Assessing Marine Endocrine Disrupting Chemicals in the Critically Endangered California Condor: Implications for Reintroduction to Coastal Environments

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ABSTRACT

Coastal reintroduction sites for California condors (*Gymnogyps californianus*) can lead to elevated halogenated organic compound (HOC) exposure and potential health impacts due to the consumption of scavenged marine mammals. Using nontargeted analysis based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS), we compared HOC profiles of plasma from inland and coastal scavenging California condors from the state of California (CA), USA; and marine mammal blubber from CA and the Gulf of California off Baja California (BC), Mexico. We detected more HOCs in coastal condors (32±5, mean number of HOCs ± SD, n=7) than inland condors (8±1, n=10), and CA marine mammals (136±87, n=25) than BC marine mammals (55±46, n=8). ΣDDT-related compounds, ΣPCBs, and total tris(chlorophenyl)methane (ΣTCPM) were, respectively, ~7, ~3.5, and ~148 times more abundant in CA than BC marine mammals. The endocrine-disrupting potential of selected PCB congeners, TCPM, and TCPMOH was determined by *in vitro* California condor estrogen receptor (ER) activation. The higher levels of HOCs in coastal condors compared to inland condors, and lower levels of HOC contamination in Baja California marine mammals compared to those from the state of California are factors to consider in condor reintroduction efforts.

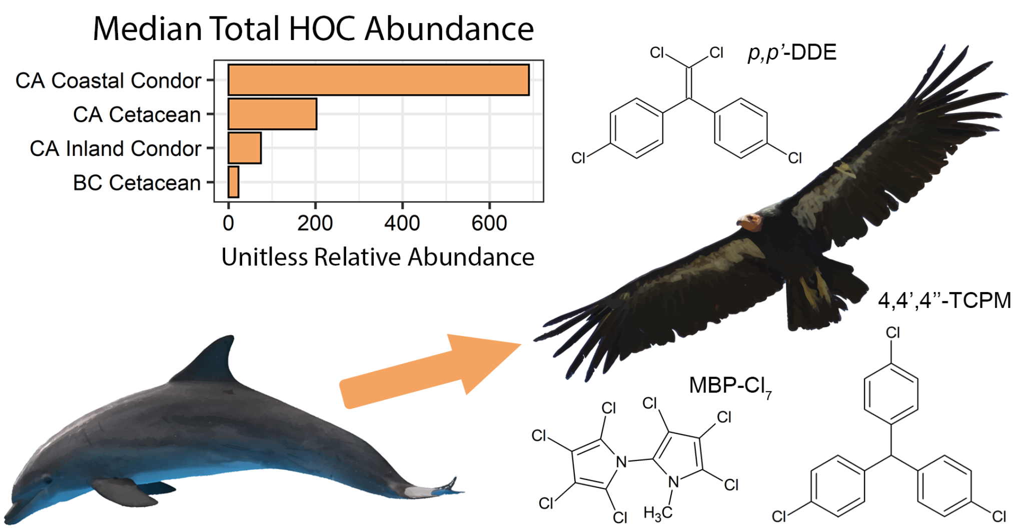
SYNOPSIS

California condor organic contaminant exposure is higher in flocks that scavenge coastal vs. terrestrial carrion, and higher in the state of California than the Gulf of California.

KEYWORDS

Halogenated organic compounds, nontargeted chemical analysis, California condor

TOC ART



INTRODUCTION

California condors (*Gymnogyps californianus*) are among the most critically endangered birds in North America. In 1987, due to a combination of environmental stressors, condors were driven to near extinction and the remaining 27 birds were placed in captive breeding facilities.[1,2,3,4] The population has since grown to more than 500 individuals, with more than half occupying wild habitats in California, Arizona, Utah, and Baja California.[5,6,7] Within California, condors live in coastal and inland regions. Condors from the central California flock near Pinnacles National Park (PNP) and Ventana Wilderness (VWS) scavenge in both terrestrial and coastal areas. In contrast, condors from the southern California flock near Bitter Creek do not currently access coastal habitats (**Figure 1**).[8,9] For the purposes of this paper the central California flock is referred to as "coastal," while the southern California flock is identified as "inland."

Condor diets are composed almost entirely of mammalian carrion, but the type depends on occupied habitat.[1,8] Inland populations scavenge terrestrial mammals, such as deer and domestic cattle, while coastal condors add dead-stranded marine mammals to their diet.[1,5,8] Time spent in coastal areas is associated with higher condor survival because it is believed to reduce lead exposure by increasing marine mammal scavenging and limiting terrestrial mammal consumption.[10,11,12] Condors are primarily exposed to lead by ingesting lead fragments from terrestrial carrion that has been shot with lead ammunition.[11] Given that lead poisoning is the leading cause of condor mortality[13], increasing reintroduction efforts to coastal environments may be advantageous.[6]

However, there is concern regarding negative health effects of a coastal diet because marine mammals contain high levels of persistent organic pollutants, many of which are halogenated organic compound (HOCs).[8,14] Despite decades-long bans on the production and use of some HOCs, these compounds are highly resistant to environmental degradation and continue to bioaccumulate in marine food webs with the potential to cause physiological harm to marine fauna.[14,15,16,17,18] Many of these compounds, such as dichlorodiphenyl trichloroethane and its metabolites (DDTs) and polychlorinated biphenyls (PCBs), are endocrine-disrupting chemicals and some have been implicated in condor reproductive impairment.[19,20] There is evidence that coastal condors are experiencing eggshell thinning associated with exposure to HOCs in scavenged marine mammal carcasses.[5,8] HOCs can disrupt hormone actions and ultimately impair reproduction through a number of mechanisms, including interacting directly with an organism's estrogen receptors (ERs).[21] Recent *in vitro* ER assays using cloned California condor ERα and ERβ found that all DDTs and most PCBs tested could activate condor ERs at varying potencies and at environmentally relevant levels.[20] This supports the hypothesis that coastal condors are exposed to levels of endocrine-disrupting chemicals capable of causing reproductive effects.

Ideally, condors would be reintroduced to coastal areas with low environmental contamination to prevent exposure to both lead and HOCs. One such potential site is Baja California, Mexico, along the northwestern coast of the Upper Gulf of California, where reintroduction efforts have successfully established a small, but growing, condor population. Although this flock currently feeds on pro-offered sheep carcasses and occasional terrestrial mammal carcasses in the Sierra de San Pedro Mártir, they will have access to the Gulf of California when their range expands. However, data on the contaminant profiles of the Gulf's resident marine mammals is scarce. An evaluation of potentially relevant endocrine-disrupting chemicals in marine mammals inhabiting the Upper Gulf of California is thus warranted to assess the suitability of this condor food source.

