically monitored yet demonstrate substantial contributions to ∑DDT+ concentrations (Buser, 1995; Jarman et al., 1992), suggesting the prevalence and bioavailability of these underreported DDT+ impurities within the southern California marine food web.

Table 3
Concentration levels (μg/g lw) for the 33 target analytes in the brown pelican samples (n = 7). The table is ordered by class.

Target Analyte	Concentration (μg/g lw)						
	LA-SY-F-0578	LA-TY-M-0568	LA-ATY-F-0054	LA-SY-F-0606	SF-HY-M-1285	SF-ATY-F-1859	LA-ATY-F-0564
% lipid	1.08	1.59	3.71	1.94	3.60	4.21	1.22
p,p'-DDE	173.02	37.03	2.16	96.49	0.49	2.03	134.35
o,p'-DDE	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p,p'-DDD	0.76	0.79	0.05	0.39	0.00	0.07	0.35
o,p'-DDD	0.05	0.04	0.00	0.03	0.00	0.00	0.04
p,p'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00
o,p'-DDT	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p,p'-DDMU	16.08	4.45	0.16	10.42	0.00	0.12	11.81
p,p'-DDNU	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p,p'-DBP	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DDOH	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p,p'-DDM	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,4′,4″-TCPM	5.05	14.99	0.16	50.42	0.06	0.11	12.22
4,4′,4″-TCPMOH	2.12	8.44	0.06	23.59	0.00	0.00	4.10
∑DDT+	**197.07**	**65.73**	**2.60**	**181.34**	**0.56**	**2.33**	**162.89**
PCB-52	1.45	0.21	0.02	0.52	0.01	0.07	0.58
PCB-66	1.26	0.20	0.01	0.53	0.01	0.05	0.61
PCB-101	8.95	0.87	0.08	2.90	0.02	0.47	3.65
PCB-70	0.11	0.03	0.00	0.03	0.00	0.00	0.06
PCB-118	9.78	1.05	0.10	3.59	0.02	0.87	5.30
PCB-153	24.46	1.99	0.34	7.84	0.07	2.18	14.36
PCB-138	17.09	1.65	0.21	5.62	0.06	1.36	9.59
PCB-192	0.73	0.06	0.02	0.22	0.00	0.09	0.55
PCB-180	10.52	0.84	0.22	3.04	0.03	1.34	7.65
PCB-169	0.12	0.01	0.00	0.04	0.00	0.01	0.08
PCB-170	3.94	0.35	0.07	1.14	0.01	0.34	2.59
PCB-194	1.55	0.16	0.05	0.48	0.01	0.24	1.29
PCB-206	0.91	0.09	0.02	0.23	0.00	0.11	0.76
Decachlorobiphenyl	0.51	0.05	0.01	0.10	0.00	0.09	0.35
∑PCBs	**81.38**	**7.57**	**1.16**	**26.28**	**0.25**	**7.22**	**47.43**
BDE 154	0.90	0.27	0.02	0.46	0.00	0.00	0.54
BDE 153	0.64	0.13	0.03	0.44	0.00	0.10	0.91
BDE 28 + 33	0.15	0.04	0.00	0.08	0.00	0.00	0.08
BDE-47	13.29	2.60	0.14	7.73	0.00	0.25	7.68
BDE 100	4.18	0.75	0.00	2.24	0.00	0.00	2.71
BDE 99	1.12	0.50	0.00	1.00	0.00	0.00	0.84
∑PBDEs	**20.27**	**4.29**	**0.20**	**11.96**	**0.00**	**0.35**	**12.76**

3.4. Historical comparisons

At the height of the pelican population decline, *p,p'*-DDE concentrations in eggs collected in southern California were upwards of 1100 μg/g lw (Table S7). Trends show that *p,p'*-DDE concentrations decreased through time, with the most recent concentrations at 19 μg/g lw. ∑PCB follow a similar trend of decline, but ∑PCB were at higher concentrations than *p,p'*-DDE in 2005 (Zeeman et al., 2010). Additionally, ∑PBDE concentrations did not significantly decline between 1993 and 2005 (Zeeman et al., 2010).

