TESIS DOCTORAL

# ASSESSMENT OF ANTHROPOGENIC CONTAMINANTS IN BOTTLENOSE DOLPHINS *(Tursiops truncatus)* FROM THE CANARY ISLANDS

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**DOCTORADO EN SANIDAD ANIMAL Y SEGURIDAD ALIMENTARIA**

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# Anexo II

## UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Departamento: Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

Programa de Doctorado: **"Sanidad Animal y Seguridad Alimentaria"**

**Título de la Tesis**

**"Assessment of anthropogenic contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Canary Islands"**.

Tesis Doctoral presentada por **Dña. Natalia García Álvarez**

Dirigida por **el Dr. Octavio Pérez Luzardo, el Dr. Antonio J. Fernández Rodríguez y el Dr. Luis Domínguez Boada.**

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Las Palmas de Gran Canaria, 2017

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Legnaro (PD), June 5, 2017

**TO WHOM IT MAY CONCERN**

This is to inform you that I, Sandro Mazzariol, Assistant Professor of Veterinary Pathology at the University of Padova - Department of Comparative Biomedicine and Food Science (Italy), have carefully read the PhD Thesis entitled ***"Assessment of anthropogenic contaminants in Bottlenose Dolphins (Tursiops truncatus) from Canary Islands",*** written by Natalia Garcia Alvarez, at the Institute of Animal Health of the University of Las Palmas de Gran Canaria.

The above PhD thesis fulfills all the scientific requirements to be presented for being public evaluated by the doctoral commission of the University of Las Palmas de Gran Canaria.

Sincerely

Dr. Sandro Mazzariol, DVM, PhD
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"…con quien cruzó el infinito abismo
del ser o no ser, del cuerpo y del alma."

(G. Echevarría, L. M.)

PARA ÁFRICA

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Fuente foto portada: SECAC

# ASSESSMENT OF ANTHROPOGENIC CONTAMINANTS IN BOTTLENOSE DOLPHINS (*Tursiops truncatus*) FROM THE CANARY ISLANDS

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# 1. SUMMARY / RESUMEN

## SUMMARY

In the last decades, pollution has become a global problem affecting different species of marine animals. Particularly cetacean populations accumulate high concentrations of anthropogenic contaminants due to their top trophic position, which made them especially vulnerable to long-term toxicity including immune suppression, reproductive impairment and carcinogenic effects. Extensive research on pollutant levels in marine mammals has been reported worldwide, however, little information is available concerning cetaceans from the Canary waters.

The present thesis focused on bottlenose dolphin (*Tursiops truncatus*), species proposed as a bioindicator of the contamination of the Canary Islands as some groups are considered as resident local populations of this archipelago.

The main objective of this study is to gain a greater understanding of the toxicological status of common bottlenose dolphin in the Canary Islands, as part of the international Macaronesia Cetacean Health Network.

To address this objective, the assessment of the levels of 57 persistent organic pollutants (POPs) and 12 toxic trace elements in blubber and liver of bottlenose dolphins stranded from 1997 to 2013 and also POPs in biopsies from live individuals collected during the period of 2003-2011 were conducted. The samples from stranded dolphins were taken from the cetacean tissue bank available at the Institute of Animal Health and Food Safety (IUSA) of the University of Las Palmas de Gran Canaria (ULPGC). The blubber biopsy samples were collected by the Society for the Study of Cetaceans in the Canary Archipelago (SECAC). The toxicological studies were conducted at the laboratory of the Clinical and Analytical Toxicology Services (SERTOX) of the ULPGC. Soxhlet method and gel permeation chromatography (GPC) for the sample's extraction and gas chromatography interfaced with a mass spectrometer (GC-MS) for the detection and quantification of organic compounds was carried out. For heavy metals and other toxic trace elements all samples were freeze-dried for a subsequent microwave digestion to be mineralized. After digestion, the analysis of the elements was performed with an inductively coupled plasma-optic emission spectrometry method (ICP-OES).

This thesis aimed to compile three articles published in peer-reviewed journals describing the results and interpretation of the toxicological analyses conducted.

The present study represents the first determination of pollutants in live free-ranging cetaceans from the Canary Islands. It also provides the first data of polycyclic aromatic hydrocarbons (PAHs) in bottlenose dolphins from the Eastern Atlantic Ocean and doubles the number of organochlorine compounds and toxic elements previously analysed in stranded cetaceans on Canary coasts (Carballo et al., 2008), also extending the study period to 15 years. According to the results of this thesis, the bottlenose dolphin inhabiting the Canary waters is facing a significant exposure to anthropogenic pollutants, highlighting the need for on-going monitoring of contaminant accumulation in cetaceans from this marine area. In general, xenobiotic levels in this species were slightly higher than those from the North Sea and comparable to Western Atlantic Ocean, several locations in the Pacific Ocean and the waters surrounding the UK. As expected, the results obtained were much lower than those observed in the Mediterranean Sea where bottlenose dolphins accumulate great burdens of chemical residues. Further studies are needed to investigate potential associations between contaminants and health status to determine the impact of anthropic environmental pollution on these animals.

# RESUMEN

En las últimas décadas, la contaminación se ha convertido en un problema mundial que afecta a diferentes especies de animales marinos. En particular, los cetáceos, reciben altas concentraciones de contaminantes antrópicos como consecuencia de su posición en la cadena trófica lo que les hace especialmente vulnerables a una toxicidad crónica incluyendo inmunosupresión, problemas reproductivos y efectos carcinogénicos. Se han realizado múltiples investigaciones sobre el nivel de contaminantes en mamíferos marinos en todo el mundo, sin embargo, hay poca información disponible en cetáceos de las Islas Canarias.

Esta tesis se centra en la especie del delfín mular (*Tursiops truncatus*), propuesta como bioindicador del estado de contaminación de las Islas Canarias, ya que algunos grupos se consideran poblaciones residentes en este archipiélago.

El principal objetivo de este estudio es obtener un mayor conocimiento del estado toxicológico del delfín mular común en las Islas Canarias, como parte de la red internacional de Salud de Cetáceos de la Macaronesia. Para llevar a cabo este objetivo, se analizaron 57 contaminantes orgánicos persistentes (COPs) y 12 elementos traza tóxicos en blubber e hígado de delfines mulares varados de 1997 a 2013 y COPs en biopsias muestreadas de individuos vivos en libertad de 2003 a 2011. Las muestras de los delfines varados se tomaron del banco de tejidos de mamíferos marinos, disponible en el Instituto Universitario de Sanidad Animal (IUSA) de la Universidad de Las Palmas de Gran Canaria (ULPGC). Las biopsias de piel fueron tomadas por la Sociedad para el Estudio de los Cetáceos del Archipiélago Canario (SECAC). Los estudios toxicológicos se efectuaron en los laboratorios del Servicio de Toxicología Clínica y Analítica (SERTOX) de la ULPGC. Para la extracción de las muestras se utilizó el método Soxhlet y una cromatografía de permeación en gel (GPC). La detección y cuantificación de compuestos orgánicos se realizó mediante cromatografía de gases acoplada a un espectrómetro de masas

(GC-MS). Para el análisis de metales pesados y otros elementos tóxicos se procedió a la digestión ácida en microondas de las muestras previamente liofilizadas para posteriormente determinar los elementos mediante el método de espectrometría de emisión óptica con fuente de plasma de acoplamiento inductivo (ICP-OES).

Esta tesis reúne tres artículos publicados en revistas científicas en los que se describen los resultados y discusión de los análisis toxicológicos realizados.

Este estudio representa la primera determinación de contaminantes en cetáceos vivos en libertad de las Islas Canarias. Además presenta los primeros datos de hidrocarburos aromáticos policíclicos (PAHs) en delfines mulares del océano Atlántico Oriental y duplica el número de compuestos organoclorados y elementos tóxicos analizados previamente en cetáceos varados en las costas canarias (Carballo et al., 2008), extendiendo el periodo de estudio a 15 años. Según los resultados obtenidos en esta tesis, el delfín mular que habita las aguas canarias está expuesto a niveles importantes de contaminantes de origen antrópico, lo que evidencia la necesidad de realizar un control permanente de la acumulación de residuos químicos en cetáceos de este área marina. En general, los niveles de xenobióticos en esta especie fueron algo superiores a aquellos encontrados en el Mar del Norte y comparables a los del océano Atlántico Occidental, algunas localizaciones en el océano Pacífico y las aguas circundantes a Reino Unido. Como era de esperar, los resultados fueron mucho menores a los observados en el Mar Mediterráneo donde los delfines mulares acumulan grandes cantidades de residuos químicos. Se precisan futuros estudios que investiguen posibles asociaciones entre contaminantes y el estado sanitario para determinar el impacto real de la contaminación ambiental antrópica sobre estos animales.

# 2. LITERATURE REVIEW

## 2.1. Introduction

After decades using seas and oceans as endless sinks of our domestic, urban and industrial wastes, there are no more pristine marine environments. We have been releasing anthropogenic pollutants for years to the air, soil and water, from the tropics to the poles, affecting different marine ecosystems all along the food chain, from the plankton to top predators. Particularly cetaceans are receiving high concentrations of anthropogenic pollutants arising out of a worldwide contamination.

Important levels of persistent, bioaccumulative and toxic (PBT) xenobiotic such as the already banned organochlorines (OCs) can still be measured. The outstanding chemicals for their abundance and known toxicity are the polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), several heavy metals (HMs) and other toxic elements. All of them have been found in tissues from marine mammals (MMs) worldwide, which are especially vulnerable to long-term toxicity of these chemicals. However, little information is available from this marine area surrounding the Canary archipelago.

The study of cetaceans may reflect the state of the environmental health to include in the global puzzle of contamination for a long-term conservation plan to preserve our marine biodiversity.

## 2.2. The Canary Islands: the study place

### 2.2.1. Features of the archipelago

The Canary Islands are a Spanish archipelago that belongs to the outermost regions (OMR) of the European Union. The 7 main islands are (from east to west) Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma and El Hierro. They are located in the North Atlantic Ocean near Europe and North Africa, within the biogeographical region of the

Macaronesia, which also includes the islands of Azores, Madeira, Savage Islands and Cape Verde (Carrillo, 2007). Four of the Canary Islands were declared a Unesco World Biosphere Reserve with several protected areas. The Canary current is in the transition zone between the upwelling coastal waters and the oligotrophic waters of the open ocean, perturbing the flow of ocean currents and trade winds (Barton et al., 1998). The biodiversity of this archipelago has designated the Canary Islands as a Particularly Sensitive Sea Area (PSSA) for the International Maritime Organization (IMO) in 2005. In addition, 12 Special Areas of Conservation (SACs) are currently designated, under the European Habitats Directive, for the conservation of the bottlenose dolphin (*Tursiops truncatus*) and the loggerhead sea turtle (*Caretta caretta*) in the Canary Islands (Tobeña et al., 2014).

## 2.2.2. Cetaceans and local populations of bottlenose dolphins

The oceanographic characteristics (e.g. mild temperatures, great depths near the coasts, calm regions in the southwestern islands) and its strategy situation make possible the presence of many species of cetaceans, being the Canary Islands a major hotspot for whale watching in Europe (Carrillo, 2007). More than 30 species have been identified (approximately one third of the cetacean species in the world), some of them migratory (occasionally-occurring species), others with sedentary local populations (Culik, 2004; Martín et al., 2009). The narrow continental shelf leads to the presence of both oceanic and inshore cetacean species close to the coast (Martín et al., 1995).

Most of the cetacean odontocetes have a worldwide distribution such as Sperm whales (*Physeter macrocephalus*), pygmy sperm whale (*Kogia breviceps*), bottlenose dolphin, Risso's dolphin (*Grampus griseus*), common dolphin (*Delphinus delphis*) and striped dolphin (*Stenella coeruleoalba*). Moreover, Macaronesia constitute the northern limit for several species such as the Atlantic spotted dolphin (*Stenella frontalis*), rough-toothed dolphin (*Steno bredanensis*), or Fraser's dolphin (*Lagenodelphis hosei*) (Carrillo, 2007). Other warm-water species frequently seen in the Canary Islands are the Gervais' beaked whale (*Mesoplodon europaeus*), which inhabits the North Atlantic Ocean (Pitman, 2009) and the short-finned pilot whale (*Globicephala macrorhynchus*). Besides all these species (Fernández et al., 2009), the Cetacean Unit of the Institute of Animal Health (IUSA) has handled other stranded species such as those among the Ziphiidae family, the Cuvier's beaked whale (*Ziphius cavirostris*)*,* Blainville's beaked whale (*Mesoplodon densirostris*), Sowerby's beaked whale (*Mesoplodon bidens*) and the True's beaked whale (*Mesoplodon mirus*)*.* Other odontocetes have been also attended such as the spinner dolphin (*Stenella longirostris*), the dwarf sperm whale (*Kogia sima*), the false killer whale (*Pseudorca* crassidens), the killer whale (*Orcinus orca*) and the Harbour Porpoise (*Phocoena phocoena*), the latter two species only stranded once to date. Among the mysticetes, the species stranded in the Canary Islands have been the fin whale (*Balaenoptera physalus*), the minke whale (*Balaenoptera acutorostrata*), the sei whale (*Balaenoptera borealis*), Bryde's whale (*Balaenoptera edeni*) and Humpback whale (*Megaptera novaeangliae*) (in decreasing order of findings).

The most frequent inshore cetaceans are the bottlenose dolphins and the pilot whales, and to a lesser extent the oceanic sperm whales. The pilot whale seem to inhabit the south-western coasts protected from the trade wind from the islands of La Gomera, Tenerife and Gran Canaria (Plasencia et al., 2001) and the sperm whale between Tenerife and Gran Canaria. However, they can be observed in the entire archipelago.

The common bottlenose dolphin is considered resident (or regularly occurring specie) in the Canary waters. As commented before, there are 12 SACs which require special conservation due to the presence of this species, 5 of them represent important feeding and reproduction areas. Preliminary studies revealed a structured population strongly associated to the islands, particularly to the south western coasts, with a weak linkage between islands; thus the populations of each SEC should be considered as independent groups of conservation (SECAC, 2013). In contrast, a recently published study (Tobeña et al., 2014) assures that 10.2% individuals travelled between the western islands of the archipelago. They suggested that this could be explained for the need of high mobility to search for food due to the oligotrophic regimen of these waters. Besides, it is important to note that almost all field observations were conducted in these south-western areas for the trade wind protection which could lead to underestimate the data; in fact, stranding events have occurred along the entire coastline. Thus, despite the field study efforts, there is a lack of population abundance estimates for the archipelago and further investigations of bottlenose dolphin residency are required.

## 2.3. The common bottlenose dolphin (*Tursiops truncatus*)

### 2.3.1. Characteristics

The common bottlenose dolphin *(Tursiops truncatus)* is one of the most well-known species of cetacean probably due to near-shore habits, use in captivity worldwide, and frequent appearance on television. Several scientific books of MMs provide great amount of information about these cetaceans (Carwardine, 1995; Perrin et al., 2009; Reddy et al., 2001; Reeves et al., 2003; Wells and Scott, 1999).