DDTs and PCBs are not the only halogenated organic compounds found in marine mammals that could be causing reproductive health effects in condors. Recent studies identified hundreds of additional HOCs that accumulate in common bottlenose dolphins (*Tursiops truncatus*), as well as other cetacean and pinniped species, from the Southern California Bight.[15,18] These additional contaminants were identified by a non-targeted analytical (NTA) method using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS). [22,23] Previous studies established the viability of this method to identify and compare HOC profiles among various biota.[15,17,18,24] Furthermore, these methods can assess unknown or unrecognized contaminants in condors that are not routinely monitored and have the potential to cause physiological harm.[25]

Our overall study objective was to identify endocrine-disrupting HOCs accumulating in the coastal California condor population via marine mammal consumption and to assess the risk of endocrine-disrupting chemical exposure for the expanding Baja California condor flock. Specifically, we aimed to: (1) evaluate the accumulation of both known and novel contaminants by coastal California condors scavenging stranded marine mammals through the comparison of southern California marine mammal blubber HOC profiles vs. coastal California condor plasma HOC profiles vs. inland California condor plasma HOC profiles using the non-targeted GC×GC/TOF-MS method; (2) determine the prevalence of HOCs in marine mammal carcasses stranded along the northwestern coast of the Upper Gulf of California and predict HOC exposure and endocrine disruption potential in the Baja California condor population; and (3) determine the endocrine-disrupting potential for prioritized HOCs using an *in vitro* California condor ER activation assay.

MATERIALS AND METHODS

Sample Information. Condor blood samples were collected by field researchers at PNP, VWS, and Hopper Mountain Wildlife Refuge Complex (USFWS) in California, USA, between June 2014 – October 2015 (**Figure 1**). There were 19 individual coastal condor samples (PNP and VWS) and 20 individual inland condor samples (USFWS). Plasma was isolated from the blood of each sample and stored at -80 °C until analysis. Individual coastal samples contained ~750 µL of plasma and individual inland condors contained ~1.5 mL. A minimum of 2 mL plasma was required for analysis; therefore, samples were pooled with three individual samples per pool for coastal condors, and two for inland condors (**Table 1**). Pools were determined by aggregating based on similar population, sex, and age resulting in 7 pooled coastal condor samples and 10 pooled inland condor samples.

Figure 1: Map of the coastal and inland California condor release sites and the marine mammal sample collection locations along the coast of California and in the Gulf of California.

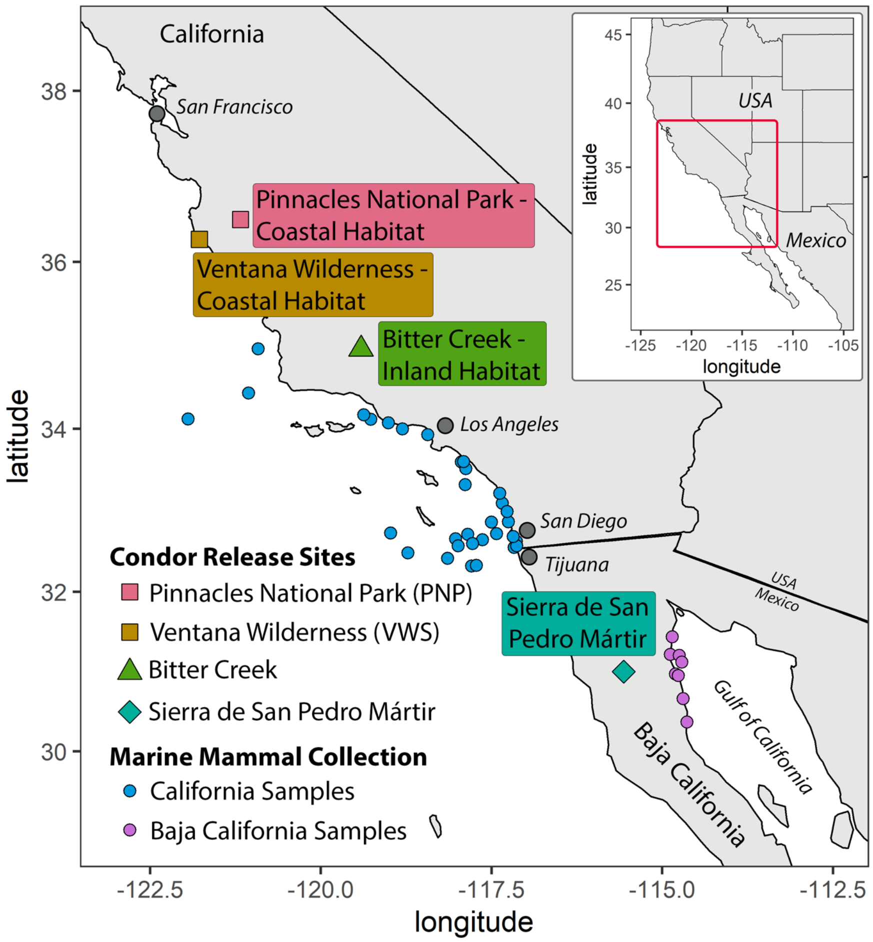


Table 1: Sample information for pooled coastal and inland California condor plasma.

Coastal condors[+]	*Pooled sample ID*	*Condor Studbook ID*	*Sample Population*	*Sex*	*Ages*	*% lipid*
	Sample 1-1	631, 688, 700	VWS, VWS, PNP	M	4, 2, 2	0.16
	Sample 1-2	470, 567, 615	VWS, VWS, VWS	M	7, 5, 4	0.36
	Sample 2-6	209, 340, 477	VWS, PNP, VWS	M	16, 11, 7	0.40
	Sample 2-7	692, 704, 729	PNP, PNP, PNP	M	2, 2, 1	0.26
	Sample 3-11	547, 626, 684	VWS, VWS, PNP	F	6, 4, 2	0.33
	Sample 4-13	543, 583*	PNP, PNP	F	6, 5	0.52
	Sample 4-14	606, 663*	PNP, PNP	M	4, 3	0.18
Inland condors[++]						
	Sample 1-3	636, 637	Bitter Creek	M	3, 3	0.32
	Sample 1-4	648, 654	Bitter Creek	F	2, 2	0.25
	Sample 1-5	509, 542	Bitter Creek	M	5, 5	0.25
	Sample 2-8	683, 694	Bitter Creek	M	1, 1	0.23
	Sample 2-9	482, 489	Bitter Creek	M	6, 6	0.36
	Sample 2-10	563, 584	Bitter Creek	F	4, 4	0.33
	Sample 3-12	568, 585	Bitter Creek	M	4, 4	0.25
	Sample 4-15	192, 255	Bitter Creek	F	16, 13	0.14
	Sample 4-16	596, 604	Bitter Creek	F	3, 3	0.17
	Sample 4-17	326, 452	Bitter Creek	M	10, 7	0.27

[+] Coastal condors are pooled with 3 individual condor samples.
[++] Inland condors are pooled with 2 individual condor samples.
* Two aliquots of one individual condor sample were used.