While the decline of *p,p'*-DDE concentrations in eggs has been extensively documented, historical data on liver concentrations for pelicans remain limited. Previous reports account only for ∑DDX—six of the thirteen DDT+ analytes included in our study. A study from 1979 measured ∑DDX in two brown pelicans from Los Angeles, CA and reported individual *p,p'*-DDE concentrations of 1.6 and 12.7 μg/g wet weight (ww) and ∑DDX concentrations of 1.8 and 13.8 μg/g ww (Table 4a) (Young et al., 1979). Additionally, the study measured ∑DDX in two other marine birds from Los Angeles (cormorants and gulls). These birds had *p,p'*-DDE and ∑DDX concentration ranges of 2.8–37 μg/g ww and 3.1–39 μg/g ww, respectively. In comparison, our study found *p,p'*-DDE and ∑DDX concentrations ranging from 0.02 to 1.87 μg/g ww and 0.02–1.88 μg/g ww (Table S8), indicating the significant contribution of *p,p'*-DDE to ∑DDX. In contrast, ∑DDT+ levels ranged from 0.02 to 3.52 μg/g ww, demonstrating that up to 47 % of total DDT-related contaminant burden may be unaccounted for when

Table 4a
Historic and current concentrations (μg/g ww) of p,p'-DDE, ∑DDX, and ∑DDT+ in California seabird livers.

Location	Species	Year	n[a]	Concentration (mean μg/g ww)			Citation
				p,p'-DDE	∑DDX[b]	∑DDT+[c]	
Los Angeles, Southern California	Brown Pelican	1976	1	1.6	1.8	–	Young et al., 1979
			1	12.7	13.8	–	
	Cormorant	1977	1	2.8	3.1	–	
			1	6.7	7.2	–	
	California Gull	1977	1	8.4	8.8	–	
			1	3.1	3.4	–	
			1	37	39	–	
Southern California	Brown Pelican	2022	7	0.879	0.885	1.27	current study

[a] *n* refers to the number of samples included in the corresponding concentration.
[b] ∑DDX includes only *p,p'*- and *o,p'*- DDT, -DDE, -DDD.
[c] ∑DDT+ includes DDX, *p,p'*-DDM, -DDMU, -DDNU, -DBP, DDOH, and 4,4′,4″-TCPM and -TCPMOH.

analyzing only ∑DDX. The maximum levels of *p,p'*-DDE and ∑DDT+ in our study fall within the lower range of historical liver data reported by Young et al. (1979) and support the overall declining trend observed in the pelican egg samples (Table S7) (Young et al., 1979). However, the considerable contribution of unaccounted DDT+ to total DDT burden further emphasizes the underestimated magnitude of DDT contamination in the SCB marine ecosystem.

In addition to temporal trends, geographical differences could reveal spatial patterns in contaminant availability and exposure. Liver and egg data from other seabirds (terns and gulls) demonstrated that *p,p'*-DDE and DDX concentrations decrease with distance from Los Angeles, the presumed epicenter of DDT contamination, while PCB and PBDE concentrations often increase. The trend is seen in seabird livers moving northward to San Francisco Bay (Table 4b) (Herring et al., 2010) and in seabird eggs moving southward to San Diego Bay (Clatterbuck et al., 2018), away from Los Angeles. While these differences in contaminant levels could be attributed to species specific accumulation—as influenced by differences in dietary trophic level, nesting habitat, and foraging areas during migratory movements—it also suggests regional differences associated with the historical discharge of legacy pollutants at different urban centers along California's coast (Clatterbuck et al., 2018).

3.5. Limitations

The results of this study are subject to two main limitations. First, analyses were conducted using a small sample size (n = 7). Results in this study may therefore be limited in its explorations in demographic, spatial, and temporal trends, as well as the generalizability to the broader pelican population. However, small sample sizes of seabirds have been found to provide sufficient statistical power in tracking marine pollution, in part due to smaller variances in organochlorine concentrations in seabirds than in fish or marine mammals (Elliott and Elliott, 2013). Additionally, previous applications of nontargeted analysis using relatively small sample sizes have proven sufficient in detecting a large suite of HOCs in other upper trophic level marine species in the SCB (Millow et al., 2015; Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2022; Mackintosh et al., 2016; Hoh et al., 2012). While more complex investigations of population HOC trends are beyond the scope of this study, we highlight the novelty of nontargeted analysis in characterizing the large suite of HOCs that pelicans may be exposed to.