#### 2.3.1.1. Taxonomic status (Montagu, 1821)

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Cetartiodactyla (Cetacea group)

Suborden: Odontoceti

Family: Delphinidae

Genus: *Tursiops*

Species: *Tursiops truncatus*

<u>Common name(s)</u>:

- English: common bottlenose dolphin, bottlenose dolphin, bottle-nosed dolphin, porpoise (often used in south-eastern USA).
- Spanish: delfín mular, delfín nariz de botella, tursión, tonina.

In 1821, Montagu (1821) classified this species as *Delphinus truncatus* referring to the flattened teeth to be definitely named *Tursiops truncatus* years later (Gervais, 1855).

The taxonomy of the genus *Tursiops* is controversial (IUCN, 2012). The bottlenose dolphin was previously recognized as one single species, *T. truncatus*, but a few years ago the genus was split into two species: *T. truncatus* (the common bottlenose dolphin) and *T. aduncus* (the Indo-Pacific bottlenose dolphin). *T. truncatus* was originally described for the North Atlantic Ocean, being currently considered a widespread species (Hoelzel et al., 1998). Additionally, some scientists recognize other dolphins from the Pacific Ocean (*T. gillii* and *T. nuuanu*) and from western South Atlantic Ocean (*T. gephyreus*) as subspecies but others considered synonyms of *T. truncatus*, which is increasingly accepted (IUCN, 2012; Rice, 1998).

In addition, in the Black Sea a subspecies is recognized (*Tursiops truncatus* ssp. *Ponticus*) which possesses morphological differences from Atlantic and Pacific populations.

Moreover, the Burrunan dolphin (*Tursiops australis*), from South Australia has been recently described as a new species by Charlton-Robb et al. (2011). However, the Society for Marine Mammalogy does not include it in their taxonomy list because they found it questionable and recommend a rigorous re-evaluation (Perrin, 2017; Taxonomy, 2016).

Their updated list definitely classifies the bottlenose dolphin as follows:

- *Tursiops aduncus* (Indo-Pacific bottlenose dolphin)
- *Tursiops truncatus* (Common bottlenose dolphin)
    - *T. t. ponticus* (Black Sea bottlenose dolphin)
    - *T. t. truncatus* (Common bottlenose dolphin)

Hence, the taxonomy of bottlenose dolphins remains unclear, due to geographical variation, and additional species may be assigned in the future. This thesis is focused on the common bottlenose dolphin, hereinafter referred as bottlenose dolphin, BND.

### 2.3.1.2. Distribution

The BND is a species of a worldwide distribution, through temperate and tropical regions, sometimes entering rivers and estuaries (Santos et al., 2007; Wells and Scott, 1999); although they are usually found in latitudes lower than 45° in both hemispheres, some studies have observed these dolphins at other latitudes (Wells and Scott, 1999; Mann and Watson-Capps, 2005). Through the SCANS II Life project for the small cetacean abundance in European Atlantic waters and North Sea, it was estimated a total population of BND to be 12,645 (SCANS-II, 2006). This abundance includes both offshore and inshore dolphins, being difficult to attribute an estimate number for the resident coastal populations (Davison et al., 2011). Moreover, before 1970 there were more strandings of BNDs in UK than what it is currently recorded (Jepson et al., 2005). This fact leads scientists to suggest that the resident UK populations of BNDs have strongly declined.

Concerning the Spanish waters, the BND is present in the Atlantic and Mediterranean coasts of the mainland and also surrounding the Balearic and the Canary Islands. There are two different ecotypes of BNDs, the coastal populations and offshore found in pelagic waters (Wells and Scott, 1999). In some areas, both forms can be distinguished by the size, colour, morphological characteristics, behaviour, etc. (Reeves et al., 2003). In the Mediterranean Sea only coastal groups are known but both ecotypes are present in the Atlantic waters, being the coastal form more abundant. These two forms have also showed genetic differences. Thus, Reeves et al. (2003) suggest that they could be divided into different species in the future.

The BND is characterised by herd behaviour with a highly variable group structure and size, ranged from a few individuals up to thousands in offshore populations (Lusseau et al., 2006; SECAC, 2013). The social structure is based on the sex, age, and reproductive status. However, there are considerable movements between different groups, which difficult the study of these social groups (INDEMARES, 2014). Moreover, BNDs are usually associated with pilot whales and other cetacean species (NOAA, 2015b).

### 2.3.1.3. Morphology

BNDs have a fusiform body, together with their flippers, fluke, and dorsal fin, allow them to adapt to an aquatic environment.

They have a medium-size and robust body with the caudal peduncle laterally compressed. The melon is well marked and convex. Their rostrum is thick and relatively short. The dorsal fin is moderately falcate and the pectoral fins and fluke proportionally large. The coloration varies from light to dark grey, being darker dorsally and laterally, with a clear ventral side. A light brush marking is often observed on their sides (Wells and Scott, 2009). Offshore dolphins, adapted for cooler waters, are usually darker and larger with smaller flippers than inshore animals (NOAA, 2015b). In contrast, Eastern Pacific offshore dolphins tend to be smaller than inshore individuals (Wells and Scott, 2009). Additionally, the size seems to be inversely proportional to water temperature, so bigger dolphins are found in free marine areas (Tejedor, 2016).

In the literature reviewed, the reported size of BNDs is highly variable depending mainly on geographical locations and ecotypes (see table 1). Moreover, the offshore ecotype is difficult to distinguish from the coastal ecotype in the field (Fernandez and Hohn, 1998).

The growth curve based on total length generally shows two-stage with a growth spurt around the sexual maturity age to later reach an asymptotic length (Mattson et al., 2006). However, (Read et al., 1993) suggested that in BNDs the growth spurt was not reflected in total length but in animal mass and/or girth. The growth spurt leads to the physical maturity based on epiphysis fusion, described in a recently presented thesis (Tejedor, 2016).

The table below shows a compilation of different data of lengths, mass or age of BNDs reported in the literature related to different life times such as birth, maturity and adult size (Table 1).

The males of BNDs seem to be slightly larger than females. In general, the maximum length of adults is 381 cm for males and 350 cm for females, with a weight range of 150 to 650 Kg, and regarding the Canary Islands, the longest male and female measured 340 cm and 303 cm, respectively (Tejedor, 2016).

This species has 16-24 teeth on each hemi-maxilla and 18-24 on each hemi-jaw. Atlas and axis are fused together. Moreover, an osteological study on BNDs inhabiting the Canary Islands has been recently presented in a doctoral thesis (Tejedor, 2016).

Table 1. Lengths and reproductive parameters estimations found in the literature consulted.

<table>
<tr><th>PARAMETERS</th><th>MALE</th><th>FEMALE</th><th>References</th></tr>
<tr><td rowspan="8">Length at birth</td><td>100 cm</td><td>100 cm</td><td>Sergeant, et al., 2011</td></tr>
<tr><td>109.4 cm</td><td>109.4 cm</td><td>Fernandez and Hohn, 1998</td></tr>
<tr><td>100-107 cm</td><td>98-103 cm</td><td>Mattson et al., 2006</td></tr>
<tr><td colspan="2">119 cm</td><td>Stolen et al., 2002</td></tr>
<tr><td colspan="2">180 cm</td><td>NOAA, 2015b</td></tr>
<tr><td colspan="2">100 cm</td><td>Kinze, 2002</td></tr>
<tr><td colspan="2">90-120 cm</td><td rowspan="2">Tejedor, 2016</td></tr>
<tr><td colspan="2">118-135 cm (Canary Is.)</td></tr>
<tr><td rowspan="12">Length/age at sexual maturity</td><td>245 cm; 13 yr</td><td>235 cm; 12 yr</td><td>Sergeant et al., 2011</td></tr>
<tr><td>237-280 cm;</td><td>219-253 cm; 9.8-15 yr</td><td>Fernandez and Hohn, 1998</td></tr>
<tr><td>9-14 yr</td><td>5-13 yr;<br>12 m (P); 18-20 m (W)</td><td>NOAA, 2015b</td></tr>
<tr><td>8-13 yr (US C Atlantic)<br>12-15 yr (SAF)</td><td>5-13 yr (US C Atlantic)</td><td rowspan="5">Wells and Scott, 1999</td></tr>
<tr><td>245-260 cm; 10-13 yr (F</td><td>220-235 cm, 5-12 yr</td></tr>
<tr><td>12-15 yr (SAF)</td><td>9-11 yr (SAF)</td></tr>
<tr><td colspan="2">8-10 yr (Sarasota)</td></tr>
<tr><td></td><td>267 cm; 7 yr (Japan)</td></tr>
<tr><td colspan="2">> 12 yr (Sarasota)</td><td>Well et al., 2004</td></tr>
<tr><td>265 cm (259 Kg) (GM)</td><td>249 cm (197 Kg) (GM)</td><td>Read et al., 1993</td></tr>
<tr><td>245-290 cm; 10-13 yr.</td><td>220-280 cm; 5-12 yr.</td><td rowspan="2">Tejedor, 2016</td></tr>
<tr><td>260-290 cm (Canary Is.)</td><td>228-265 cm (Canary Is.)</td></tr>
<tr><td>Length at physical maturity</td><td>306 cm (Canary Is.)</td><td>270 cm (Canary Is.)</td><td>Tejedor, 2016</td></tr>
</table>

| | | | |
|---|---|---|---|
| **Asymptotic length** | 270 cm | 250 cm | Sergeant et al., 2011 |
| | 255 cm | 250 cm | Mattson et al., 2006 |
| | 264 cm | 245 cm | Fernandez and Hohn, 1998 |
| | 255 cm | 246 cm | Stolen et al., 2002 |
| | 263 cm | 250 cm | Read, 1993 |
| | 260 cm | 240 cm | Kinze, 2002 |
| | 283 cm (EA) | 279 cm (EA) | Hale et al., 2000 |
| | 313 cm (CS) | 291 cm (CS) | |
| | 294 cm (SAF) | 279 cm (SAF) | |
| | 301 cm (SA) | 286 cm (SA) | Siciliano et al., 2007 |
| | 260 cm; > 10 yr (US A) | 250 cm; 10 yr | Wells and Scott, 1999b |
| | 12-15 yr (SAF) | | |
| | 380 cm (136-635 Kg) | | NOAA, 2015b |
| | 380 cm | | Wells and Scott, 1999a |
| | 282 cm | 279 cm | Hale et al., 2000 |
| | 245-381 cm | 240-350 cm | Tejedor, 2016 |
| | 340 cm (Canary Is.) | 303 cm (Canary Is.) | |
| **Reproductive rate** | 1 calf/yr | | Sergeant et al., 2011 |
| | 1 calf/3-6 yr (to 45 yr of age) | | NOAA, 2015b |
| | Calves all year. 2 peaks on summer and winter (Canary Is.) | | SECAC, 2013 |
| | 1 calf/3-6 yr; 1-2 yr (W) Sarasota | | Wells and Scott, 1999 |
| | 1 calf/3 (SAF) | | |
| **Lifespan** | 25 yr | | Sergeant et al., 2011 |
| | 40-45 yr | > 50 yr | NOAA, 2015b |
| | > 40 yr | > 50 yr | Wells and Scott, 1999 |
| | 39 yr | 49 yr | SECAC, 2013 |
| | 50 yr | 50 yr | Wells et al., 2004 |
| | 40-45 yr | 50 yr | Tejedor, 2016 |

## 2.3.1.4. Reproduction and life cycle:

They are long-lived dolphins with a lifespan of more than 40 years for males and more than 50 years for females (Wells and Scott, 1999). Wells et al. (2004) described three age ranges in BND from Sarasota, 0 to 4.9 years for calf/juvenile, 5 to 11.9 years for subadult and >12 years for adult category.

The age of sexual maturity of BNDs is highly variable mostly depending on different populations worldwide. Based on the literature reviewed (see references in table 1), the puberty is reached in a range of 8-15 years for males and 5-15 years for females. The mating system seems to be promiscuous (Wells and Scott, 1999). Calves born after a 12-month pregnancy period with length and weight at birth ranging from 85 to 130 cm and 15 to 30 Kg, respectively (see table 1), being 90-120 cm a more appropriate range for the species (Tejedor, 2016). The calves are weaned at 12 to 20 months and reach 160-180 cm (60-80 Kg) at post-weaning. Data from Sarasota indicate that calves typically remain with their mothers for 3-6 years, when dolphins reach approximately 225 cm in length. Separation generally coincides with the time of the next birth, resulting in a calving interval of at least three years (Wells and Scott, 1999).

The females can give birth until the age of 45 years (NOAA, 2015b). The seasonal reproduction often shows a single of bimodal birth occurrence in spring/early summer and fall (wells and Scott, 1999). However, dolphins inhabiting Canary waters appear to give birth in summer and winter (SECAC, 2013).

#### 2.3.1.5. Diet:

BND are generalists and opportunistic predators, preying upon benthic and nektonic species, including a wide variety of fish and squid. Several studies have found a locally abundant prey in their stomachs including fish, cephalopods and crustaceans (Fernández et al., 2009; Santos et al., 2007). A cuttlefish (Sepia spp.) and garfish (*Belone belone*) were found in one stomach of this species from the Canary waters (Fernández et al., 2009), which the author related to inshore feeding. However, these dolphins seem to feed also in offshore waters from other North-Eastern Atlantic marine areas (Santos et al., 2007).

Like other odontocetes, BNDs echolocate for feeding, employing several feeding strategies, such as striking the fish with their flukes and throw it out of the water, which is known as "fish whacking" (NOAA, 2015b). The active foraging can be a social or a solitary behaviour (Mann et al., 2007).

### 2.3.2. Anthropogenic threats and conservation

There is a growing concern over the last decades about the impact of anthropogenic stressors on the marine environment. The anthropogenic threats are represented by industrial, agricultural, maritime and recreational activities, causing the hole in the ozone layer, climate change, overexploitation of marine resources and acoustic and chemical pollution, among others (Bellas, 2014). Besides, emerging diseases are newly described due to what some authors called as “environmental distress syndrome”, caused in part by anthropogenic activities (Bossart, 2007).

Thus, MMs are exposed to a multiple threats, which individually or combined, directly or indirectly, affect the survival of these animals. In fact, MMs are especially susceptible to these stressors, due to their life history characteristics such as a long lifespan, low reproductive rate, slow growth rates, late maturity and their high position in the food chain (Fair and Becker, 2000).