All marine mammals were analyzed individually, not as pools (**SI Table 1**). Baja California (BC) cetacean and pinniped samples were collected between 2017-2019 along the western Gulf of California in Mexico (**Figure 1**). The BC marine mammal samples (n = 8, **SI Table 2**) consisted of 4 common bottlenose dolphins (*Tursiops truncatus)* and a single sample each of striped dolphin (*Stenella coeruleoalba*), vaquita (*Phocoena sinus*), common dolphin (*Delphinus sp.*), and California sea lion (*Zalophus californianus*). All samples were collected as dead strandings, except for the vaquita and one common bottlenose dolphin that died from directed and incidental capture, respectively.[26] All non-bycatch BC samples were decomposing (**SI Table 1**), and blubber tissue could not be separated from muscle tissue. Thus, all analyzed BC samples contained both blubber and muscle tissues, except for the vaquita and single common bottlenose dolphin which were collected freshly dead. Permit information is provided in the **SI Methods**.

HOC data for the southern California marine mammals was previously acquired, and individual sample information can be found in Shaul et al. (2014) (SI Table 5) and in Cossaboon et al. (2019) (SI Table 2). The Shaul *et al.* (2014) data set consisted of 8 dead stranded common bottlenose dolphin (*Tursiops truncatus*) samples collected between 1995-2010. The Cossaboon *et al.* (2019) data set consisted of three cetacean species (n = 5 individuals each) and two pinniped species (n = 5 individuals each) that were dead stranded or bycatch, collected between 1990-2014. The cetaceans were long-beaked common dolphin (*Delphinus delphis bairdii*), short-beaked common dolphin (*Delphinus delphis delphis*), and Risso's dolphin (*Grampus griseus*). The pinnipeds were California sea lion (*Zalophus californianus*) and Pacific harbor seal (*Phoca vitulina richardii*). All CA marine mammal samples were comprised of blubber only. We used the CA marine mammal HOC datasets as proxies for the coastal condors' dietary items. Although the CA marine mammals were not sampled specifically from the coastal condors' habitat, the marine mammals' habitat ranges extend throughout the coast of California and overlap with that of the coastal condors.[27, 28]

Materials, sample preparation, and instrumental analysis. Sample preparation followed the methods described by Shaul *et al.* (2014) and Cossaboon *et al.* (2019). A detailed description is in the **SI Methods**. Briefly, samples were spiked with internal standards, and both condor plasma and marine mammal blubber were extracted with 1:1 ethyl acetate:cyclohexane. Sample extracts were injected into a gel permeation chromatography system (GPC, J2 Scientific, Columbia, MO). Extracts were then concentrated to 100 µL and injected into a LECO 4D GC×GC/ TOF-MS. A procedural blank (n = 7) was processed with each batch of samples and compounds identified in the blanks were excluded from the subsequent data analysis.

HOC screening and identification. GC×GC/TOF-MS data was processed using LECO ChromaTOF software (version 4.72.0.0) and followed methods described in Cossaboon *et al.* (2019). A detailed description is in the **SI Methods.** Compounds were named and structurally classified based on Cossaboon *et al.* (2019) and Shaul *et al.* (2014).

The set of compounds identified in the California condor and BC marine mammal samples was then merged with the previously acquired CA marine mammal datasets.[15,17,18] Compounds were matched between datasets based on assigned name and retention times. To account for GC×GC retention time shifts, we compared internal standard retention times across samples. Non-detected compounds were assigned a normalized abundance of zero. The final dataset consisted of 415 unique HOCs (excluding PCBs) across the CA condor, BC marine mammal, and CA marine mammal samples. Four structural classes contained isomeric compounds that could not be matched between samples because of identical mass spectra and similar retention times: DMBPs (DMBP Br_4Cl_1 isomer, DMBP Br_2Cl_4 isomer, and DMBP Br_3Cl_2 isomer); MeO-PBBs (the MeO-PBB isomer); MBP (MBP Cl_7 isomer); and PCBs (most congeners could not be accurately aligned).

The previously acquired CA marine mammal data sets did not assess PCBs. To identify PCBs in this study, the original GC×GC/TOF-MS data files were reviewed by extracting ions with indicative PCB *m/z* values (e.g., *m/z* 292, 255, 220 for PCB 4Cl). We searched PCBs with 2-10 degrees of chlorination. If a peak was identified, the complete mass spectrum was reviewed to confirm the identity. An attempt was made to merge the PCB data with the condor and BC marine mammal data as described above, however, most PCBs could not be aligned across samples. Therefore, using the elution order and retention times for all 209 PCB congeners run on the same column (Restek RTX-5) described in Frame (1997), we tentatively assigned the identity of the most abundant PCB congeners in the samples. From those assignments, we selected 42 for confirmation with authentic PCB standards (AccuStandard, New Haven, CT, USA). We confirmed the identity of 9 of the 42 PCB congeners (PCB 52, 101, 110, 118, 138, 153, 170, 180, and 187), and these 9 congeners were accurately aligned across all samples.

Tran *et al.* (2020) estimated the limit of detection (LOD) for the GC×GC/TOF-MS method, defined as the lowest concentration giving an identifiable mass spectrum for nine model compounds, as 10 to 100 ng/mL (concentration of the injected standard solution). This corresponds to a condor sample estimated LOD of 120 – 300 ng/g lipid (0.39 – 0.68 ng/g wet weight plasma) and a marine mammal estimated LOD of 0.20 – 2.54 ng/g lipid (0.14 – 0.50 ng/g wet weight blubber).