Second, the health conditions of the pelicans (i.e. emaciation and/or other afflictions) may distort the contaminant burdens of the pelicans due to the depletion and/or mobilization of lipids—and thus lipid soluble HOCs—during periods of stress (Malcolm et al., 2003; Wienburg and Shore, 2004; Hela et al., 2006). While this may result in elevated contaminant levels, relative abundance and concentration measurements used in analyses are normalized to individual liver lipid content to minimize effects associated with lipid variations between samples. Additionally, liver samples may be beneficial in the analyses of unhealthy birds in that liver concentrations are contextualized with body condition at the time of analysis, thus incorporating current vulnerabilities in relation to both the chemicals of concern and current environmental conditions (Newton, 1988). Pelicans and wild animals alike are subject to various environmental and physiological stressors that may initiate mild to intense lipid mobilization which may produce harmful synergistic effects between elevated contaminant levels and environmental stressors (Bustnes et al., 2010; Bogan and Newton, 1977; Holmstrup et al., 2010; Debier et al., 2006). Given the prevalence of nutritional stress in pelican populations—as observed in recent mass stranding events—nontargeted HOC screening in unhealthy birds may provide useful information regarding highly vulnerable members of the population (Duerr et al., 2023a; Duerr et al., 2023b; Jaques, 2016; Nevins et al., 2011).

3.6. Implications for California brown pelican

DDT+ and other historically unmonitored HOCs have now been detected in southern California sediment and across a range of trophic levels to top predators, indicating widespread contamination (Millow et al., 2015; Shaul et al., 2015; Cossaboon et al., 2019; Stack et al., 2024; Stack et al., 2022; Mackintosh et al., 2016). These HOCs were consistently found in California brown pelicans, further demonstrating their ubiquity in the California marine ecosystem. DDTs, PCBs, PBDEs and other organochlorines are associated with a range of sublethal effects in wildlife, including urogenital carcinomas in sea lions (Gulland et al., 2020), eggshell thinning in California condors (Burnett et al., 2013), declining body condition in glaucous gull chicks (Bustnes et al., 2003), and liver toxicity and reduced long-term survival in marine birds (Zeeman et al., 2010; Kuzyk et al., 2003). Moreover, recent studies show the importance of including DDT+ in environmental monitoring to truly understand the impacts of DDT contamination. Lesser studied impurities, such as TCPM and TCPMOH, have limited toxicological data in birds, but what is available suggests their potential toxicity as endocrine disruptors (Wilson et al., 2023; Navarrete et al., 2021; Kimbrough et al., 1973; Foster et al., 1999; Schrader and Cooke, 2002). Additionally, the high contribution of TCPM and TCPMOH to ∑DDT+ concentrations (≤41 %) in this study highlights their prevalence and further emphasizes the need to expand upon traditional monitoring of DDX. Similarly, limited investigations on PCTs, which are considered toxicologically similar to PCBs, have demonstrated hepatotoxic effects, including hypertrophy and tumor development, and intestinal metabolic interferences in PCT-fed rats and primates (Gallagher et al., 1995; Shirai et al., 1978; Carlos Sosa-Lucero et al., 1973; Allen and Norback, 1973; Madge, 1977). PCTs may also act synergistically with other contaminants, as suggested by their amplified hepatotoxic effects by hexachlorobenzene, another HOC found in all of our pelican samples (Shirai

Table 4b
Recent levels of *p,p'*-DDE, ∑DDX, ∑PCB, and ∑PBDE in California seabird livers. Mean concentrations are reported in μg/g ww.