Particularly the BND is considered one of the cetaceans suffering higher impact of anthropogenic activities. This species faces different threats and human disturbance (Fair and Becker, 2000; NOAA, 2015b):

- Fishery interaction: injury and mortality derived from fishing gear, such as gillnet, seine, trawl, and longline fishing and marine debris entanglement in lost and discarded fishing gear. BNDs sometimes interact with fish-farm cages or take fish from commercial or recreational fishing gears (Read et al., 2006; Wells and Scott, 1999). In contrast, it has been recently reported that, in the Spanish Mediterranean Marine Protected Areas (MPAs), BNDs avoid encounters with fishermen and recreational activities, suggesting that human presence may displace these animals (Castellote et al., 2015). Additionally, overfishing leads to a consequent loss of food resources.
- Hunting: dolphin catches are conducted for bait in fisheries, human food, to remove competition with industrial fishing and for captive display (Wells and Scott, 1999). Despite bans on hunting of cetaceans implemented in the mid-90s, dolphin meat and blubber are still used as shark bait and also for human food (Robards and Reeves, 2011). Even despite the international criticism, and the possible public health risk for the consumption of highly contaminated meat, thousands of dolphins are caught in drive hunts each year. In addition, the common BND is the species most often held in captivity (Reeves et al., 2003).
- Exposure to toxic compounds: xenobiotic chemicals (e.g., industrial and agricultural spills, oil pollution) and biotoxins (toxic algal blooms). Ingestion debris.
- Climate change
- Habitat degradation: reduced prey availability caused by environmental degradation…
- Noise/acoustic pollution: in fact, it is considered another form of habitat destruction and degradation.
- Human disturbance from ship traffic to whale watching activities.
- Biological factors: diseases (viral outbreaks), parasites, algal blooms...

Coastal populations of cetaceans, like the BND, are especially vulnerable to hunting, incidental catch, and habitat degradation. The contribution of anthropogenic pressure to an increasing trend of Unusual Mortality Events (UME) involving this species remains unclear (IUCN, 2012; NOAA, 2015a).

Several places worldwide present conservation problems concerning this species (Reeves et al., 2003):

- Mediterranean and Black Sea: past hunting, incidental by-catch and environmental degradation caused significant population declines (Bearzi et al., 2008; Reeves et al., 2003). The largest direct kills have traditionally been conducted by Russian and Turkish in the Black Sea, reducing local populations. Commercial hunting of Black Sea cetaceans was banned within the period of 1966 to 1983 (Jefferson et al., 1993). Additionally, anthropogenic OCs strongly affect MMs in the North-western Mediterranean Sea (Pinzone et al., 2015).

- Adriatic Sea: populations of BNDs likely have dramatically declined over the last decades, mainly due to past killing to reduce competition for fish, but also to habitat degradation and overfishing (Bearzi et al., 2008; IUCN, 2012). Additionally, there is a great concern about the high levels of contaminants affecting this marine area and therefore the health status of cetaceans.
- Gulf of Mexico and U.S. southeast coast: live capture removals have strongly affected some populations (Jefferson et al., 1993).
- Peru (and Chile): direct and incidental killing of both inshore and offshore forms (Van Waerebeek and Reyes, 1994). Peru is considered one of the principal marine areas over the world of concern for bycatch of small cetaceans to be consumed (BlueVoice, 2013; Mangel et al. 2010).
- Sri Lanka: BNDs are taken by harpoon and gillnet (Reeves et al., 2003).
- Taiwan: recent use of drive and harpoon fishery on the Penghu Islands and commercial whaling for meat appear to continue on the east coast (IUCN, 2012).
- Japan: thousands of dolphins have been caught in the drive and harpoon fisheries and culling for fishery protection. The culling has decrease in recent years but the take in drive and harpoon has increase (IUCN, 2012).
- Faroe Islands: tens of BNDs are killed during the pilot whale drives.

Concerning the Canary Islands, the BNDs is threatened mainly by maritime traffic of transport and recreational craft, by poorly regulated whale watching activity, interaction with artisanal fishing and fish-farm cages, and also by contamination with chemicals and solid debris (SECAC, 2013). All these factors contribute to the degradation of the marine environment surrounding this archipelago, habitat of these cetaceans.

All the SACs based on the presence of BNDs are placed at the South-Western areas of the islands, with good climatology conditions for diverse human activities. In fact, in some locations, the urban and touristic development has reached significant levels, making necessary to ensure the survival of these marine animals (SECAC, 2013).

This thesis focuses on the presence of environmental contaminants in BNDs inhabiting the Canary waters. We consider, as other authors do, that anthropogenic chemical compounds present a major threat and are the most insidious (Fair and Becker, 2000), particularly since ongoing exposure to high levels of pollutants constantly affects coastal BND populations, despite being banned for decades (Davison et al., 2011).

Concerning the state of conservation, all cetaceans are included in Annex A of EU Council Regulation 338/97; in particular BND was listed on Appendix II of the Convention on Migratory Species (Bonn Convention), and Appendix II of the Bern Convention (1979) to be further afforded special protected status under Annex II of the European Union's Habitats Directive (currently proposed to be included in Annex IV in the future). It is listed on Appendix II of CITES and considered as a "Least Concern (LC)" species by the IUCN Red List (IUCN, 2012), with the exception of the subspecies *Tursiops truncatus ponticus* which is listed as endangered (EN).

The BND is also classified as vulnerable on the Spanish National Catalogue of Threatened Species and under the special protection group of the Annex V on the Canary Catalogue.

Hence, even if the widespread BND seems to be abundant, the scientific experts clearly recommend its protection throughout all these lists and conventions coverage. As explained before, several marine protected areas, designed as SACs, exist in the Canary archipelago for the presence of this marine mammal.

The intense human activity in the Northeastern Atlantic Ocean places the marine environment under an enormous pressure (www.ospar.org). The importance of the anthropogenic impact and the interaction between different stressors and MMs should be properly understood for conservation purposes.

### 2.3.3. Bioindicator of the health of marine ecosystems

Human health depends on the well-being of ocean environment, and MMs are considered significant bioindicators of ecosystem and public health (Reif et al., 2015). Particularly cetaceans accumulate high concentrations of persistent OCs arising out of a worldwide contamination. They can concentrate greater levels of toxic compounds through feeding and also pass them to next generations through lactation. Thus, these animals have been proposed as sentinels to evaluate aquatic ecosystem health and identify damaging environmental trends.

Additionally, many marine mammal species share the coastal habitat and food with humans, so serving as effective sentinels for public health problems (Bossart, 2011; de Moura et al., 2014). They are also good sentinels for studying metabolism and possible toxic effects of chronic exposure to pollutants. This assessment will finally lead in evaluating possible effects in exposed humans to OCs from seafood consumption (Fair and Becker, 2000).

BND has been selected for the present thesis as a bioindicator of this marine ecosystem because, from an ecotoxicological point of view, this cetacean is considered a prime sentinel for the study of contaminant accumulation due to various features (Bossart, 2011; Fair and Becker, 2000; Kucklick et al., 2011; Wells and Scott, 1999):

- They are top predators, situated at high trophic level with a worldwide distribution (MMC, 1999). Thus, these animals accumulate high concentrations of organic pollutants in their tissues through bioaccumulation and biomagnification processes, presenting a large absorption capacity and slow elimination.
- They have long lifespan, which increases the accumulation of contaminants.
- They are long-term coastal residents in tropical and temperate regions throughout the world. Therefore, this inshore species occupies areas closer to domestic, urban and industrial sewage as sources of anthropogenic pollutants (Kucklick et al., 2011).
- They feed a wide variety of fishes and squids, thus they provide information on chemicals with the greatest risk to consumers at the top of the food chain, better

than using laboratory models. They may serve as good predictive models for human pollutant exposure from seafood consumption.

- They are homeotherms; therefore they need to keep the body temperature constant through a greater intake rate, which may lead to higher exposition to PBTs
- They have unique fat stores (blubber), where lipophilic toxic compounds are deposited.
- Low detoxification and elimination capacity. These animals have a poor ability of xenobiotics degradation due to their specific cytochrome P-450 enzyme systems.

Additionally, regarding the surrounding waters of the Canary Islands, the present study has focused on this species for the pollutant assessment considering several important aspects:

- The existence of 12 SACs in the Canary Islands due to the presence of BNDs.
- Particularly, some groups are considered as year-round resident populations of Canary Islands, which may reflect the contamination of local marine areas where anthropogenic activities are abundant (Aguilar et al., 2000; Wells et al., 2004).
- The large amount of studies carried out in this species, related to biology and pathology issues (Culik, 2004) and pollutant burdens worldwide (Kucklick et al., 2011; Law et al., 2012; Yordy et al., 2010b, 2010c, 2010d).
- Additionally, clinicopathologic data collected from free-ranging BNDs can be used to study possible relations between pathogens and chemicals (Reif et al., 2008).

## 2.3.4. Strandings along the Canary Islands coasts (1997-2016)

Seventy BNDs have stranded on the Canary coasts over the last 20 years (from 1996 to 2016). Out of these strandings, 51 have been necropsied and samples from 44 were available for toxicology studies in the tissue bank of the Institute of Animal Health (IUSA). Out of them, 31 stranded BND were available at the moment of this doctoral thesis (see publications in section 5).

Location of sampling sites for the present study is shown in figure 1. Lanzarote (LZ), Fuerteventura (FV), Gran Canaria (GC), Tenerife (TF), La Gomera (LG). This map indicates the number of specimens included in the publications of this thesis (in white) as a proportion of the total number of strandings (in red).

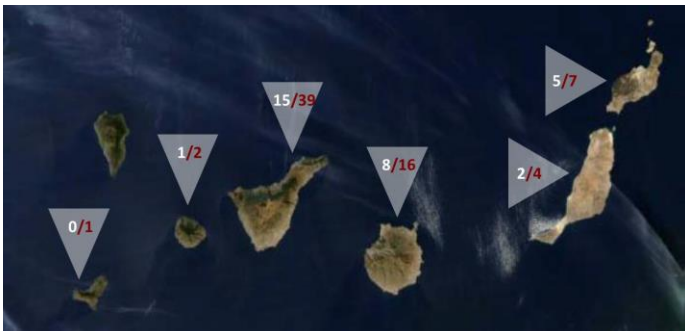



Figure 1. Location of the studied samples versus the total stranded BNDs from 1997 to 2016.

The following graph (Fig. 2) shows the temporal tendency of strandings, with a mean value of 3.3 strandings of BNDs per year. On 2000, 2003, 2005, 2008, 2010, 2011, 2014 and 2015 the number of strandings were over the mean value, which appears to happen every 2-3 years; additionally 2005, 2008 and 2014 are noted by peaks of stranding events, what should be thoroughly investigated. For instance, in 2008, 6 out of 7 dolphins stranded on TF coasts and regarding the gender, 5 were females and 1 was a male (plus 1 undetermined).

This graph also shows the gender distribution of BNDs stranded on the Canary Islands by year with no significant differences (total of 33 females and 30 males in this study period).

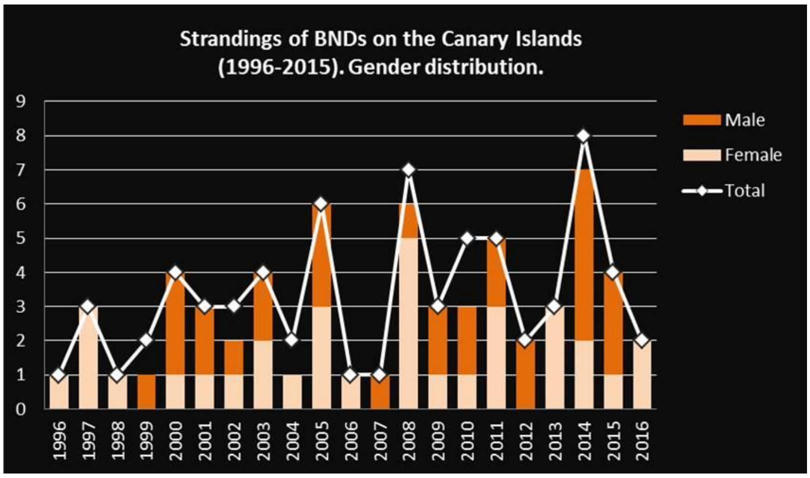
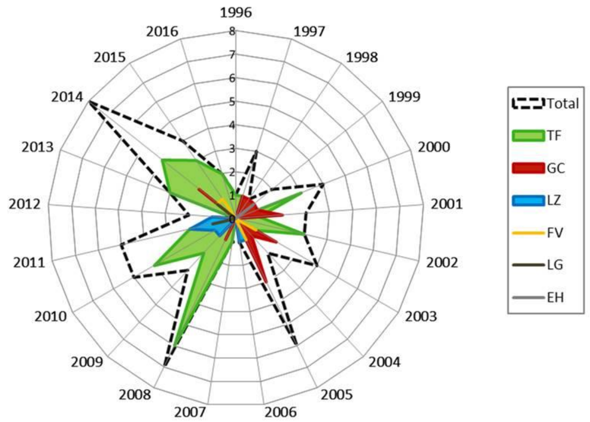



Figure 2. Temporal trend of strandings of BNDs from 1996 to 2016.

Regarding spatial distribution, a geographical profile was found in the density of strandings (see Fig.3), since the majority of animals stranded on TF (39/70; 57%) and GC (16/70; 23%), followed by LZ (7/70; 10%), FV (4/70; 6%), La Gomera (2/70; 3%) and EH island (1/70; 1%). This finding highlights the need to further investigate possible residency patterns (Tobeña 2014).



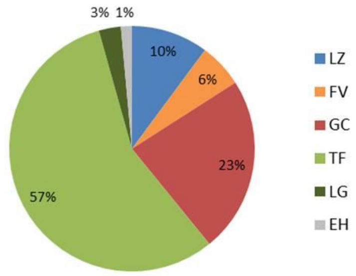




Figure 3. Spatial-temporal distribution (left) and geographical percentage distribution (right) of BNDs stranded on the Canary Islands (1996-2016).

The spider graph on the left shows that the 2005 stranding peak mainly occurred in GC, in contrast with 2008 and 2014 where the dolphins mostly stranded on TF coasts.

Regarding age categories of stranded BNDs, a criterion have been established based on the literature review of this thesis, so some categories have been updated after having published the articles (see sections 8.1 and 8.3). Most of the dolphins stranded from 1996 to 2016 were adults (32/70; 46%) and 33 were included in the sexually immature category ages (11 calves, 5 juveniles and 17 subadults). Among the 11 calves (16%), 9 stranded on TF coast (82%), and the 2 remaining stranded on LG and GC islands. Seven of these animals were necropsied, and 2 of them (CET 584 and CET 592) were included in the published articles of the present thesis (see section 5). It is remarkable to note that 4 of these calves stranded in 2014 on TF coasts. Unfortunately most of the carcasses were at autolytic conservation state.