Estrogen receptor activation assays. Prior to use, 17β-estradiol (E_2, Steraloids, Newport, RI), PCBs 101, 110, 118, 170, 180, 187 (Accustandard, New Haven, CT, purity >99% for all), TCPM and TCPMOH (Matrix Scientific, Columbia, SC) were dissolved in DMSO. Activation of California condor ERs α and β by PCBs, TCPM, and TCPMOH were assessed as described previously.[20] HEK293 cells were plated in 96-well plates in minimum essential media (MEM) with 10% fetal bovine serum (FBS). After 24 h, cells were co-transfected with pCMX-β-galactosidase, pGL2-3xERE reporter plasmid (Addgene plasmid 11354)[31] and California condor ESR-pcDNA3.1(+) expression plasmid (Invitrogen) using TransIT 2020 (Mirus Bio LLC, Madison, WI) and incubated for 24 h. Cells were then treated with 1 pM to 10 μM of test compound (17β-E_2 or HOC) or 0.01% DMSO (vehicle control). Co-treatments with 0.1 nM 17β-E_2 were also performed at all HOC concentrations tested in MEM with 10% of Char/Dex FBS (HyClone, Logan, UT) for 24 h. Cells were lysed and assayed for luciferase and β-galactosidase activity and all data were normalized to maximal E_2 activation as reported previously.[20,32]

Statistical analysis. The normalized chromatographic peak abundance of each compound was determined using methods from Shaul *et al.* (2014) where the compound peak area was divided by the peak area of the internal standard $^{13}C_{12}$-PCB-169, then divided by the lipid weight (g) of each sample. Contaminant abundance data was not normally distributed, therefore we used non-parametric statistical analyses. The statistical methods are described in the **SI Methods**.

RESULTS AND DISCUSSION

In total, 415 unique HOCs, excluding PCBs, were identified across all condor and marine mammal samples. A total of 238 unaligned chromatographic features representing PCB congeners were identified among all samples; however, only 9 PCB congeners could be accurately aligned across samples through use of authentic standards (described above). The maximum number of unique PCB congeners in a single sample was 67, implying that at least 67 unique PCB congeners existed across all samples. Further details on PCB analysis are available in **SI Methods.** Overall, the HOC compounds comprised 43 structural classes, including 9 unknown classes (**SI Table 3**). Eight of these unknown classes (referred to as "Unknown-1" to "Unknown-8") were comprised of compounds with similar fragmentation patterns or identical mass spectra but varying retention times, as described by Shaul *et al.* (2014) (**SI Table 4)**. If a compound's mass spectra did not match any of these groups, it was assigned to the ninth general unknown class (referred to as "Unknown").

Six structural classes comprised ~55% of the identified compounds across all samples. The general Unknown structural class contained the most compounds (n = 83), followed by PCBs (n = 67), DDT-related compounds (n = 42), polychlorinated terphenyls (PCTs) (n = 37), chlordane-related compounds (n = 27), and toxaphenes (n = 26). Most of the identified compounds were from anthropogenic sources (70.8%), followed by unknown sources (20.4%), natural sources (8.6%), and mixed sources (0.3%)[15,18] (**SI Table 3)**. The number of HOCs identified in each sample varied by sample type. On average (± SD), we detected more HOCs in coastal condors (32 ± 5) than in the inland condors (8 ± 1). CA marine mammals contained more HOCs (136 ± 87 across all species) than the BC marine mammals (55 ± 46 across all species).

Data considerations. A single BC pinniped was collected, and prior work observed differences in HOC load between CA cetaceans and CA pinnipeds, possibly due to varying diets or metabolism[15]; therefore, we excluded pinnipeds from the subsequent data analyses. Note that although the size for the BC cetaceans was n = 7, previous GC×GC/TOF- MS based NTA studies demonstrated that n = 4 could generate a consistent and representative contaminant profile for a regional population.[15,18,24,33]

To simplify the presentation of the data analysis, we selected the structural classes that comprised at least 95% of the total normalized abundance for each of the four sample types (coastal CA condor, inland CA condor, CA cetacean, and BC cetacean) (**SI Table 5, SI Table 6**). This resulted in 13 selected structural classes: DDT-related, PCB, PBDE, TCPMOH, chlordane-related, TCPM, MBP, DMBP, MeO-BDE, brominated indole, chlorinated benzene, toxaphene, and Unknown (i.e. the general unknown class). For each sample type, the number of structural classes that comprised > 95% of the total abundance were: coastal condors = 2, inland condors = 1, CA cetaceans = 8, and BC cetaceans = 11. Abundance data for all structural classes is available in **SI Table 7**

HOC profile comparison

HOC profiles (the set of identified contaminants' normalized abundances) were compared between species and habitats to (a) determine if evidence suggested coastal CA condors acquire contaminants from scavenging stranded marine mammals (Aim 1) and (b) qualitatively estimate the potential for HOC exposure in the Baja California condor population relative to the coastal CA population by comparing marine mammal prey HOC profiles from the two regions (Aim 2). Below, we compare coastal CA condor and inland CA condor HOC profiles to examine differences based on habitat and diet, then compare coastal CA condor and sentinel CA marine mammal HOC profiles to establish that exposure is derived from a marine mammal diet. Last, we compare CA marine mammal profiles with BC marine mammal profiles to compare potential dietary HOC exposure for condors in the two regions.

Comparison of coastal CA condor and inland CA condor HOC profiles. Coastal condors contained a significantly larger number of individual HOCs than inland condors (Mann-Whitney U test, $p = 0.001$) and a greater diversity of structural classes. Coastal condors had 57 unique HOCs identified across 15 structural classes. Inland condors contained 19 unique HOCs in 8 structural classes. **Figure 2** shows the summed normalized abundances for each structural class across each sample group. Inland condors did not contain compounds from the following structural classes: methyl bipyrrole (MBP), dimethyl bipyrrole (DMBP), tris(4-chlorophenyl)methane (TCPM), hexachlorocyclohexane (HCH-related), heptachlor epoxide, mirex, and toxaphene. Mann-Whitney U tests evaluated differences in total structural class abundance between condor populations (**SI Table 8**). The normalized abundances of eight of the structural classes were significantly higher in coastal CA condors compared to inland CA condors, with DDT and PCB 7 times and 40 times more abundant in coastal CA condors, respectively.