Location	*Species*	*Year*	*n*[a]	Concentration (mean μg/g ww)				*Citation*
				p,p'-DDE	*∑DDX*[b]	*∑PCB*	*∑PBDE*	
San Francisco Bay, California	Caspian Tern	2005	5	0.71	0.72	1.52[c]	0.22[e]	Herring et al., 2010
San Francisco North Bay, California	Forster's Tern	2005	1	0.19	0.21	0.83[c]	0.05[e]	
San Francisco Central South Bay, California	Forster's Tern	2005	2	0.56	0.62	11.31[c]	0.64[e]	
San Francisco Lower South Bay, California	Forster's Tern	2005	4	0.37	0.41	4.08[c]	0.24[e]	
Southern California	Brown Pelican	2022	7	0.879	0.885	0.349[d]	0.099[f]	current study

[a] *n* refers to the number of samples included in the corresponding concentration.
[b] ∑DDX includes only *p,p'*- and *o,p'*- DDT, -DDE, -DDD.
[c] 101 PCBs quantified.
[d] 14 PCBs quantified.
[e] 38 PBDEs quantified.
[f] 6 PBDEs quantified.

et al., 1978). Such effects can have broader implications for population health, manifesting as reproductive impairment, embryotoxicity, and overall population decline (Blus et al., 1970; Bustnes et al., 2003; Fry, 1995). Although the toxicological interactions and potential synergism of diverse suites of contaminants are not yet fully understood, characterizing HOC exposure in sentinel species is an essential first step.

Bioaccumulation of these toxic HOCs is of particular concern for pelicans given their comparatively high sensitivity to contaminants such as DDT (Blus, 2011). In addition to this sensitivity, their high trophic level, piscivorous diet, and long lifespan make pelicans good bioindicators of ocean health. This is seen throughout pelican history, in which declines in population health strongly reflected underlying issues within their regional ecosystem: severe reproductive decline due to excessive DDT exposure (Blus et al., 1970; Blus, 2011), increased pelican morbidity due to domoic acid poisoning (Work et al., 1993), and changes in pelican reproductive rates in response to anchovy abundance (Anderson et al., 1980). Biomonitoring of pelicans as a sentinel species could therefore provide indication of underlying health concerns within their ecosystem (Millow et al., 2015; Clatterbuck et al., 2018; Burger and Gochfeld, 2004). This is highlighted by the increased incidences in breeding failure and pelican mass stranding events observed in 2009, 2010, and 2022 of which the cause(s) remains unknown, but chemical stressors may be a contributing factor (Anderson et al., 2017; Duerr et al., 2023b; Nevins et al., 2011). Long-term exposure to a complex suite of HOCs may impose sublethal physiological effects, such as a weakened immune system, which could pose additional threats to wildlife and ecosystems already strained by climate-related stressors (e.g., harmful algal blooms) and anthropogenic impacts (e.g., overfishing). This concern over contaminant exposure is compounded by the recently rediscovered offshore dumpsites in the San Pedro Basin off Los Angeles, which may be a significant secondary source of DDT due to bulk dumping of DDT waste in the region (Kivenson et al., 2019; Wu et al., 2025; Schmidt et al., 2024). While any connection between contaminant exposure and the mass stranding events is currently inconclusive, this study indicates the need for a larger monitoring study to further explore any potential relationships.

The diverse suite of bioaccumulative HOCs observed in this study demonstrates the importance of nontargeted chemical analysis methods. As legacy pollutants persist and new chemicals are identified in the environment, more comprehensive methods are needed to characterize modern contaminant profiles. Together with targeted quantitative analysis, nontargeted methodology can identify unmonitored and unknown potentially harmful contaminants (e.g., TCPM, TCPMOH, PCTs) for future monitoring and provide a more complete assessment of total contaminant burden. As such, we emphasize the importance of nontargeted chemical analysis in developing a more comprehensive understanding of contaminant exposure and observed health effects in the marine ecosystems. Addressing the source, toxicological impacts, and ecological consequences of HOCs is critical for protecting vulnerable marine species, such as the pelican, and ensuring long-term ocean health.

CRediT authorship contribution statement

A.N. Agas: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **M.E. Stack:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **W.H. Richardot:** Writing – review & editing, Investigation, Formal analysis, Data curation. **R.S. Duerr:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **N.G. Dodder:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **K.E. Sant:** Writing – review & editing, Funding acquisition, Conceptualization. **E. Hoh:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Author disclosures

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve the readability of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

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