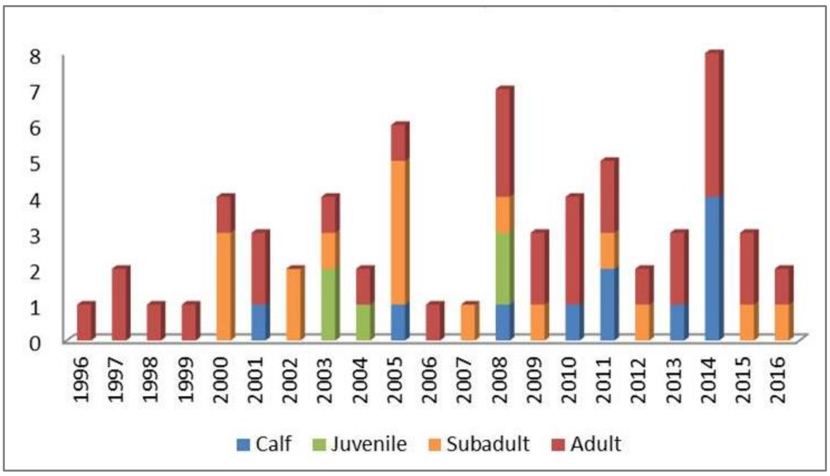



Figure 4. Temporal and age category distribution of BNDs stranded on the Canary Islands (1996-2016).

It is remarkable that all calves and juveniles were found stranded on the Western islands (12 in TF, 3 in GC and 1 in LG). On the Eastern islands of LZ and FV 11 BNDs stranded in the study period (3 subadults and 8 adults). This finding is also mentioned in the publications included in the thesis (see section 5).

## 2.4. Anthropogenic contaminants in the marine environment

The water is a finite and indispensible able resource for life. Yet, for decades, we have used the oceans as dumping grounds for our sewage and chemical waste. Human activities release large amounts of chemical contaminants which will eventually end up in marine environments. This chemistry pollution represents one of the many anthropogenic stressors affecting the survival of MMs, and it has been widely discussed by the scientific community. It is thought that anthropogenic pollution has dramatically changed the chemical composition of the biosphere, and that chemicals have the potential to alter the biodiversity of ocean habitats (Evans, 2011).

In the 1960s the bioaccumulation of these persistent pollutants in tissues of MMs was first demonstrated. Thus, there is a widespread concern over their possible negative impact on their health, which has yet to be fully understood (Fair and Becker, 2000).

The impact of hazardous pollutants may be aggravated by climate change which may alter the dispersion of these compounds causing the release of old contaminants from sediments to the aquatic environment, bringing them back to the water column to upper layers (Hilscherova et al., 2007) and increases coastal erosion with a clear potential to contaminate near waters (EEA, 2015).

The term *environmental contaminants* refers to harmful chemicals which today are distributed throughout the world, found in soil, air and water; such as oceans, deserts and polar areas. They also accumulate in organisms of all trophic levels, from plankton to whales. They have been identified even in human tissues of people inhabiting polar regions, where it seems to have no sources of pollution. These compounds may come directly from human sources such as urban, industrial or agricultural run-off and wastewater discharge (anthropogenic contaminants), or they may be naturally formed, such as toxic substances generated by algae blooms.

The term pollution and contamination are often used interchangeably but really, they have subtle differences. For example, contamination may be used when the content of a natural or synthetic compound in the environment is above the usual level but it is not necessarily harmful. On the contrary, pollution refers to a higher concentration of the chemical which could cause detrimental effects to organisms (Chapman, 2007). Others claim that pollution is generally man-made and contamination occurs naturally (FAO, 1999).

There are also several definitions for environmental contaminant, pollutant or xenobiotics. For instance, the European legislation defines contaminants as: *"substances (i.e. chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bio-accumulate and other substances or groups of substances which give rise to an equivalent level of concern"* (EC, 2016).

Thus, all these definitions may be dynamic and for that reason contaminant, pollutant or xenobiotics are referred without any distinction throughout this thesis.

For the present thesis a wide range of Chemicals grouped by Persistent Organic Pollutants (POPs) and a second group of HMs and/or trace elements has been selected (Table 2). The 57 POPs have been divided into three categories: PCBs, OCPs and PAHs. Although PAHs are not strictly persistent, they are usually considered as semi-persistent pollutants because of their environmental prevalence.

Table 2. POPs and trace elements analysed in the present thesis.

| OCPs | | PAHs | PCBs | | Trace elements |
|---|---|---|---|---|---|
| 23 | | 16 | 18 | | 12 |
| | | | 7 markers | 12 DL | |
| **Cycloalkanes** | HCH alpha | Naphtalene | PCB 28 | PCB 77 | Aluminium (Al) |
| | HCH beta | Acenaphtylene | PCB 52 | PCB 81 | Arsenic (As) |
| | HCH gamma (Lindane) | Acenaphtene | PCB 101 | PCB 105 | Cadmium (Cd) |
| | HCH delta | Fluorene | PCB 118 | PCB 114 | Chromium (Cr) |
| **Cyclodienes** | Heptachlor | Phenanthrene | PCB 138 | PCB 118 | Copper (Cu) |
| | Aldrin | Anthracene | PCB 153 | PCB 123 | Iron (Fe) |
| | Endrin | Fluoranthene | PCB 180 | PCB 126 | Lead (Pb) |
| | Dieldrin | Pyrene | | PCB 156 | Manganese (Mn) |
| | Chlordane Trans | Benzo (a) anthracene | | PCB 157 | Mercury (Hg) |
| | Chlordane Cis | Chrysene | | PCB 167 | Nickel (Ni) |
| | Mirex | Benzo (b) fluranthene | | PCB 169 | Selenium (Se) |
| | Endosulfan alpha | Benzo (k) fluranthene | | PCB 189 | Zinc (Zn) |
| | Endosulfan beta | Benzo (a) pyrene | | | |
| | Endosulfan sulphate | Indeno (1,2,3-c,d) pyrene | | | |
| **Diphenyl-aliphatics** | op-DDE | Dibenz (a,h) anthracene | | | |
| | pp-DDE | Benzo (g,h,i) perylene | | | |
| | op-DDD | | | | |
| | pp-DDD | | | | |
| | op-DDT | | | | |
| | pp-DDT | | | | |
| | Dicofol | | | | |
| | Metoxychlor | | | | |
| **Polychlorinated benzene** | HCB | | | | |

All these chemicals may be released to the environment from anthropogenic activities such as tourism, agriculture, small-scale industries or fishing carried out in the Canary Islands. Natural sources are also important due to the volcanic nature of this archipelago, but this issue remains unexplored.

## 2.4.1. Persistent Organic Pollutants (POPs)

These POPs were not present in the natural environment before the first quarter of the 20th Century (Fair and Becker, 2000). After the Second World War there was a boom in the chemical industry which resulted in a large-scale production of OCs such as PCBs and pesticides. For instance, the dichlorodiphenyltrichloroethane (DDT) was first used as pesticide for military purposes and after widely used in the population to eradicate transmission vectors of malaria, thereby saving millions of people (Paul H. Müller received the Nobel Prize for Medicine in 1948). However, the long-term effects of environmental pollutants were observed two decades later, when these chemicals began to be banned, but unfortunately they still persist in the environment.

These xenobiotics are mainly of anthropogenic origin known as PBTs and also very lipophilic therefore with high ability to cross biological membranes. Thus, these chemicals accumulate in lipid-rich tissue and build up along trophic levels, therefore affecting populations of MMs all over the world with potential immune suppression, reproductive impairment and carcinogenic effects (De Guise et al., 1994; Lahvis et al., 1995; Martineau et al., 1994; Schwacke et al., 2002, 2012; Wells et al., 2005; Yap et al., 2012).

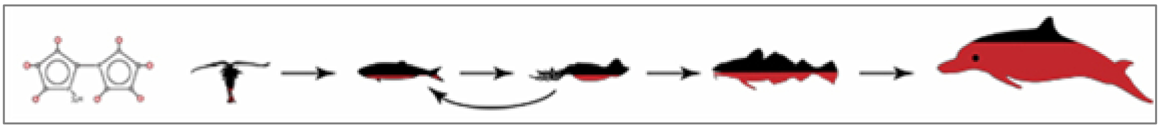


Figure 5. Bioaccumulation of POPs along the marine food chain

These environmental contaminants encompass a large variety of compounds whose main characteristics are the following:

- High stability and persistence. Many compounds are resistant to physical, chemical or biological degradation. They are considered persistent compounds that can remain for years.
- Atmospheric transport and redistribution. They can be spread over large distances from their emission sites through repeated deposition and evaporation, therefore contaminating biota until remote locations.
- Most of the substances are highly lipophilic, thus they accumulate in fatty tissues like marine mammal blubber. This tissue is their main reservoir of POPs but it also may become a source through the mobilisation of POPs into the circulation during periods of energy imbalance; to date, little is known about this issue (Louis et al., 2014).
- Bioaccumulation in organisms with limited capacity to metabolise and eliminate these chemicals.
- Biomagnification as a result of a combination of persistence and resistance to biodegradation, with its high lipophilicity. Pollutants can build up along the food chain, reaching higher concentrations in animals at higher trophic levels. Thus, the concentrations in marine biota were higher than found in terrestrial ecosystems probably due to their longer food chains (Dietz et al., 2000). In addition, it has been reported that pollutant burdens were positively correlated with increasing trophic level but inversely with body size (Borrell, 1993), thus, dolphins accumulate higher concentrations of POPs compared to whales, which may be also due to diet differences.
- Highly toxic for humans and wildlife.

It should be noted that in marine environment, a decreasing gradient of pollutant concentrations from coastal waters to the open ocean is commonly observed, and that these compounds have high affinity for the organic soils and sediment, which can be source and reservoir.

These pollutants are present in the environment as many different congeners and chemical mixtures which may have distinct kinetic behaviours and toxicities (ICES, 2010). Many of them are considered as endocrine disrupters, acting as hormone agonists of antagonists, and they may also impair reproductive functions and cause immunosupresion. It must be highlighted that the exposure to complex mixtures of compounds may also have additive or synergistic effects on the animal health, making it difficult to assess.

Among the organohalogenated compounds, the OC chemicals (PCBs and pesticides) are by far the best investigated (ICES, 2010) together with petroleum aromatic hydrocarbons (PAHs).

## 2.4.1.1. Polychlorinated Biphenyls (PCBs)

PCBs are man-made toxic OC chemicals widely manufactured and use between 1930s and 1980s; they began to be banned in the late 70s but still persist in the environment and accumulate in humans and wildlife.

They are synthetic organic compounds with industrial application for their dielectric characteristic as transformer coolants, insulation products, adhesives, oil additives, paint, sealant plasticizers, thermal paper etc. (Jepson and Law, 2016; Storelli et al., 2007).

The chemical structure consists in a biphenyl with 1 to 10 chlorine atoms.

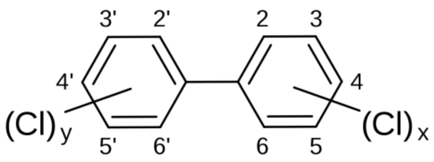



Figure 6. Chemical structure of PCBs.

There are 209 congeners divided into 2 categories depending on the position of chlorines (Duffy et al., 2003):

- Coplanar (DL-PCBs): non-*ortho* or mono-*ortho* substitution. Their two phenyls are in the same plane which provides a rigid structure.
- Noncoplanar (marker-PCBs): chlorine atoms at the *ortho* positions.

PCBs were frequently used as mixture of congeners, known as Aroclors, which are very dangerous to the environment (Safe, 1994).

Despite been banned decades ago, PCBs continue to pose an important toxicological threat to MMs (Gulland and Hall, 2007).

Their toxicity is closely related with their chemical structure; the coplanar PCBs are the most important congeners from an environmental point of view and their similar toxicity to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), known as dioxin-like (DL) compounds.

Non-*ortho* congeners contribute more to the 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) toxic equivalents than mono-*ortho* congeners. Thus, PCB 126 is the major contributor, being the most potent coplanar congener with dioxin-like activities (Storelli and Marcotrigiano, 2003).

The dioxin compounds are not manufacture or used, they are by-products as a result of incomplete combustion of organic matter. There are 210 congeners, 75 PCDD and 135 PCDF, being the TCDD the most toxic one. In fact, a series of toxic equivalency factors (TEFs) have been developed for toxicity assessment of PCBs relative to TCDD, TEQ-PCBs (Corsolini et al., 1995).

## 2.4.1.2. Organochlorine Pesticides (OCPs)

These OC chemicals were widely manufacture in the 40s for application in agriculture as pesticides and for the control of vector transmitted diseases.

There are several groups of OCPs depending on their chemical structure:

- Cycloalkanes: hexachlorocyclohexanes (HCHs). Used as insecticides.
- Polychlorinated benzenes: hexachlorobenzene (HCB). Used as a fungicide, in fireworks etc.
- Cyclodienes: heptachlor, aldrin, endrin, dieldrin, chlordanes, mirex, endosulfanes. They were used in agriculture for pest control.
- Diphenyl-aliphatics: DDTs, dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), Metoxychlor. Used as pesticides for agricultural application and controlling the vectors of CFP transmission.

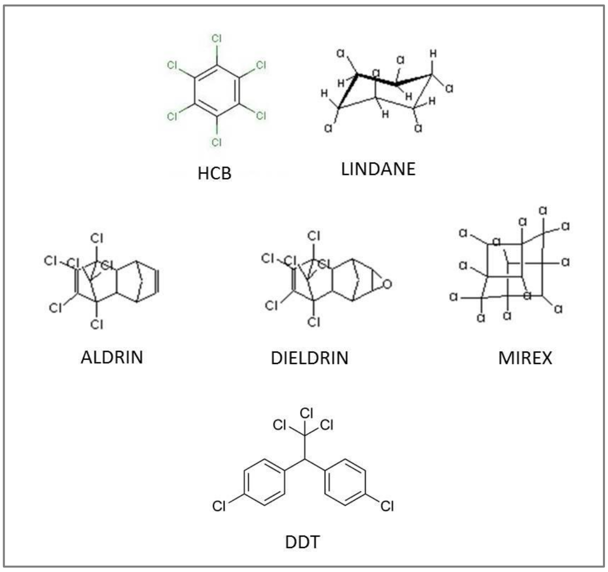



Figure 7. Chemical structure of some OCPs.

DDT ant its metabolites are the most representative compounds of all pesticides. DDTs have been widely used in the past in agriculture and for the control of diseases transmitted by vectors such as malaria and typhus (ATSDR, 2002).

In the early 40's, DDT and dieldrin were associated to great mortalities of vertebrates, and decades later direct adverse effects specially in reproduction were demonstrated in birds (Carson, 1962).

There is no information about contaminants in MMs in the bibliography at that time, but retrospective studies showed that pinnipeds and whales accumulated DDTs in the 50's (Vos et al., 2003). Although all forms of DDTs were banned by the United States Environmental Protection Agency (USEPA) in 1972, DDT is currently found in the blubber of MMs and is one of the most studied POPs worldwide.