Identification of HOCs in inland condors was expected, as some legacy contaminants, such as *p,p'*-DDE, are ubiquitous in most biota including terrestrial birds and humans.[34,35] Consistent with our study, DDTs, PCBs, PBDEs, and other chlorinated pesticides have been previously detected in inland condors, but at concentrations 12-100 times lower than coastal condors.[8] Notably, in our analysis inland condors lacked the halogenated natural product (HNP) classes MBP and DMBP that are produced by marine organisms and detected regularly in cetacean species due to their bioaccumulative nature.[14,15.36] The presence of these natural products in only coastal condors indicates their HOC exposure is marine-derived.

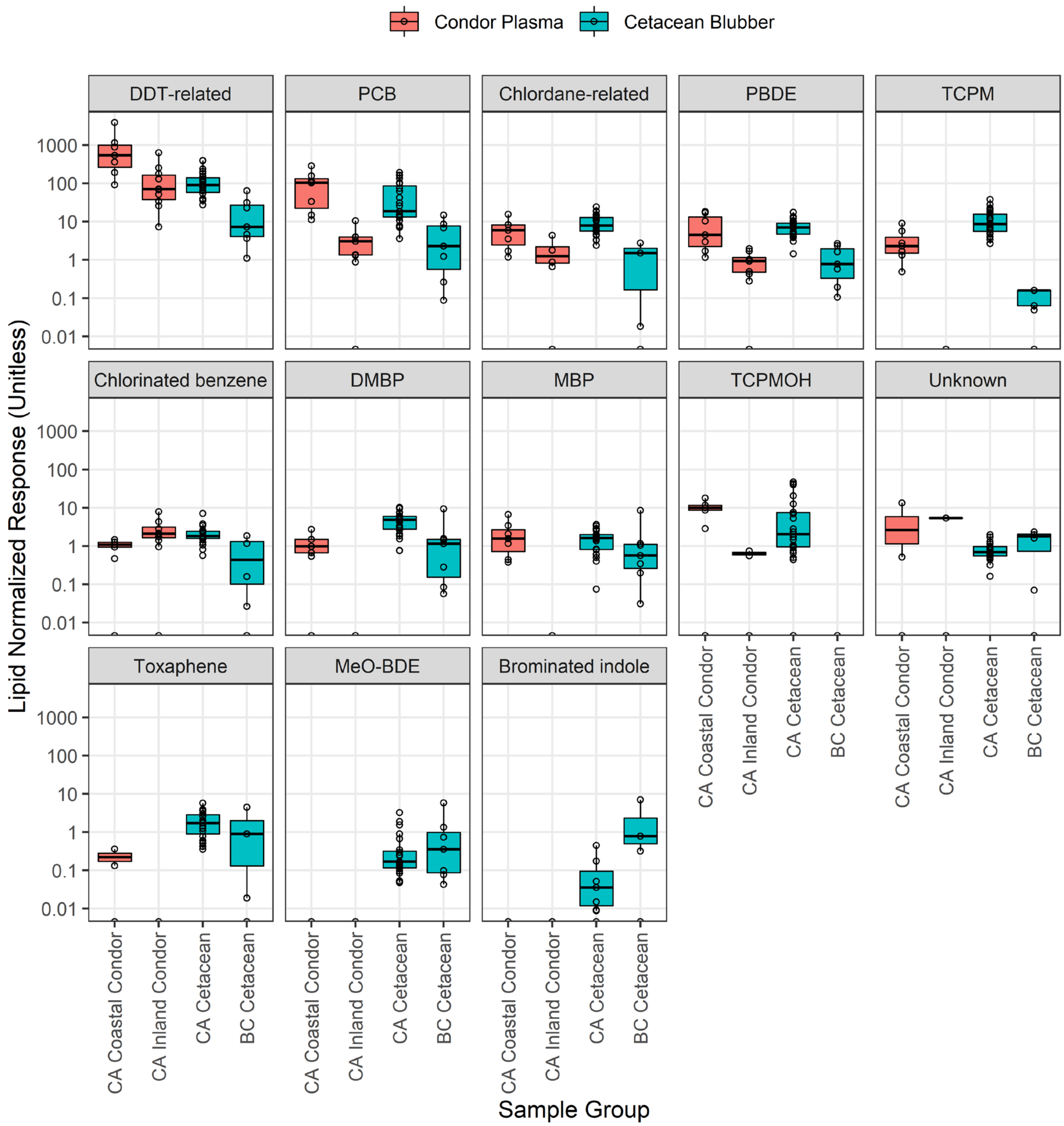


Figure 2: Normalized GC×GC/TOF-MS peak area abundance of select structural classes among sample groups.

Comparison of coastal CA condor and CA cetacean HOC profiles. We used dead stranded marine mammals as sentinels for lipophilic contaminants in the region[14,15,37]. Overall, there was similarity in the HOC profiles of coastal CA condors and CA cetaceans – evidence that HOC exposure in coastal CA condors is from the consumption of marine mammals. All HOCs detected in coastal CA condors were identified in CA marine mammals, except for one (benzoic acid, 2,6-dichloro, methyl ester). DDT-related compounds and PCBs were the most abundant structural classes in both coastal CA condors and CA cetaceans (**Figure 2**). Two classes (DDT-related and Unknown) were significantly more abundant in the coastal CA condors than the CA cetaceans, indicating potential biomagnification (Mann-Whitney U test, $p < 0.05$) **(SI Table 9)**. CA cetaceans contained more diverse HOCs than the coastal CA condors, with HOCs from 40 structural classes (including 9 unknown subclasses), compared to 15 structural classes in the coastal condors (including 2 unknown subclasses).

There are two caveats to this comparison. First, condor samples were plasma whereas marine mammal samples were blubber. Blubber contains more lipid than plasma (avg. marine mammal blubber % lipid = 58.4 %; avg. condor plasma % lipid = 0.3 %, **SI Table 1**). This difference in lipid content could affect detection limits, such that higher lipid content could allow lower detection limits and a better likelihood of compound detection. Second, coastal CA condors primarily consume pinniped species rather than cetacean species because pinnipeds strand more often than cetaceans in this region, although individual condors may consume different proportions of marine mammal species.[5,8,38] Hierarchical clustering by individual contaminants in all samples, including CA pinniped species, shows that coastal CA condors and CA pinnipeds cluster together more closely than coastal CA condors with CA cetaceans (**Figure 3**). However, the abundance of HOCs common to both pinnipeds and cetaceans generally follow the same rank-order patterns (**SI Table 11**). Since Cossaboon *et al*. (2019) found CA pinniped HOC profiles are generally a subset of CA cetacean HOC profiles, cetaceans may better characterize potential dietary HOC exposures for condors under a variety of conditions.

