ASSESSMENT OF CONTAMINATION BY POPs IN BLUE WHALES *(Balaenoptera musculus)* FROM THE SOUTH PACIFIC OCEAN

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Introduction

Marine mammals and in particular cetaceans have long been employed as sentinel organisms to biomonitor persistent organic pollutants (POPs) in marine ecosystems[1]. Given the lipophilic nature of POPs, blubber biopsies have routinely been chosen to investigate the exposure to these contaminants. On top of their compositional suitability, bubbler biopsies can be obtained in a relatively easy, non-lethal and minimally invasive way from free-ranging animals by means of darts or poles[2,3].

As their role as sentinel species, it is known that levels of contaminants in cetaceans are dependent on several factors such as age, sex, and trophic level among others. It also known that these species present a lower capacity for degradation of xenobiotics such as POPs in comparison to birds and terrestrial mammals[4]. Thus, high levels of POPs have been documented in numerous marine mammal species from different geographical areas (mostly from the northern hemisphere). Furthermore, possible associations between POPs and adverse health effects such as for instance endocrine disruption, reproductive impairment or immunosuppression have been argued[1,5].

Worldwide populations of blue whale (*Balaenoptera musculus*) are currently very scarce, primarily due to the decimation of their numbers as a consequence of the massive hunt carried out during the whaling era. Hence, the blue whale is today classified as "endangered" in the Red List of Threatened Species of the IUCN[6]. Being the biggest animal that has ever lived and despite their low trophic level as strictly feeders on krill, blue whales may hold a valuable potential as sentinel organisms owing to 1) their long lifespan (up to ninety years) and 2) the sheer magnitude of bioconcentration processes taking place in their fatty tissues as a result of the daily massive amount of ingested prey. However, and most likely owed to their above mentioned scarcity, there exist very few studies reporting on any levels of POPs in blue whales, and none -to the best of our knowledge- focused on specimens sampled in southern hemisphere waters.

The main objective of this work was to assess for the first time the degree of contamination by certain POPs in blue whales from the South Pacific Ocean.

Materials and methods

Sampling

A total of thirty six integument biopsies (epidermis, dermis and blubber) of blue whale (*Balaenoptera musculus*) were obtained in or around March 2011 (n=13), 2012 (n=2) and 2013 (n=21). All of them stemmed from free-ranging specimens sampled at Chilean waters using biopsy darts fired by means of a crossbow. Samples were immediately placed in liquid nitrogen and stored at -80°C until residue analysis.

Analytical procedure

The analysis of PCBs, HCB, DDTs and PBDEs was carried out on freeze-dried blubber biopsy samples. In brief, samples were spiked with isotopic labeled standards of PCBs, HCB, DDTs and PBDEs prior to the soxhlet extraction for 24 h with a mixture of n-hexane:dicloromethane (9:1, v:v). A subsequent clean-up process was achieved by low pressure chromatography on acidic and basic silica gel multilayer. Final extracts were evaporated using a TurboVap® system until ~1 mL, transferred to vials, and dried under a gentle nitrogen steam. Samples were reconstituted in a solution of different ^{13}C-labeled standards in nonane as internal standards for instrumental analysis.

Instrumental determination

Twenty *ortho* and mono-*ortho* PCB congeners (# 28, 52, 95, 101, 105, 114, 118, 123, 132, 138, 149, 153, 156, 157, 167, 170, 180, 183, 189, 194), six DDTs (*p,p'*- and *o,p'*- DDT, -DDE and -DDD) and HCB were analyzed by high resolution gas chromatography low resolution mass spectrometry (HRGC-LRMS) using a 7890N gas chromatograph coupled with a 5975C quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in selected ion monitoring mode (SIM) and electronic impact (EI) as ionization mode. Quantification of the target analytes was based on the isotope dilution technique. Fifteen brominated BDE congeners, from tri- to deca-substituted (# 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, 209), were analyzed HRGC-LRMS using a 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in SIM with negative chemical ionization (NCI) as described elsewhere[7].

Results and discussion

All investigated POPs were detected in the blue whales samples analyzed in this study. The concentration values obtained are summarized in Table 1 on a lipid weight basis (l.w.). The considerable variation found for concentration values could be likely ascribed to unknown differences in age, sex and reproductive status of the sampled specimens. Additionally, data did not follow a normal distribution for any study group of POPs.

Table 1. Mean, median, range, detection frequencies (% > LOQ) of total PCBs, DDTs, HCB and PBDEs in blubber biopsies from blue whales. Concentrations are expressed in ng/g l.w.