Due to the degradation of DDT in the environment and because DDE is more persistent an bioaccumulative, this metabolite is the most frequently observed in MM. Thus, the DDE/tDDT rate has been proposed to estimate the exposure and degradation time of DDT (Aguilar, 1984).

### 2.4.1.3. Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs occur as a result of incomplete combustion of fossil fuels or organic materials during industrial, urban or rural activities. Due to their continuous emission to the environment and because they are ubiquitous for their semi-volatile and hydrophobic characteristics, these compounds are considered as POPs (Shen et al., 2013).

The sources of PAHs can be both natural (forest fires or volcanic activity) or anthropogenic (industrial processes, domestic heating, emission in the transport, waste incinerators, electric generation plants or tobacco). The chemical structure of these hydrocarbons is formed by several benzene rings (Fig. 8).

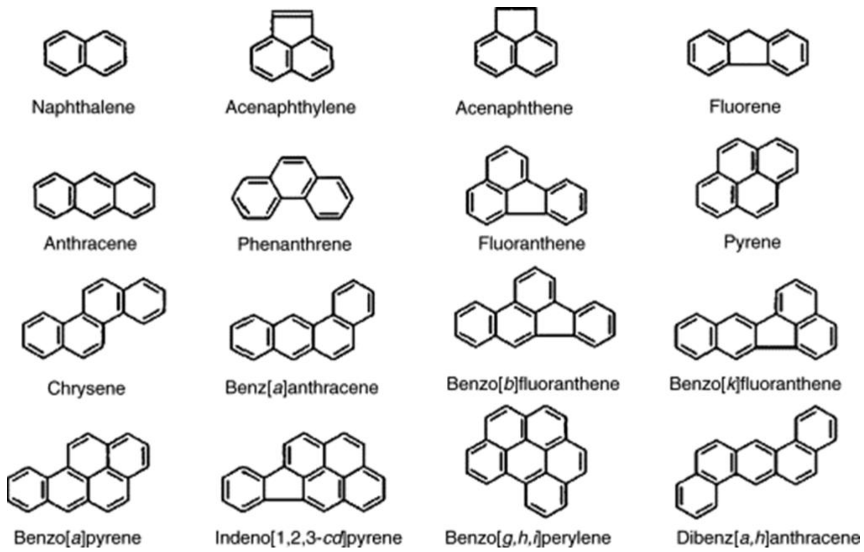



Figure 8. Chemical structure of the 16 PAHs listed by the EPA.

In the late 70s, the USEPA considered 16 PAHs as priority environmental pollutants for their potent bioacumulative and toxic capacity (Fig. 8).

PAHs are recognized as toxic to marine organisms which act as agonist of the aryl hydrocarbon receptor (AhR) (2005, Mastandrea). In MMs the cytochrome P450 (MFO) is responsible for the metabolism of PAHs, and many intermediate products are highly toxic. Additionally, PAHs of higher molecular weight as benzo(a)pyrene or dibenzo(a,h)anthracene are more persistent and considered as potent carcinogens (Martineau et al., 1994).

The Canary Islands has a significant oil-tanker and maritime traffic which resulted in the IMO of 2007 designating the Canary Islands as a PSSA. Despite of this recognition, studies of PAHs in this marine area are scarce.

## 2.4.2. Toxic trace elements

Toxic elements are usually named with the term of “heavy metals”, but in fact not all of them are metals. This term is referred to chemical elements with high density and most of them being toxic at low levels.

To avoid any confusion, they are named as toxic trace elements in the present study. Strictly speaking the 12 elements analysed in this thesis are included in the following chemical groups:

- Transition metals: iron (Fe), mercury (Hg), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), cadmium (Cd) and chromium (Cr).
- Post-transition metals: aluminium (Al) and lead (Pb).
- Metalloids: arsenic (As).
- Non-metals: selenium (Se).

Additionally, these elements can be divided in two groups:

- Essential elements: Se, Fe, Zn, Cu, Mn, Ni, Cr, Al.
- Non-essential elements: Hg, Cd, Pb.

The second group includes the most dangerous elements. Thus Hg can harm the nervous system among others, Cd mainly damages the kidneys and Pb affects mental development of offspring. In addition, many of the vital trace elements can become toxic at high concentrations or cause deficiencies at low levels.

These elements can be released to the environment from natural and anthropogenic sources but metal pollution is most commonly associated to industrial activities and combustion of fuels (Zaccaroni et al., 2011). MMs are exposed to toxic elements through skin, water and mainly via diet.

Toxic elements are also persistent and bioacumulative. Hg appears to be the metal of most concern, actually is related to several dramatic human poisonings (Bakir et al., 1973; Powell, 1991). The anthropogenic sources of this metal can be mostly mining and industrial activities like incinerators and coal plants (UNEP, 2008), and it may occur as elemental, inorganic or organic form, methyl-mercury (MeHg). The inorganic Hg can be transformed to MeHg by microorganisms in the marine environments being this organic Hg considered as the most toxic form which can cross the blood-brain and placental barrier. It is a potent neurotoxic and also harmful for kidneys, lungs, thyroid gland and immune system.

The levels of Hg are positively correlated to Se concentrations for its role in the detoxification of Hg mostly in liver tissue. This mechanism seems to protect the MM against adverse health effects and making them tolerant to certain metal exposures (see third publication of this thesis).

## 2.5. Impact of environmental contamination on cetacean populations

### 2.5.1. Strandings, contaminants and infectious diseases

In the last decades, several species of MMs and birds have been affected by diseases and unusual mortalities, which have raised concerns about the damage to ocean's health (Gulland and Hall, 2007). Although it is known that morbillivirus was the primary cause in most outbreaks, it was also speculated that pollutants played an important role inducing a previous immunosuppression state. Thus, these immunodepressed animals were more vulnerable to infections (De Swart et al., 1994). In fact, the International Whaling Commission (IWC) (Reijnders et al., 1999) and the Marine Mammal Commission (MMC, 1999) concluded that mortality events of MMs with high levels of OCs are increasingly common.

A mass stranding poses the most dramatic response of an animal population to a stress impact (Fair and Becker, 2000). Therefore, when several die-offs occurred in different marine areas worldwide within a short period of time, there was a raised awareness about the toxicity effects of chemicals in the physiology of the individuals (Houde et al., 2005).

The most important mortality events involving MMs worldwide are listed below, showing the year of the event, the species involved and the number of individuals affected, when possible:

- 1987-1988: 14 humpback whales off Cape Cod, Massachusetts, Northeastern coast of the United States (USA) (Geraci, 1989).
- 1987-1988: more than 7000 BND stranded along the central and southern Atlantic coastline of the United States (Geraci, 1989). Significant levels of PCBs in tissues from these dolphins were reported (Houde et al., 2005; Kuehl et al., 1991).
- 1988-1989: 23000 harbour or common seals (*Phoca vitulina*) in the Wadden Sea (Gulland and Hall, 2007; Ludes-Wehrmeister et al., 2016), hundreds of grey seals (*Halichoerus grypus*) (Osterhaus and Vedder, 1988), and thousands of Baikal seals (*Phoca sibirica*) from the Lake Baikal, Russia (Likhoshway Ye et al., 1989). It

represented the first dramatic phocine distemper virus (PDV) epidemic. High concentrations of pollutants were also found in seals from the mass mortality compared to the survivors (Hall et al., 1992).

- 1989: Exxon Valdez oil spill in Prince William Sound, Alaska (Morris and Loughlin, 1994). Hundreds of marine animals were affected. This event, together with the stranding of humpback whales and the BND die-off, led to the development of a legal framework to monitor marine mammal UMEs (Gulland and Hall, 2007).
- 1990-1991: more than 1000 striped dolphins in the Mediterranean Sea. Although Morbillivirus was found to be the primary cause, PCB levels in dead individuals were up to three-fold higher compared to samples from live animals collected before and after the outbreak (Aguilar and Borrell, 1994; Houde et al., 2005).
- 1990-1992: 360 BNDs in the Gulf of Mexico (Kuehl and Haebler, 1995).
- 1996: 150 manatees (*Trichechus manatus*) along southwest Florida. It is thought that was due to a brevetoxin produced by Harmful Algal Bloom (HAB).
- 1997: 200 Mediterranean monk seals (*Monachus schauinslandi*) off northwest Africa.
- 1998: 1600 New Zealand sea lion pups (*Phocarctos hookeri*) in the Auckland Islands, New Zealand. HAB as a possible cause (brevetoxin).
- 1998: more than 400 California sea lions (*Zalophus californianus*) in California. HAB as a possible cause (domoic acid).
- 1983-1998: 246 belugas (*Delphinapterus leucas*) from the Saint Lawrence Estuary, Quebec, Canada, one of the most industrialized areas in the world.
- 1999-2000: 651 gray whales (*Eschrichtius robustus*) in the Eastern North Pacific. Only 3 of stranded animals were examined. Equine encephalitis was detected in one of them and other whale was intoxicated with domoic acid. Due to the small number of the animals tested, no evidence is available for the actual cause of this mass mortality (Gulland and Hall, 2007; Gulland et al., 2005).
- 2000: thousands of caspian seals (*Phoca caspica*) from the Caspian Sea (Kennedy et al., 2000). Although deaths were attributed to a morbillivirus epizootic (Aguilar and Raga, 1993; Kennedy et al., 2000), chemical xenobiotics were suggested to contribute to the development of this outbreak (Houde et al., 2005).
- 2002: 30000 harbour seals in the North Sea (Ludes-Wehrmeister et al., 2016). Another PDV outbreak.
- 2002: 14 beaked whales from the Canary Islands, comprising mainly Cuvier’s beaked whale species. This event was linked to anthropogenic sonar signals (in particular, a mid-frequency naval sonar used in the area) which triggered a "Gas and fat embolic syndrome" found in the animals from this mass stranding (Fernandez et al., 2005; Jepson et al., 2003).
- 2004: 4 Cuvier's beaked whales in the Canary Islands. They stranded after an international naval exercise. The Spanish government imposed a moratorium on naval

exercises in this marine area and there have been no mass beachings since (Fernández, 2013).

- 2007-2008: dozens of dead striped dolphins on Mediterranean beaches. They showed the same symptoms presented in the animals of the epizootic in 1990, but seemed to be a new strain of the morbillivirus (Raga et al., 2008). The virulence of this new outbreak and the levels of OCs observed in the dolphins were much lower. Thus, this mass mortality appeared not to be strengthened by pollutants (Castrillon et al., 2010).
- 2012: 747 dead dolphins in 135 kilometres of northern coast of Peru. Two species were affected, mainly the long-beaked common dolphin *(Delphinus capensis)* (91%) but also the Burmeister's porpoise (*Phocoena spinipinnis*) (9%). According to scientists in charge, dolphins died due to acoustical trauma and decompression syndrome (Alava, 2012). Moreover, the authors highlight the importance of the accumulation of POPs that could lead the dolphins to a vulnerable state to infection, also exacerbated by the lack of food cause by "El Niño" phenomenon and possible HABs.
- 2013: 46 false killer whales in the Strait of Magellan, Chile. The cause of the stranding event remains unknown; however, the coastal morphology could be a possible explanation (Haro et al., 2015).
- 2014: 7 sperm whales in the Adriatic coasts, Mediterranean Sea (southern Italy). Greater levels of Hg and Se in the brain and liver were observed compared to previous strandings (Squadrone et al., 2015).
- 2010-2015: 1441 stranded cetaceans in Northern Gulf of Mexico, affecting mainly BNDs. *Brucella* spp. was found in several individuals but it must be considered that overlapping in time and space with this UME was the Deepwater Horizon (DWH) oil spill (Venn-Watson et al., 2015). The impact with oil can cause chemical burns, irritation, ulcers and internal bleeding. The cetaceans can also breathe the fumes emitted by the floating oil, interfering with the filter feeding of whales and bioaccumulate toxic chemicals that could damage the health of the animals.
- 2013-2015: 1827 BNDs along the Atlantic coastline of USA from New York to Florida. Based upon preliminary diagnostic testing the National Oceanic and Atmospheric Administration (NOAA) experts think that this mortality event may be caused by cetacean morbillivirus (NOAA, 2015a). Besides, *Brucella* spp. bacteria were found in joint, brain and reproductive organ lesions in these dolphins. Additional contributory factors to this mass mortality are under research.
- 2015: aproximately 40 Guadalupe Fur Seals in California. Seven times higher than the anual average. They were mainly calves and juveniles, most of them showed malnutrition, dehydratation and secondary bacterial and parasitic infections.
- 2015: 10 long-finned pilot whales (*Globicephala melas*) on Calais Beach (France).
- 2015: 200 long-finned pilot whales in New Zealand.
- 2015: 337 sei whales in the southern patagonian channels of Chile. The carcasses were observed along 500 km of channels and archipelagos. Two hypotheses have been

suggested: whales reached the bay to feed, suffering a disorientation or due to poisoning by eating contaminated fish by biotoxin from HAB.

- 2015: 23 dolphins stranded in Baja California Sur State, Mexico. Twentyone were rough-toothed dolphins and 2 common dolphins. All of them showed disorientation and weakness. Seven were rescued and released into the sea. Three specimens were necropsied at the University of Baja California Sur (UABCS). Under investigation.
- From 1991 to the present, 62 recognized UMEs have occurred along the coasts of USA, mainly affecting California and Florida states and involving mostly BNDs, California sea lions and manatees.
- 2015-2016: more than 70 large whales stranded in the Gulf of Alaska (fin whales, humpback whales, gray whale and unidentified cetaceans). This data represents 3 times de annual historical average, according to experts. Most carcassess were found floating and only a few specimens were available to analyzed. The causes are unknown, but it is possible that changes in climate and water temperatures contributed to the death of these animals.
- 2016: hundreds of pilot whales have been stranded on the coast of the south of India. Several live sperm whales have stranded around the Wadden Sea. Seven grey whales and one humpback whale stranded on Baja California Sur. Experts point out the phenomenon "El Niño" as a main cause, as it modifies the temperature of ocean currents and could alter the migration of MMs. Climate change contributes to the development of this phenomenon.

Causes of mass die-offs can be multiple and are not fully understood (Reeves et al., 2003); several causes have been attributed to these events such as biological agents (biotoxins from HABs, viruses, bacteria, parasites), changes in oceanographic conditions, complex coastal topography, geomagnetic disturbances, stranding during pursuit of prey or escape from predators and due to human interactions (e.g. anthropogenic pollution, acoustic contamination, oil spills and climate changes) (Gulland and Hall, 2007; Starr et al., 2017). For instance, causes of 32 UMEs occurred in USA have been determined, including infections, biotoxins, human interactions, and malnutrition. UMEs associated with biotoxins have increased dramatically since 1996, particularly involving domoic acid and brevetoxin (Twiner et al., 2012).