Furthermore, in our field sampling stranded BC cetaceans were more frequent that BC pinnipeds. Thus, in this study we use cetaceans as indicators for regional environmental pollution.

Because coastal condors primarily consume pinnipeds, it is important to assess pinniped HOC burden before making reintroduction recommendations.[5,8] While the BC marine mammal dataset only included a single pinniped sample, the contaminant loads were lower in the BC pinniped than the CA pinnipeds. Only 9 known structural classes were identified in the BC pinniped, whereas CA pinnipeds contain 18 known structural classes. CA pinnipeds had higher abundances of DDT-related compounds, PCBs, PBDEs, and other contaminant classes than the BC pinniped but statistical significance could not be tested due to the small sample size.

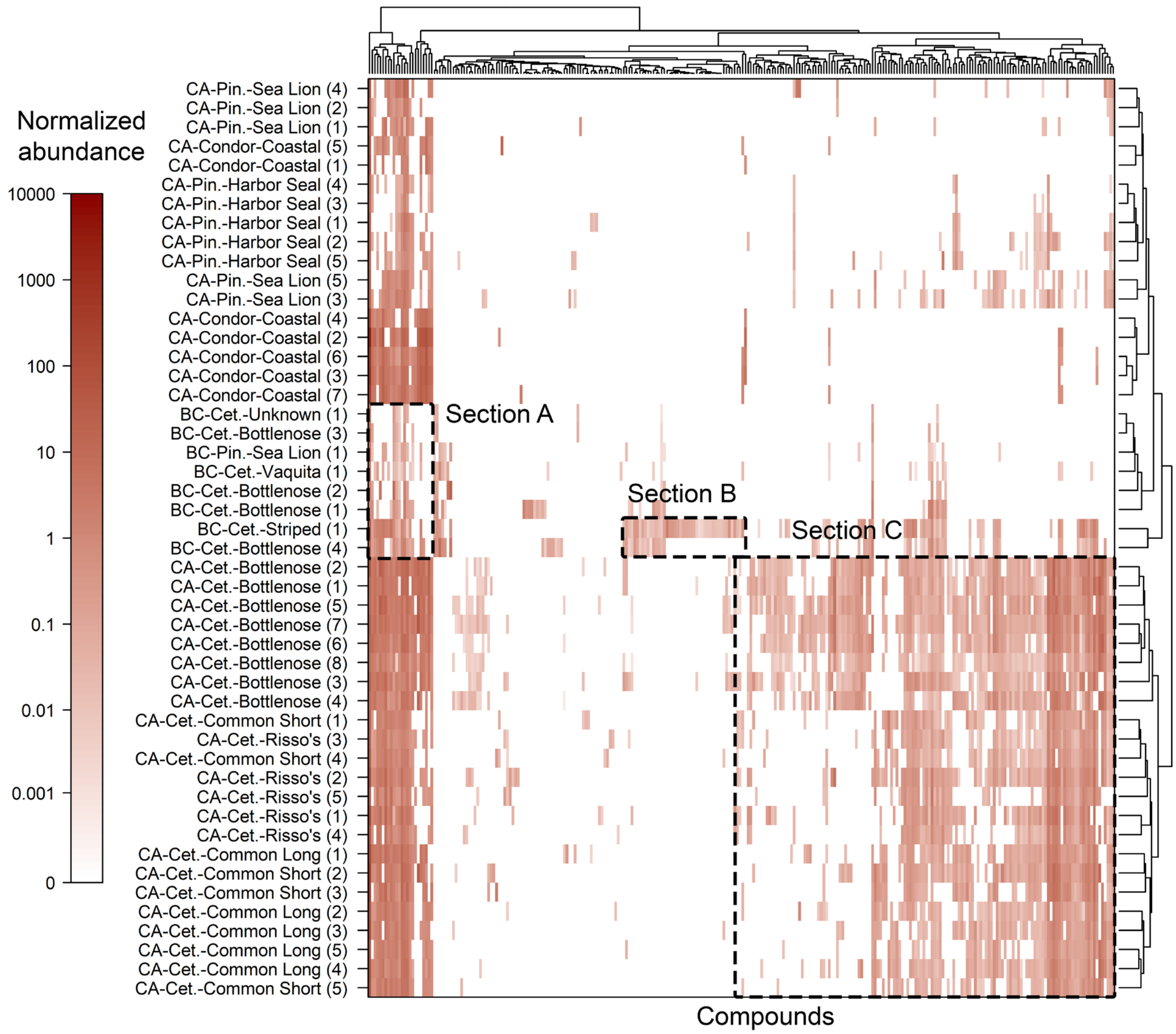


Figure 3: Hierarchical clustering heatmap of the log-transformed normalized GC×GC/TOF-MS peak area abundance of individual compounds identified in coastal and inland CA condors, CA marine mammals, and BC marine mammals (cet = cetacean, pin = pinniped). Sections A, B, and C are discussed in the text. Compounds identified exclusively in CA bottlenose dolphins were excluded. Abbreviated names for select marine mammals are used: sea lion (California sea lion), bottlenose (bottlenose dolphin), striped (striped dolphin), common short (common short-beaked dolphin), Risso's (Risso's dolphin), and common long (common long-beaked dolphin).

Comparison of CA cetacean and BC cetacean HOC profiles. BC cetaceans contained fewer HOCs (13-139 HOCs/sample) than CA cetaceans (105-317 HOCs/sample). The HOCs detected in BC cetaceans comprised 26 structural classes (including 6 unknown subclasses). The most abundant structural classes were DDT-related compounds and PCBs, but DDT-related compounds were ~7 times less abundant in BC cetaceans than CA cetaceans, and PCBs were ~3.5 time less abundant. **Figure 2** shows nearly all structural classes were less abundant in the BC cetaceans than the CA cetaceans. No structural classes were significantly more abundant in the BC cetaceans than the CA cetaceans (**SI Table 10**). The condition of the BC cetacean samples (freshly dead to mummified/skeletal, **SI Table 1**) did not appear to influence the observed contaminant profile.

Using these cetacean groups as sentinel species to compare environmental contamination, our data suggests the Gulf of California is less polluted with HOCs than the coast of the state of California. Data on organic contaminants in Baja California is scarce, but HOCs including DDT, lindane, and hexachlorobenzenes have been detected in coastal sediment of the Gulf of California[39,40], but organochlorine concentrations in California sea lions were found to be 1-2 orders of magnitude lower in animals from the Gulf of California compared to those from the Pacific coast of California, Oregon, and Washington.[41,42] This is consistent with our findings, demonstrating that HOC burdens are lower in BC marine mammals than in CA marine mammals.