	Mean	Median	Range	>LOQ (%)
PCBs (ng/g)	139	92.4	2.97 – 975	100
DDTs (ng/g)	49.0	28.0	3.50 – 537	100
HCB (ng/g)	16.2	12.9	<LOQ – 63.1	95.0
PBDEs (ng/g)	7.11	5.32	<LOQ – 31.3	90.0

The relative abundance of the study contaminants followed the order PCBs>DDTs>HCB>PBDEs. The lack of studies on POPs in blue whales or any species of cetaceans from the South Pacific Ocean for that matter, makes it challenging to analyze the measured contamination in a comparative way. The very few data available in the literature on the same POPs for blue whales' blubber biopsies are shown in Table 2. A comparison between the data obtained in this study with that reported in the literature must be exerted with caution given the important differences in the number of sampled specimens, years and geographical areas. However, blue whales from Chilean waters seemed to present notably lower levels –of at least one order of magnitude- for all study contaminants. This difference was the widest in the case of DDTs, for which concentrations found in Chilean specimens were up to two orders of magnitude lower than those reported in blue whales from Canada and Mexico. The present study might support evidence for a lower degree of contamination by POPs in southeastern Pacific waters in comparison to Canadian north Atlantic ones in the 90's and to the Gulf of California area in recent years. Not surprisingly, this scenario is also in agreement with the reduced degree of contamination found by numerous studies in marine mammals from south Pacific waters relative to those from northern hemisphere regions[8].

Table 2. Mean reported concentrations in the literature for PCBs, DDTs, HCB and PBDEs in blubber biopsies from blue whales.

Samples	Mean PCB concentration (ng/g l.w.)	Mean DDT concentration (ng/g l.w.)	Mean HCB concentration (ng/g l.w.)	Mean PBDE concentration (ng/g l.w.)	Sampling area & year	Reference
unknown sex (n=3)	2220	3130	125	-	Gulf of St. Lawrence (Canada) 1992	9
males (n=38)	2020	3420	226	-	Gulf of St. Lawrence (Canada) 1992-1997	10
females (n=27)	1220	1350	90.0			
males (n=3)	4910	3930	-	32.9	Gulf of California (Mexico) 2010	2
females (n=3)	2550	902	-	19.7		

In terms of abundance profiles, the average PCB content was mostly dominated (~15%) by those congeners with a medium-low chlorine content such as PCB 95 and 101. Important contributions (5 – 10%) were also found for congeners 153 > 52 > 28 > 149 > 138~132. This picture is somewhat similar to that described by Gauthier et al.[9] in Canadian blue whales, at least in the predominance of PCB 101 (with PCB 95 not analyzed) over congeners 153, 138 and 180. This is interesting since the latter ones are typically examples of the most recalcitrant PCB congeners and therefore generally found dominant as direct consequence of their high resistance to biodegradation[11]. However, it is consistent with the fact that surface oceans are enriched in the lower chlorinated PCB congeners[12] and also consistent with the shortness of the blue whale's food chain. This last fact could account for the absence of a clear biomagnification of higher chlorinated congeners.

As for DDTs, the relative contribution to the average total content followed the pattern: *p,p'*-DDE (~72%) >> *p,p'*-DDD (~15%) > *p,p'*-DDT (~7%) > *o,p'*-DDT (5.5%) > *o,p'*-DDD (0.5%) > *o,p'*-DDE (non detected). There exist a study reporting DDT levels, back in 1983, in blubber from specimens of Bryde's whale (*Balaenoptera edeni)* and fin whale (*Balaenoptera physalus*) in waters from the Chilean cost[13]. These both species are phylogenetically close to the blue whale. The average DDT levels (∑DDTs) established in that study were 589 ng/g (fresh weight, n=2) and 54.4 ng/g (fresh weight, n=2), respectively. The heighten decrease of DDT levels in the area along with the prevalence of *p,p'*-DDE among DDTs point out towards a likely reduction in the use and input of this pesticide in the environment accordingly to its worldwide ban that Chile implemented in 1984[14].

Lower-medium brominated BDE congeners such as 47, 28 and 99 noticeably dominated the average PBDE content with median contributions of 38%, 26% and 10%, respectively. These results are once again consistent with the low trophic level of the blue whale, given that those same congeners have been found prevalent in zooplancton from an Artic foodweb[15] and in Antarctic krill[16,17].

The present study provides valuable information about the current status of the POPs occurrence in a geographical area such as the South Pacific Ocean for which there is today a marked shortage of scientific information in comparison to other areas of the globe. Moreover, is one of the very few documented works reporting data on blue whales. Considering the distinct characteristics in the bioaccumulation patterns shown for certain congeners, blue whales could represent valid and useful sentinel organisms of the ocean contamination.

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