Regarding anthropogenic pollutants, high tissue levels of PCBs presented in the dolphins affected by the morbillivirus epizootic in the Mediterranean Sea (Aguilar and Borrell, 1994), higher levels of contaminants in the harbour seal mass mortalities compared to not affected individuals (Hall et al., 1992) and high OC concentrations observed in several BND die-offs (Geraci, 1989; Kuehl and Haebler, 1995), leads to suspect that these chemicals may be linked with immunosuppresion and be responsible for vulnerability to infection. Thus, in the last decades, many researches have focused in the potential role of contaminants in marine mammal outbreaks and mass mortalities (Ross et al., 1995).

In this respect, Aguilar and Borrell (1994) suggested three possible scenarios for the association between high PCB burdens and disease:

1. A weakened immune system leads to increased susceptibility to infections.
2. Increasing of PCBs levels in blood from a mobilization of fat reserves, which may cause a hepatic lesion and a consequently increased of susceptibility to disease.
3. Unspecific lesion in the liver producing both PCB increase and susceptibility.

## 2.5.2. Xenobiotics in marine mammals

More studies have been focused on pinnipeds compared to cetaceans due to logistical, ethical, and legal constraints. In cetaceans, most xenobiotic studies have been conducted on members of the suborder Odontoceti mainly involving the superfamily Delphinoidea, which include the families Delphinidae, Phocoenidae and Monodontidae.

After the 1990-1992 morbillivirus epizootic occurred in striped dolphins of the Mediterranean sea (Domingo et al., 1995), many publications have been published about the influence of pollutants on the development of infectious diseases (Ross, 2002). It has been reported that PCBs were involved in the morbillivirus epizootic in that marine area (Aguilar and Borrell, 1994; Kannan et al., 1993; Storelli et al., 2012; Wafo et al., 2005). Extensive information is also available from other places in Europe (Berrow et al., 2002; Jepson et al., 2005; Law et al., 2012) and worldwide as North America (Balmer et al., 2011; Kucklick et al., 2011; Yordy et al., 2010c), South America (Dorneles et al., 2013; Lailson-Brito et al., 2012), Asia (Moon et al., 2010; Ochiai et al., 2013; Wu et al., 2013), Australia and New Zealand (Stockin et al., 2007; Weijs et al., 2013).

### 2.5.2.1. Factors affecting pollutant levels in cetaceans:

Several factors affect variability of xenobiotic concentrations found in MMs apart from the trophic position; some of these are described as follows:

- Age and body size: pollutant concentrations tend to increase with age in males. Additionally, excretion rate and activity of drug metabolism decrease with age, making old animals more susceptible to contaminant accumulation. On the contrary, a high metabolic rate is linked to high xenobiotic levels which may result in greater residue concentrations in smaller individuals (Reijnders et al., 1999).
- Sex: although before sexual maturity, males and females show similar accumulation patterns, higher levels of pollutants are found in adult males. Females mobilize their reserves in situations of high requirements, releasing chemicals during pregnancy and lactation to the offspring through the placenta and milk. Therefore, animals are exposed to high levels of lipophilic xenobiotics from a critical stage of development (Martineau et al., 1994). After the first calf, females reach a balance between intake and loss of contaminants, thus, the offspring of primiparous females have greater pollutant levels than subsequent calves and are especially vulnerable (Aguilar and

Borrell, 1994; Krahn et al., 2003; Wells et al., 2005). However, the pollutant levels depend on transfer rates and length of lactation (Reijnders et al., 1999).

- Diet: a great variety of pollutant levels is observed in cetaceans depending on species of prey and eating habits of these predators, also providing different patterns of accumulation. Diet may explain inter-specific differences in xenobiotic levels and intra-specific variation among different populations or inter-individual when diet is age or sex-related (Reijnders et al., 1999). Thus, a broader range of contaminant levels is usual to find among studied individuals, possibly due to different degrees of dietary exposure (Hobbs et al., 2003). Thereby, whales typically accumulate lower pollutant concentrations compared to dolphins (Borrell, 1993; Torres et al., 2015).
- Body composition: as most of these xenobiotics are lipophilic pollutants, residue levels will closely depend on the relative mass of blubber of the individual (Reijnders et al., 1999).
- Disease: affects negatively physiological functions, damages nutritive condition and reproductive system, usually resulting in greater residue levels and redistribution of pollutants in tissues of unhealthy animals (Reijnders et al., 1999).
- Nutritive condition: lipid mobilization results in an increase of lipophilic pollutant concentrations in lipid, blood and other tissues. However, when a residue level rises in a tissue, the detoxification process also increases, what makes this factor not as influential as may be expected (Reijnders et al., 1999).
- Geographical distribution: there are differences of pollutant burdens depending on certain marine habitats (Pinzone et al., 2015). Coastal species are particularly exposed to human activities, therefore they may show higher anthropogenic contaminants levels.
- Historical reference: the presence of OCs has varied over the years. These persistent pollutants have decreased mainly in those areas where there is an established regulation. In this regard, an overview of contaminant time trends affirms that trends for those chemicals under regulation regarding their manufacture and use (e.g. OCPs, PBDE and HBCD flame retardants, butyltins) are downwards, however, this earlier downward trend observed for PCBs, has remained flat in many regions (Law, 2014).

All these factors should be taken into account when comparing sample groups or assessing toxicological impact of a population.

### 2.5.2.2. Contaminated marine areas based on studies on marine mammals:

The first studies on contaminants in MMs were published in 1966, particularly related to DDTs in seals from Antarctica and Netherlands (Koeman and Van Genderen, 1966; Sladen et al., 1966), until the present where a large database on chemical residues in MMs exists. However, there are gaps to fill to our knowledge, for instance the majority of the studies are focused in just several species out of dozens pinnipeds and cetacean species and they are concentrated in the northern hemisphere, extremely limited in Africa and most regions of the southern hemisphere (Aguilar et al., 2002).

Studying pollutants in tissues of MMs may provide useful information to determine geographical habitats of these animals, classify populations and for the monitoring environmental contamination (Cardellicchio, 1995).

In general, the most relevant marine areas showed in literature (Aguilar et al., 2002; Esperón, 2005), related to pollutant loads in MMs are indicated in red on the map (Fig. 9).

The review of Aguilar et al., (2002), focused particularly on PCBs and DDT in four species, the BND, the harbour porpoise, the fin whale, and the harbour seal. They observed that fish-eating species from mid-latitudes of Europe and North America showed the highest OC loads, highlighting certain areas on the western coasts of the United States together with the Mediterranean Sea known for its extremely high levels of pollutants due to the highly industrialized regions that surround this semi-enclosed sea. Therefore, these polluted marine areas are highlighted in the map, also in accordance with some other publications which studied other pollutants and marine mammal species:

Mediterranean Sea:

POPs in long-finned pilot whales, sperm whales and fin whales (Pinzone et al., 2015); toxic elements in striped dolphins, BNDs and Risso's dophins from the Adriatic Sea (Bilandzic et al., 2012); OCs in striped dolphins, BNDs, common dolphin and fin whale from the western Ligurian Sea (Fossi et al., 2006); PCBs in striped and BNDs (Storelli et al., 2012; Storelli and Marcotrigiano, 2003), PCBs and DDTs in common BND y long-finned pilot whale (Marsili et al., 2011), PCBs in striped dolphins affected by the 1990-92 Mediterranean epizootic (Aguilar and Borrell, 1994), Hg and Se in striped and BNDs from Tyrrhenian Sea (Leonzio et al., 1992), comparison of Hg in striped dolphins stranded on French Atlantic and Mediterranean coasts (Andre et al., 1991).

Southwestern coast of the Iberian Peninsula:

In a large pan-European meta-analysis of stranded and biopsied cetaceans, three species were found to have PCB levels over toxicity thresholds (striped dolphins, BNDs and killer whales), being both, the Southwestern Iberian Peninsula and western Mediterranean Sea PCB hotspot areas (Jepson et al., 2016).

United Kingdom waters:

An overview that stresses the role of contaminants mainly in harbour porpoises, BNDs and killer whales (Jepson et al., 2016; Law, 2014), where authors postulated that PCBs in cetaceans from Europe waters are among the highest worldwide, probably causing declines of local populations; study of POPs in common dolphins and harbour porpoises from western European seas (Pierce et al., 2008) and PCBs in harbour porpoises (Jepson et al., 2005).

Baltic and North Sea:

There are many researches based on MMs inhabiting both seas well known for their high xenobiotic contamination. Study of association between endocrine disruptors and pathology found in Baltic grey seals (Bergman et al., 2003), Hg in harbour porpoises and white-beaked dolphins (*Lagenorhynchus albirostris*) from the German waters of the North and Baltic Seas (Siebert et al., 1999), organohalogenated in harbour porpoises form the North Sea (Covaci et al., 2002), HMs and POPs in sperm whales stranded on the North Sea (Holsbeek et al., 1999) and harbour seals feeding on environmentally contaminated herring from Baltic Sea (De Swart et al., 1995b) among others.

St. Lawrence River estuary (SLE):

Many studies exist related to the beluga population from the SLE, for instance, OCPs and PCBs in blubber biopsies from belugas (Hobbs et al., 2003); OCs, PAHs and HMs in belugas (Martineau et al., 1994, 2003), metals and OCs in belugas, in vitro assays (De Guise et al., 1996, 1998).

Northeastern Pacific coast:

Hg and Se in harbour seals from Central California (McHuron et al., 2014), PCBs in killer whales from British Columbia and Washington waters (Alava et al., 2012; Hickie et al., 2007), polybrominated diphenyl ether (PBDEs) and PCBs in killer whales (Mongillo et al., 2012), OCs in California sea lions (Ylitalo et al., 2005), HMs and OCs in several species from northern waters (Wagemann and Muir, 1984), PCBs and DDTs mainly in common dolphin, BND and northern elephant seals (*Mirounga angustirostris*) from California coast (Schafer et al., 1984).

Southeastern coast of USA:

PCBs in northwest Atlantic harbor seals (Shaw et al., 2014), health assessment of BNDs in Barataria Bay, Gulf of Mexico, following the Deepwater Horizon Oil Spill (Balmer et al., 2015; Schwacke et al., 2014), trace elements in BNDs from South Carolina coastal area and the Indian River Lagoon, Florida (Schaefer et al., 2015; Stavros et al., 2011), PCBs in BNDs near Georgia coasts (Balmer et al., 2011; Schwacke et al., 2012), legacy POPs and PBDE in BNDs from U.S. Atlantic and Gulf of Mexico coasts (Kucklick et al., 2011), POPs in BNDs from South Carolina and Florida (Fair et al., 2010), PCBs in BNDs from the Western Atlantic and the Gulf of Mexico (Houde et al., 2006), POPs in liver from small cetaceans stranded along Florida coasts (Watanabe et al., 2000).

Marine regions from Asia, such as Bay of Bengal:

DDT, HCB and PCBs in BNDs, spinner dolphins, humpback dolphin (*Sousa chinensis*) indicating lower results compared to data obtained in cetaceans from the Sea of Japan or China Sea (Karuppiah et al., 2005). China Sea: OCs, mainly DDTs in Indo-Pacific humpback dolphins (*Sousa chinensis*) from the Pearl River Estuary, China (Wu et al., 2013); PBDEs and OCs in finless porpoises (*Neophocaena phocaenoides*) from the South China Sea, which mainly showed high levels of DDTs (Ramu et al., 2006); OCs and HM in BNDs (Parsons and Chan, 2001); role of OCs in Indo-Pacific humpback dolphins (Jefferson et al., 2006); HM in Indo-Pacific Humpback Dolphin and the Finless Porpoise in Hong Kong waters (Hung et al., 2007). Japan coasts: OH-PCBs in three porpoises species, finless porpoises, harbor porpoises and Dall's porpoises (*Phocoenoides dalli*) from Japanese coasts (Ochiai et al., 2013); trace elements in harbor porpoises from north Japan (Yasuda et al., 2012); PBDE and OCs in harbor porpoise, pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Dall's porpoise and Stejneger's beaked (*Mesoplodon stejnegeri*) (Kajiwara et al., 2006). Highest concentrations were found in animals from Hong Kong waters (China Sea), followed by Japan, and much lower levels from the Philippines and India (Kajiwara et al., 2006; Wu et al., 2013).

Finally, South of Australia (Indian Ocean):

Is an important area for Hg and Se contamination according to the results showed by Lavery et al. (2008). They studied metal and selenium concentrations in common dolphins (*Delphinus delphin*), common BNDs and Indo-Pacific BNDs (*Tursiops aduncus*) from South Australia, being *T. aduncus* the species with the greatest tissue burdens.

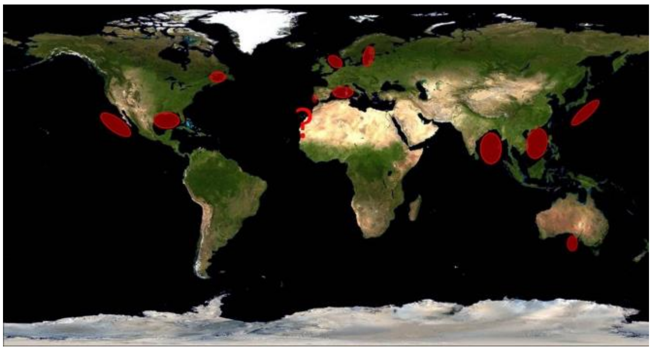


Figure 9. World map with the most contaminated marine areas based on studies on MMs (in red).

Overlapping a map of global electricity consumption (IEA, 2012), it can be noted that these hot spot areas are near highly industrialized locations (Fig. 10).

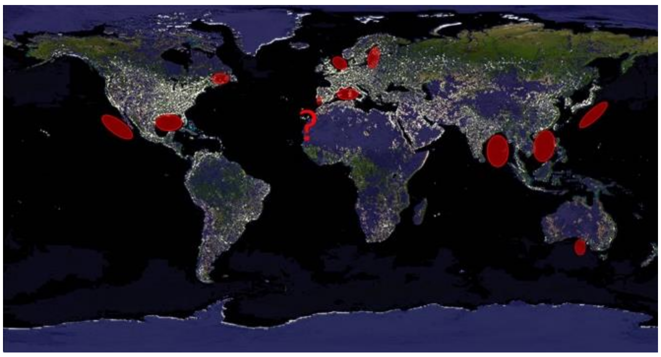


Figure 10. Map of global electricity consumption overlaying figure 9.

Although cetaceans from the industrialized regions of the Northern Hemisphere exhibit the highest levels of environmental contaminants worldwide, these xenobiotics have also been found in animals inhabiting the Southern Hemisphere (Alonso et al., 2017), even in remote areas such as the Arctic (Dietz et al., 2015; Fisk et al., 2005), reflecting the global affection of contaminants in the marine ecosystem (Houde et al., 2005).

After regulation of manufacture and release in the 1970s and 1980s of anthropogenic pollutants, a downward trend was observed in regions where the policy was implemented (Law, 2014), however, in some other areas this trend has remained flat at important levels of concern for some top predator species (Jepson et al., 2016; Law, 2014; Law et al., 2010).