The heatmap (**Figure 3**) shows all BC marine mammals cluster together (including the single BC pinniped sample) and have a common contaminant profile that is distinct from the CA marine mammals (both cetaceans and pinnipeds) and CA coastal condors. The three labeled heatmap sections (A, B, C) highlight these profile differences. Section A shows a group of 5 compounds that were completely absent in BC marine mammals, but typically identified in CA marine mammals and CA condors (4,4',4"-TCPMOH and 4 PCB congeners). These compounds, particularly TCPMOH, the presumed metabolite of TCPM, could be markers for historic chemical dumping off the coast of California (discussed below). Section B contains compounds exclusive to BC dolphin species, which is a set of unique compounds that condors could be exposed to if reintroduced to Baja California. This group consists of 32 compounds over 11 structural classes (**SI Table 12**), including two compounds previously identified in Brazilian bottlenose dolphins[33] and a new, presumed isomer of MBP Cl_7 (Q1) based on similar molecular ions and mass spectra (**SI Figure 1**).[43] The most frequently occurring structural classes in Section B are unknown compounds (n = 12), DMBP (n =5), and DDT-related (n = 4). Section C shows compounds found exclusively in CA marine mammals (not in CA coastal condors or BC mammals), consisting of 81 compounds over 21 structural classes, with DDT-related compounds as the most frequently occurring (n = 13), followed by Unknown-4 (n = 11) and the Unknown class (n = 7).

DDT and TCPM in Southern California

The Southern California Bight is one of the most highly DDT-contaminated regions in the world due to historic DDT waste dumping by the Montrose Chemical Company (MCC).[44,45] MCC released upwards of 870 tons of DDT waste through a sewage outfall. Evidence also indicates a second waste disposal method using containerized dumping further offshore.[44,46] The barrel dumping is an unaccounted and little-studied source of DDT waste in the Southern California Bight. **SI Figure 2** shows that CA cetaceans contain ~7 times the abundance of DDT-related compounds than BC cetaceans, perhaps due to inputs of DDT from multiple sources.[17] TCPM, a compound associated with the DDT technical product, has been found globally.[47,48] However, CA marine mammals have a high diversity and abundance of TCPM related compounds, with 12 TCPM isomers and 7 TCPMOH isomers detected in CA marine mammals.[15,17,18] Comparatively, 3 TCPM isomers were identified in dolphins from the northern and southern Atlantic Ocean.[23]

Additionally, our data shows that BC marine mammals, which are geographically separated from the Southern California Bight by the Baja California landmass, contain just two TCPM isomers and no TCPMOH (**SI Figure 3**). Therefore, these various TCPM/TCPMOH isomers may be markers of historic DDT pollution. Understanding TCPM/TCPMOH contamination is important because they biomagnify, are highly persistent, and can be acutely toxic.[48,49]

Estrogen Activation Assays

Potential activation of California condor ERα and ERβ by TCPM, TCPMOH, and PCBs 101, 110, 118, 170, 183 and 187 was assessed by methods described previously (**SI Figure 4**).[20] These 8 compounds were selected because they had not been previously tested, were abundant in the majority of coastal condor samples, and were identified almost exclusively in the coastal condors. Weak, but significant, activation of ERβ was observed following treatment with 10^{-5} M of PCBs 101 and 110, respectively (**Figure 4**). To determine whether the compounds tested could antagonize estrogen receptor activation (i.e., exhibit anti-estrogenic activity) cells expressing condor ERα or ERβ were co-treated with each of the compounds listed above and 10^{-10} M E_2 (**SI Figure 5**). The majority of the compounds, tested at concentrations reflecting those of other HOCs found in condors, exhibited no anti-estrogenic activity. However, at the highest concentration tested (10^{-5} M), PCB 187 ($p<0.001$) exhibited weak inhibition of Erα, while TCPM exhibited weak inhibition of condor ERβ (**Figure 4**). Note other compounds identified in the coastal condors stimulated activation of condor ERs in prior work.[20] Dieldrin, *trans*-nonachlor, and PCB 52 moderately activated both condor ERs, and *o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE significantly activated ERβ at similar concentrations to the compounds tested here (10^{-6}-10^{-4}M).[20]

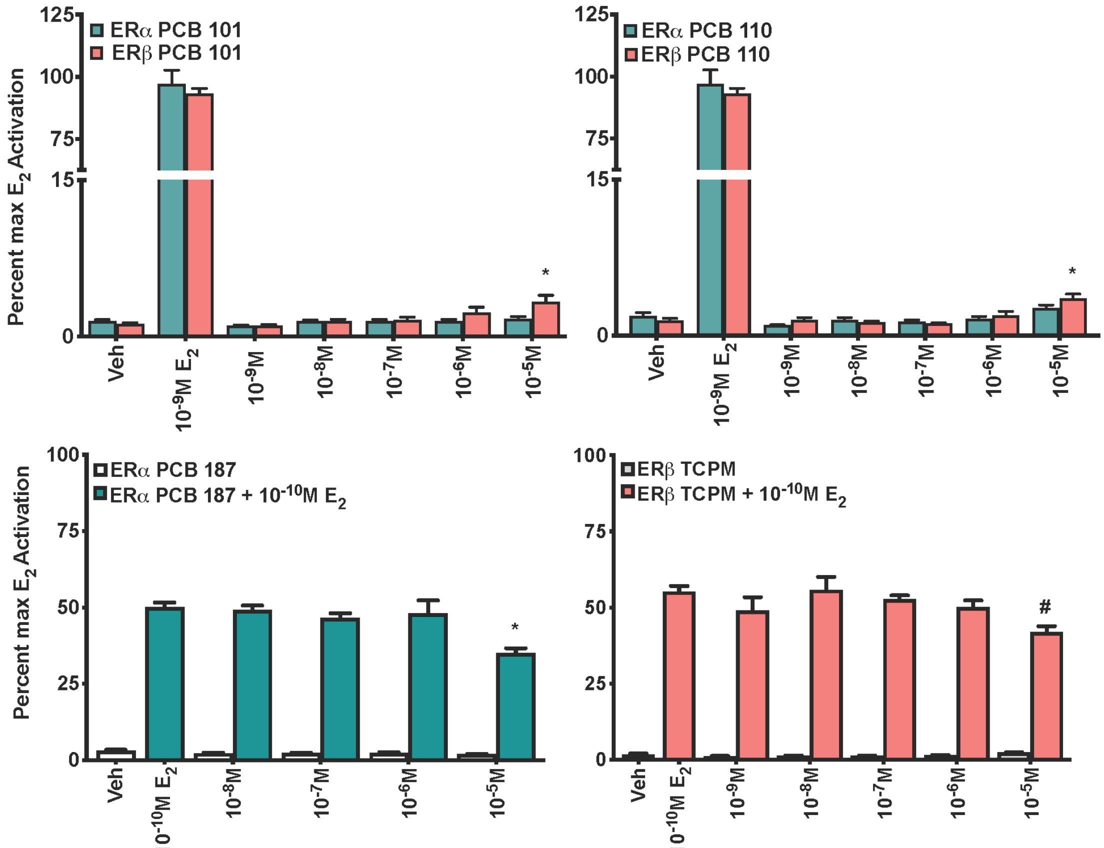


Figure 4. Activation and inhibition of California condor ERα and ERβ by various HOCs. Human embryonic kidney cells (HEK 293) were transfected separately with ERα or ERβ. Data are represented as mean ± SEM of the fold activation of each treatment relative to max E_2 activation. Significant differences in activation or inhibition (following co-treatment with E_2) of ERα and ERβ by HOCs compared to that of the vehicle were determined using a one-way ANOVA and Dunnett's post hoc test (*$p \leq 0.001$, $^{\#}p=0.04$).

Study Limitations

1) In the Shaul *et al.* (2014) bottlenose dolphin data set, some high abundance DDT related compounds and PCB congeners had saturated chromatographic peaks due to the amount of injected sample necessary to detect low abundance compounds. This may have led to an underestimation of the relative abundance of those DDT and PCB compounds. The other data sets did not have saturated chromatographic peaks. 2) Collection dates for condor plasma

samples and CA marine mammal blubber samples varied. Condor samples were collected from 2014-2015, whereas CA marine mammal samples were collected from 1990-2014 and BC marine mammal samples were collected from 2017 to 2019. However, within sample groups there was no evidence that collection year influenced contaminant profiles. 3) ER activation assays were conducted using a heterologous expression system in a human-derived cell line. Although similar systems are commonly used to predict physiological effects of EDC exposure in a wide variety of species, they may not be identical to those experiences by condors *in vivo*. In addition, ER activation assays were only performed with single compounds and not the complete mixture of chemicals to which condors are exposed.

Implications for condor reintroduction and conservation

Our study shows coastal condors have both a greater number and diversity of HOCs compared to inland condors. This indicates that coastal condors may be exposed to contaminant levels that elicit physiological responses while concentrations in the inland condors remain below these thresholds.[5,8] Comparison of CA cetacean and BC cetacean HOC profiles shows that the Upper Gulf of California aquatic food web is less contaminated with HOCs than the coast of the state of California. In particular, DDT-related compounds are lower in BC cetaceans than both CA cetaceans and CA pinnipeds. We also detected several compounds not previously identified in California condors. These included the halogenated natural products (HNPs) MBP Cl_7 and DMBP Br_4Cl_2, as well as anthropogenic TCPM and TCPMOH. HNPs have been found in whale tissues pre-dating 1925 and are consistently identified in modern marine mammals, indicating their continuous ubiquity in marine environments.[15,18,43] Little is known about HNPs, but their structural similarity to PCBs and DDT suggest that they could cause endocrine disruption.[43] TCPM has been identified in Pacific marine mammals since the 1980s and is now known to be globally distributed.[47,51] TCPMOH was not identified in any BC marine mammals; additional evidence they are not exposed to the same historic DDT-related contamination as CA marine mammals. Of the potential endocrine-disrupting chemicals tested in this study, three exhibited the ability to interact with condor ERs, but only at low micromolar concentrations that exceed circulating PCB and chlorinated pesticide concentrations previously documented in condors and therefore may not be physiologically relevant[8]. However, several of the other compounds identified in the coastal condors are capable of interfering with multiple endocrine pathways (e.g., estrogen, androgen and thyroid signaling) in other species, including *p,p'*-DDE, PCB 52, and TCPMOH, among others.[20,48] Therefore, further investigation into how HOCs may interact with other hormone receptors and potentially disrupt endocrine and reproductive function in condors is warranted.

Our study shows NTA methods can be successfully used to compare contaminant profiles acquired across multiple projects and years, with different instrument operators and data analysts.[15,17,18] Across all projects, we relied on a method for storing the NTA results in an archival yet accessible format, described in Hoh *et al.* (2012). The prior work established the usefulness of high trophic level marine mammals as sentinels of contamination in the region[15,18], and the GC×GC/TOF-MS based NTA enabled the detection of unexpected regional contaminants including TCPM, TCPMOH and other DDT-related compounds.[17] Thus, this study illustrates the potential usefulness of NTA for adaptable long term environmental monitoring. The NTA method also allowed for determination of unexpected and unknown contaminants in the coastal condor, inland condor, and BC marine mammal samples. Note, however, the contaminants observed in these three sample groups were largely subsets of those observed in the prior CA marine mammals.

Condors reintroduced to Baja California may benefit from limited HOC exposure.[5,52] The non-targeted analysis presented here indicates the largest known organic contaminant threat to coastal condors in California remains DDT and its metabolites. In 2014, ~40% of breeding age coastal condors from central California were predicted to have levels of DDE associated with eggshell thinning.[8] These exposures have significant effects on reproduction, such that coastal condor hatching success is less than 50%, whereas inland condors have rates of 70-80%.[5] Given the California condor's slow reproductive rate (1 egg/~1.5 years), survival of every egg is paramount.[9] Organochlorine exposure may also be related to increased glucocorticoid stress response, which can have physiological impacts on condors.[52] Furthermore, increased reintroduction to Baja California may limit lead exposure, since no condors in the BC flock have died of lead toxicosis in the last 5 years, compared to 17 coastal condors and 12 inland condors in California.[6,7,9,53-56] Although reintroduction site selection and successful condor recovery depends on multiple factors, the reduced potential for lead and HOC contamination in Baja California, compared to the southern California coast, highlights the value of this site.

Supporting Information: Additional experimental details, methods, figures, and tables as mentioned in the text in S-1. The mass spectra of unknown compounds identified exclusively in the BC marine mammals can be found in SI-2. This information is available free of charge via the Internet at http://pubs.acs.org.

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