### 2.5.2.3. Types of toxicological studies in marine mammals:

Down below, different types of toxicological studies of exposition to pollutants and its effects in MMs are described, mostly based on a published review (Weijs and Zaccaroni, 2015):

1- DESCRIPTIVE OR EPIDEMIOLOGICAL STUDIES:

They are related to findings observed in free populations of marine mammal from contaminated areas, such as the SLE belugas, California sea lions, striped dolphins from the Mediterranean Sea, the BND from the US Atlantic coast and Gulf of Mexico and the grey seals from the Baltic sea and UK waters. These populations are known for their high burdens of environmental pollutants, suggested to be associated with negative health effects (Baker, 1989; Borrell et al., 1996; De Guise et al., 1994; Hobbs et al., 2003; Jenssen, 1996; Kuehl and Haebler, 1995; Kuehl et al., 1991; Leonzio et al., 1992; Olsson et al., 1994; Ylitalo et al., 2005). There are also other epidemiological studies which use large samples sizes, such as several researches made on stranded harbour porpoises from UK, where PCBs and HM were related to infectious diseases (Bennett et al., 2001; Hall et al., 2006a; Jepson et al., 2005). In a large pan-European study on around 1000 stranded and biopsied cetaceans, the authors suggested that the high PCBs observed may have caused the population decrease (Jepson et al., 2016).

2- “IN VIVO STUDIES”:

Most monitoring studies are focused on stranded dead animals, however, despite the very valuable samples provided by these individuals, sometimes they are perceived as a skewed study regarding the status of their population. Thus, the scientific community attempted to use in vivo approaches.

Nevertheless, these studies are not very common due to legislation and protective measures around marine mammal’s research. However, several experimental studies have been carried out in the past mostly with animals in captivity (de Swart et al., 1996a, b; Reijnders, 1986). In these experiments, seals of different species were fed on contaminated fish. Impairment of the immune system (Ross et al., 1996) and reproductive system (Reijnders, 1986) of harbor or common seals were observed and also in the sensory system in the cochlea of harp seals (*Pagophilus groenlandicus*) (Ramprashad and Ronald, 1977). These results suggested that xenobiotics were associated with adverse effects in the animal health.

Experiments with animals in captivity are currently deprecated; thus, the term in vivo is used to describe sampling techniques for live specimens, such as biopsies or blood both from animals in captivity and in the wild, such as methods of capture and release. In fact, nowadays there is a tendency to use less invasive methods of sampling, mainly using skin, hair, stool, urine, biopsies and blood (Hunt et al., 2013).

The most common samples are the skin, blubber biopsies and blood, where pollutants and several health parameters, such as vitamin A and hormones, can be measured (Foltz et al., 2014; Hall et al., 2003); additionally, these tissues are mainly used in ex vivo or in vitro experiments (Lunardi et al., 2016).

In addition, in vivo studies would also include experiments with laboratory animals like rodents, ducks, guinea pigs etc. For instance, Friend and Trainer (Friend and Trainer, 1970) get mallard ducks into two groups, one of them exposed to PCBs. Days later they challenged them all with hepatitis virus, finding that animals previously exposed to PCBs suffered higher mortality than the not exposed group. Other studies found that mice exposed to PCB were more sensitive to malaria and bacterial infection (Loose et al., 1978; Thomas and Hinsdill, 1978), and that a study in rats fed herring from the contaminated Baltic Sea resulted in pollutant-induced immunosuppression also observed in harbour seals (Ross et al., 1996). These observations support the scientists stating that environmental contaminants impair host resistance.

3- "IN VITRO":

It refers to the assessment of toxicological effects without the use of live animals.

Most in vitro studies use cells derived from biopsies, blood and tissues from very fresh carcasses, to be exposed to individual pollutants or mixtures for further evaluation of possible effects. They may not be a perfect system due to the absence of multiorgan effect and kinetic processes. However, it is possible to study toxic effects at a cellular and molecular level, and differentiate the response in different species (Weijs and Zaccaroni, 2015).

In the case of samples from animals in captivity, the species typically used are BND, belugas, and seals; on the other hand, the chemicals most frequently analysed are organohalogen compounds such as PCBs, OCP, and brominated, and HMs.

Regarding wild animals, seals are the most commonly used to sample collection for in vitro studies because the ease way of capture (Das et al., 2008; Dupont et al., 2013).

Recently, in vitro studies are developing bioassays using engineered cells lines (Wu et al., 2013; Yordy et al., 2010a). As these bioassays are based on cell lines derived from other species such as rodents or humans, it could be argued that the observed responses are not necessarily similar to the effect in MMs. However, the routes of toxicity shown by bioassays from different species are often highly conserved (Weijs and Zaccaroni, 2015).

Other cell based systems for toxicological application are known as bacterial bioreporters (bacterial stress-gene profiling assay) which includes a stress sensitive promoter with a detectable reporter. After toxic exposure, this system is activated and the reporter gene is produced (Dardenne et al., 2007), indicative of protein disturbance, DNA and membrane integrity (Krivoshiev et al., 2015).

In vitro studies have been conducted mainly in hepatic cells, and more recently in fibroblasts and integumentary cells obtained from skin biopsies, for the study of biomarkers (Fossi et al., 2006; Marsili et al., 2000; Wilson et al., 2007), as described later in this thesis.

Examples of in vitro studies:

- Study of the effect of HMs on steroid production in grey seals (Freeman and Sangalang, 1977). They noted that low concentrations of Se and As alter the synthesis of steroid hormones, with a consequent gonadal function impairment.
- Study of enzyme induction and immunotoxicity by POPs. Levin 2007 used blood collected from free and captive sea otters (*Enhydra lutris*). The authors reported a difference of in vitro response to different mixtures of OCs between free and captive otters, free animals being the most sensitive (Levin et al., 2007).
- Ex vivo assay carried out with skin from BND cultured and treated with perfluorooctanoic acid (PFOA) and bisphenol A (BPA) to analyze gene expression. The skin transcriptome provide information on the contaminant exposure, affecting genes involved in immunity modulation, response to stress, lipid homeostasis, and development (Lunardi et al., 2016).
- In vitro assay with BND peripheral blood immune cells exposed to increasing concentrations of brevetoxins to measure effects on innate and adaptive immune functions (Gebhard et al., 2015). Phagocytosis was not affected by the brevetoxin, but neutrophil and monocyte respiratory burst increased at the highest levels of this natural toxin. These levels were within the range observed in the dolphins during the UMEs, and such immunomodulation may cause disease susceptibility.

4- “IN SILICO”

It is a computer modelling which helps the interpretation of different biomonitoring data providing a more comprehensive approach. These models depend entirely on the availability of data such as the concentration of pollutants and several biological parameters. The limiting factor is the need for greater scientific knowledge about kinetic processes, which is complicated in MMs for the lack of data. However, some studies exist about cetaceans and pinnipeds (Weijs et al., 2014) and polar bears (Dietz et al., 2015; Pavlova et al., 2014; Sonne, 2010). So far, models have been designed for POPs but not for HMs (Weijs and Zaccaroni, 2015).

## 2.5.3. Adverse health effects of pollutants on marine mammals:

POPs persist in the environment and pose a risk of adverse health effects in wildlife, first described in the early 60s (Carson, 1962). At present, POPs are linked to a wide range of adverse health effects but causal evidence for individual contaminants in wildlife is difficult to establish (Krahn et al., 2003; Vos et al., 2000) due to the lack of population data, exposure to an unknown mixture of compounds, the role of pathogens, environmental factors and nonexistent biomarkers. In addition, there is a weak knowledge about the functioning of reproductive, immune and endocrine systems in these species (Burek et al., 2008).

However, accumulating evidence indicates that xenobiotics affect physiological mechanisms which may impact marine mammal populations (Ross et al., 2000; Van Bressem et al., 2009). The combination of epidemiological and descriptive studies of effects observed in free-ranging populations inhabiting contaminated marine areas, laboratory studies where rodent are used for chemical exposures and researches with semi-field or captive animals fed on contaminated fish, have associated levels of environmental contaminants with reproductive impairment, immunosuppression and development alteration among others (Beckmen et al., 1999; De Guise et al., 1996; Houde et al., 2005; Krahn et al., 2003; Reijnders, 1986).

The toxic effect of a cocktail of xenobiotics is very complex as it depends on the toxicity profile of the chemicals, possible synergetic or antagonistic effects, bioavailability and persistence in animal tissues and how the marine mammal metabolizes these compounds (EC, 2017). In addition, sensitivity to such compounds varies by species, sex and age and also by the individual health status which is also affected by a multitude of environmental stressors (Bossart, 2007; Fair and Becker, 2000). In view of these limitations, many investigations have been conducted in attempt to fill these knowledge gaps.

As suggested by several studies shown before, many of the environmental chemicals can negatively affect the immune, endocrine, and nervous system function that may result in damage to growth, reproduction, development and resistance to disease (Fair and Becker, 2000). Among OC compounds, PCBs were found to be the main contributor for reproductive impairment, immunotoxicity and carcinogenicity in polar bears from the Arctic (Dietz et al., 2015).

### 2.5.3.1. Immunotoxicity

As commented before in this thesis, many catastrophic viral epidemics that affected populations of seals (Osterhaus and Vedder, 1988; Vos et al., 2003), porpoises (Kennedy et al., 1991; Vos et al., 2003) and dolphins (Domingo et al., 1990, 1992), where highly contaminated by anthropogenic pollutants. It is well known that the direct cause of death was the virus, but additionally to the immunosuppression caused by the morbillivirus, a susceptibility to viral infections as a result of a chronically exposition to PCBs was suggested (Lahvis et al., 1995).

The increased susceptibility to viral infections of laboratory animals after exposure to PCBs (Friend and Trainer, 1970; Koller, 1977; Thomas and Hinsdill, 1978), led to speculation about the association between environmental pollution and immunosuppression (Houde et al., 2005). Furthermore, a recently published review states that contaminant exposure is consistently depressing the immune function in MMs (Desforges et al., 2016).

The contaminants could affect both the cell viability (cytotoxicity) and cell function (immunotoxicity). There are many effects such as decreased viability, phagocytosis and lymphocyte proliferation and increased apoptosis and necrosis (Camara Pellisso et al., 2008; Dupont et al., 2016). The main studies to assess the toxic effects on immune system of MMs are focused on immune tissue histopathology, hematology, lymphocyte proliferation, phagocytosis, natural killer cell activity, immunoglobulin and cytokine production (Desforges et al., 2016).

Desforges et al. (2016) summarized contaminant-mediated effects on the immune system of MMs. The authors revealed that pollutants modulate both innate and adaptive immunity, including cellular and humoral responses, also supported by other authors (Gebhard et al., 2015). Although data are lacking related to many immune aspects, the authors suggest that these effects on the immune system may also negatively affect reproductive and endocrine systems, resulting on decreased fitness and population growth.
Examples of studies on immune effects in MMs:

- Anthropogenic pollutants may compromise the immune system leading to epidermal lesions in cetaceans (Beland et al., 1993) and facilitating the opportunistic bacteria and fungi to invade wounded tissue (De Swart et al., 1994).
- Jepson et al. (2016) suggested that high levels of blubber PCB found in several cetaceans from a European meta-analysis are likely to contribute to population declines due to reproductive impairment and immunotoxicity.
- A research investigated the immune effects of PCBs and TCDD in MMs and mice. They found that B lymphocyte proliferation was mainly induced by non-coplanar PCBs (Mori et al., 2008).
- Peripheral blood was sampled from free-ranging BNDs along the west coast of Florida. An in vitro study found that reduced lymphocyte responses were associated with greater levels of PCBs and DDT in blood. The authors affirm that this immune dysfunction may induce opportunistic infections (Lahvis et al., 1995).
- A study described an association between perfluoroalkyl compounds (PFCs) and health immune parameters in BNDs in USA Atlantic waters sampled with capture-released method (Fair et al., 2013).
- Other study attempted to correlate brucellosis with PCB exposure in BNDs from the UK; they enhanced the necessity of further studies to determine if this relationship is causal or not (Davison et al., 2011).
- Many studies on striped dolphins affected by the morbillivirus epizootic state that the high burdens of PCBs found in these animals induce a state of immunosuppression (Aguilar and Borrell, 1994; Kannan et al., 1993).
- In vitro assessment with metal exposition of beluga cells showed greater cell mortality in Con-A-stimulated thymocytes cultured with Hg, with no viability effect on

splenocytes. They also observed a reduction in the splenocyte and thymocyte proliferation with high levels of both Hg and Cd, not so with Pb (De Guise et al., 1996).

- In vitro exposure of beluga leukocytes and splenocytes to different mixtures of PCBs and DDT metabolites were assessed on phagocytosis and cell proliferation. They found a variety of effects depending on the mixture profile, but in general the decreased cell proliferation observed support the assumption that pollutants are potential immunosuppressants (De Guise et al., 1998).
- In contrast, rats were fed blubber of belugas from the contaminated St. Lawrence River estuary (SLE), Canada, and from the unpolluted Arctic area. They didn't found any immune effect, being inconsistent with many studies which relate OC with immunosuppression. They attribute this finding to possible antagonistic effects in mixtures or the short period of the experiment (Lapierre et al., 1999).
- Harbor porpoises from the German North and Baltic Sea showed a correlation between thymic atrophy or splenic depletion with increasing levels of PCBs and PBDEs. Thus the authors support that pollutants induced susceptibility of porpoises to disease (Beineke et al., 2005).
- Study on harbor porpoises of UK waters showed a significantly greater level of PCBs in the infectious disease group than the physical trauma group, in porpoises with PCB burdens over a proposed threshold for toxic effects (17 ppm). These results were consistent with a direct association of PCB exposure with disease (Jepson et al., 2005).
- Immunosuppression in harbor porpoises from UK waters when PCB exceeds toxicity thresholds. In contrast, thymic cysts were found to be age-related (Yap et al., 2012).
- Hg was associated with the severity of lesions and nutritional state of porpoises and white beaked dolphins (*Lagenorhynchus albirostris*) from German waters. Hg was also linked to parasitic infestation and other diseases (Siebert et al., 1999).
- Hepatic concentrations of Hg, Se and Zn were higher in porpoises with infectious diseases compared to presumably healthy individuals. In contrast, Pb, Cd, Cu and Cr were found at similar levels in both study groups (Bennett et al., 2001).
- Semi-field study on two groups of harbor seals fed fish from two different marine areas, the polluted Baltic Sea and the relatively unpolluted Atlantic Ocean. They found significantly lower natural killer-cell (NK) activity and T-cell (LT) responses and higher levels of circulating polymorphonuclear granulocytes in seals fed on herring from the Baltic Sea. Particularly, PCB and HCB reduced NK activity, and LT proliferation was decreased by PCB, PCDD, PCDF, Dieldrin and b-HCH. This data provided evidence of functional impairment of innate and the adaptive immune system of harbor seal after chronic exposure to environmental pollutants (De Swart et al., 1994, 1996a; Ross et al., 1995). These results also suggested that immunosuppression induced by pollutants may have contributed to the increase of bacterial infections and the severity of morbillivirus outbreaks (de Swart et al., 1995a).
- A study on harbor seals from the North Sea found immunotoxicity (compromise of lymphocyte activity, proliferation and cell survival) after in vitro exposure to MeHg of

peripheral blood mononuclear cells from free-ranging individuals. They enhanced that MeHg should be considered as an important contributor to the immunosuppression induced by environmental pollutants (Das et al., 2008).

- The results of a study on biomarkers to assess the impact of environmental stressors on harbor seals from the North Sea showed a significant correlation between interleukin-2, xenobiotic markers and trace elements in fur samples, suggesting an immune modulation by metal exposure. This relationship found may mean that immunosuppression occurs after exposure increasing the susceptibility to inflammatory disease (Lehnert et al., 2016).
- Although it appears that PCBs and DDTs did not contribute significantly to CDV mortality of Caspian seals epizootic, the authors maintain that pollutants may contribute in the immune dysfunction of the animals being the AhR mediated chemicals, PCBs, PCDDs and PCDFs, the most immunotoxic compounds (Wilson et al., 2014).
- High OC levels found in polar bear were associated with impairment of both humoral and cell-mediated immune responses (Fisk et al., 2005).
- A study in free-ranging northern fur seal pups (*Callorhinus ursinus*) observed a reduced lymphoproliferation with the increasing level of PCBs in blood. First born pups showed greater OC burdens and less immune response to vaccination which may indicate higher risk than pups of multiparous fur seals (Beckmen et al., 2003).
- Rats were fed oil extracted from fish coming from highly polluted Baltic Sea and less contaminated Atlantic Ocean. They observed a detrimental effect in the cellular immune response in rats exposed to Baltic Sea herring oil or TCDD (Ross et al., 1997).
- Thymus atrophy and immunosuppression was observed in laboratory animals after TCDD exposure due to the apoptosis induced by this pollutant (Chopra and Schrenk, 2011).

Therefore, in view of these assessments and despite all evidence, further researches are needed to ascertain if immunosuppression is caused by pollutant exposure or secondary to disease (Beineke et al., 2005).

### 2.5.3.2. Endocrine disruption

"Endocrine disruption" is a potential mechanism of action of many contaminants (McLachlan, 2001). There are several ways to define this term, three of them shown below:

- Definition by The Veterinary Toxicology book (Gupta, 2012): "endocrine disruption refers to the effects of any synthetic or naturally occurring xenobiotic which can affect the endocrine system of exposed individuals and, as a result of exposure, cause physiological alterations". They indicate that reproduction is one of the physiological functions most affected by these endocrine disrupters but also “nonreproductive” endocrine systems can also be affected by this chemical exposure.

- Definition by the National Institute of Environmental Health Sciences (NIH, 2017): “endocrine disruptors are chemicals that may interfere with the body’s endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife”
- Definition by The International Programme for Chemical Safety (WHO, 2017): “an endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations”.

Endocrine disrupters may interact with the endocrine system in three possible ways (Fair and Becker, 2000):

- by mimicking the action of a natural hormone, thus getting similar chemical reactions;
- by blocking the hormone receptors in cells, thereby inhibiting the action of normal hormones;
- by interfering in the synthesis, transport, metabolism and excretion of hormones, what alters the level of these hormones.

These interactions may result in negative effects such as (developmental malformations, interference with reproduction, increased cancer risk and harmful disorders in the immune and nervous system function (EPA, 2017). Many reproductive effects were first documented decades ago in humans after contaminated rice oil consumption in Japan (Kuratsune et al., 1972) and minks fed fish from the Great Lakes (Heaton et al., 1995). Also, masculinization (imposex) of marine snails was observed due to tributyltin exposure, egg-shell thinning of raptor birds species by DDE and distort of sex development in alligators caused by a pesticide spill into a lake in Florida, USA (Vos et al., 2000).

Thus, endocrine disruption is a potential global problem since a wide range of compounds can be considered as endocrine disruptors, including man-made chemicals (PCBs, dieldrin, aldrin, DDTs, endosulfan, methoxychlor, toxaphene, HCB and plastic additives) and also by-products of industrial processes such as dioxins, many of them found in tissues of MMs (Borrell et al., 1996; Jepson and Law, 2016).

Examples of studies on endocrine disruptions in MMs:

Endocrine and reproductive disrupting effects are closely related. In fact, Murphy et al. (2015) suggest that reproductive disorders due to PCB exposure can occur through either endocrine disrupting or disease due to immunosuppression. Endocrine disruption is also largely linked to thyroid system (Balmer et al., 2015; Techer et al., 2016), whose impact is essential in foetuses and newborns for a correct neural development. Functional and morphological alterations have been found in thyroid gland and adrenal cortex in many species after exposure to OC compounds, including seals and belugas (De Guise et al., 1995).

Reproductive dysfunction has been observed in laboratory animals after exposure to OCs (Safe, 1994) and in wildlife populations inhabiting the aquatic environment, where disturbances vary from slight to permanent alterations, such as sex differentiation, disorder in sexual behavior, and immune function impairment (Vos et al., 2000).

A reproductive impairment associated to OC compounds have been observed in MMs in several studies, involving species such as grey and ringed seals (Olsson et al., 1994), belugas in SLE (Martineau et al., 1994), California sea lions where high levels of OCs where associated with premature pup birth (DeLong et al., 1973) and common seal in western Wadden sea where PCB levels where related to decrease in the reproductive success (Reijnders, 1980, 1986). PCB and DDT were linked to the loss of fecundity and abnormalities of female sexual organs found in Baltic seals (Helle et al., 1976). Ridgway and Reddy stated that high levels of OC residues in milk of BND are correlated with reduced calf survival (Reddy and Ridgway, 2003) and other studies suggest that PCB and petroleum exposure cause low reproductive success rates in BND (Hall et al., 2006b; Kellar et al., 2017; Schwacke et al., 2002). In addition, the risk of detrimental reproductive effects due to PCB exposure where studied in females from three populations of BND (Schwacke et al., 2002). They found a high likelihood of reproductive impairment (stillbirth or neonatal mortality) in primiparous females with chronic exposure to PCBs. Jepson et al. (2016) associated some declining populations of BNDs and killer whales from the NE Atlantic with reproductive toxicity induced by PCBs.

In particular, many lesions were common to find in post mortem examination of grey and ringed seals from the highly polluted Baltic Sea (Helle et al., 1976; Olsson et al., 1994): stenosis and occlusions of the uterus and uterine leiomyomas, injuries of claws and skull bone, intestinal ulcers and arteriosclerosis. However, they highlighted the usual finding of adrenal changes such as adrenocortical hyperplasia and adenomas, stating that this hyperadrenocorticism or Cushing's syndrome could explain the existence of most of the rest of lesions (Bergman et al., 2003). In addition, it has been also reported thymic atrophy, splenic depletion, osteoporosis and decreased epidermal thickness (Das et al., 2006).

Regarding hormonal alterations, OH-PCBs show different actions, including T4-binding competition on transthyretin (TTR); inhibition of deiodinase (implicated in activation or deactivation of thyroid hormones), oestrogen receptor (ER) binding and AhR binding. The high levels of OH-PCBs found in foetus and neonatal individuals may be relevant in developmental neurotoxic and reproductive effects observed in laboratory animals. Additionally, Das et al. (2006) hypothesized an association between PCBs, PBDE, DDE and DDT chemicals with thyroid fibrosis in harbor porpoises. Furthermore, there is also a synergistic effect on the decrease of thyroid hormone and vitamin A caused by hepatic elimination enhanced by complex mixture exposures and the interaction of these compounds with the serum carrier for retinol (Fisk et al., 2005; Simms and Ross, 2000). As an example, a study on seals observed depletion in plasma retinol and thyroid hormones likelihood induced by PCB intakes (Reijnders et al., 1999). Thus, Desforges et al. (2013) identified PCBs as disruptors of concentration and profiles of vitamin A and E in beluga tissues from the Arctic region. They found that moderate levels of these chemicals may disrupt vitamin profiles, still having a global impact long after the ban of PCBs.

Regarding trace elements, blood Hg and Se concentrations in free-ranging BND has been observed to be negatively associated to total thyroxine and total number of leukocytes (Schaefer et al., 2011). Other study with laboratory rats showed that Pb and Cd produced testicular toxicity and impaired fertility after exposure (Pandya et al., 2012).

Concerning adrenal toxicology, OCs are thought to impact the adrenocortical metabolism in seals leading to pathological findings (Bergman and Olsson, 1985). Accordingly, PAHs may also affect adrenal function, particularly benzo(a)anthracene produced apoptosis and necrosis in the zona reticularis and fasciculate in rats after exposure (Fu et al., 2005). Although the functional adrenocortical suppression has been suggested as the most important toxicological effect by pollutant exposure, such as the hypoadrenocorticism observed in dolphins affected by the Deepwater Horizon (DWH) oil spill (Schwacke et al., 2014; Venn-Watson et al., 2015), the role that chronic exposures may play to adrenal function still remains unknown (Harvey et al., 2007).

As a summary, Balmer et al. (2015) stated in their review that POPs and petroleum constituents may be toxic through AhR pathways (immune suppression and reproductive impairment). Additionally, endocrine disruption can be observed, but POPs are more usually associated with the interaction with reproductive and thyroid hormone receptors and carrier proteins, while petroleum chemicals prefer the hypothalamic–pituitary–adrenal (HPA) axis as their target. After the study of the populations of BND from Gulf of Mexico that were affected by the DWH oil spill, the authors disclosed that dolphin mortalities have been linked with the DWH oil spill but as a result of their study, they asserted that POPs were likely not a primary cause of the poor health conditions observed in these populations following the DWH disaster. In addition, OCs are known to mediate their effects through AhR, particularly exerted on developing brain and lymphoid organs of young animals and on adrenal glands of adult MMs (Martineau, 2007).

### 2.5.3.3. Carcinogenic effects

In 1996, the USEPA declared PCBs to be probable human carcinogens (EPA, 1996) and an evaluation made by the International Agency for Research on Cancer (IARC) in 1998 agreed with that conclusion and also concluded that TCDD is carcinogenic to humans (McGregor et al., 1998). Laboratory animal studies have provided sufficient evidence of carcinogenicity, for instance liver adenomas or carcinomas were found in rats after exposure to different Aroclor mixtures (Brunner et al., 1996; Mayes et al., 1998) and to TCDD (Chopra and Schrenk, 2011). Case-control reports in humans also observed associations between breast cancer risk and PCB 118 and 156 (Demers et al., 2002), breast cancer and OC mixtures (Rivero et al., 2015) and between PCBs and lymphomas (Hardell et al., 2001). EPA and IARC also assigned DDT, DDE, and DDD as probable human carcinogens based on increased incidence of liver tumors in laboratory animals (Cabral et al., 1982). Additionally exposure to As present en drinking water may lead to develop skin cancer and occupational exposure by inhalation is related to lung cancer (Jarup, 2003).

Regarding MMs, it is essential to stress the case of a unique population of cetaceans which inhabits the SLE in Canada, one of the most industrialized regions of the world. The southernmost population of beluga inhabits this waters isolated from other groups with Arctic habits. The St. Lawrence population has suffered a dramatic decline from about 5000 to 600-700 individuals mainly due to past hunting until 1979, with no evidence of recovery. For that reason, this population is been investigated since 1982 to attempt to assess its health status. It has been observed that these animals are heavily contaminated by industrial and agricultural chemicals, such as PCBs, DDTs, PAHs and also with toxic elements like Hg and Pb (Martineau et al., 1994). Out of this long term research, the main causes of death were found to be respiratory and gastrointestinal infections with parasites, cancer and other infections produced by bacteria, virus and protozoa. Cancer was observed in 27% of adult animals being the population with the highest annual rate of cancer reported among all marine mammal populations worldwide (Martineau et al., 2003).

Thus, St. Lawrence belugas may represent the risk linked to chronic exposure to environmental pollutants usually compared to less contaminated Arctic belugas (De Guise et al., 1995).

Excessive accumulation of these compounds may predispose an animal to immunodeficiency states which will increase susceptibility to infectious agents and tumor development (Exon et al., 1985). In fact, exposure to environmental pollutants can cause oxidative stress increasing the susceptibility to diseases, such as cancer (Asimakopoulos et al., 2016; Bagchi et al., 1993; Krivoshiev et al., 2015). Particularly benzo(a)pyrene is known as one of the most carcinogenic chemical which may be responsible for the cancer found in SLE beluga population (Martineau et al., 1994).

Thus, PAHs exposure, possible combined with viral infections, was suggested to be related to the high rate of cancer of proximal intestine, due to the following findings (Martineau et al., 2003):

- Experimental studies with laboratory rats found that chronic ingestion of coal tar was related to intestinal cancer.
- Presence of PAHs in the SLE sediments.
- Belugas significantly fed on benthic invertebrates.
- Benzo(a)pyrene adducts with DNA were detected in tissues from SLE belugas, not found in Arctic belugas.

Wild California sea lions have a great load of contaminants together with an outstanding high prevalence of cancer (18% of stranded dead adults). A studied reported that animals with carcinoma had higher levels of PCBs (Ylitalo et al., 2005).

In this section it is important to highlight a case report of one of the animals included in this thesis, CET 78 (Jaber et al., 2005). They suggested that the high level of PCB found may have yielded to the hepatosplenic lymphoma observed in the dolphin. However, they also stated that further studies in a larger number or cetaceans were necessary to better evaluation.

Demonstrating the effects of chemicals is a difficult task due to the complex multifactorial trait that may negatively influence the health status; for instance, a recently published study of the implication of pollutants in human health (Boada et al., 2015) revealed that PAHs serum levels seem not to be related to bladder cancer. Nevertheless, the authors stressed the relevance of genes encoding xenobiotic metabolizing enzymes.

### 2.5.3.4. Other effects

Related to the DWH oil spill in the Gulf of Mexico in 2010:
After the explosion of the DWH many studies were carried out to assess the impact in the marine wildlife, particularly in the common BND. Hypoadrenocorticism and lung diseases were observed in samples from the contaminated area of Barataria Bay, suggested to be consistent with petroleum compound exposure (Schwacke et al., 2014; Venn-Watson et al., 2015) not so with POPs which were found to be likely not a primary cause (Balmer et al., 2015). However, the synergistic effects of POPs and oil exposure and the direct cause-effect link are still under discussion (Jacobs, 2014).

When an animal faces a stressor like a pollutant, generally, the nervous system is the first system to be impacted. In this way, MeHg is a potent neurotoxic (Clarkson and Magos, 2006; Magos and Clarkson, 2006; Sakamoto et al., 2013) due to its easiness of absorption and to pass the blood–brain barrier (Aschner and Aschner, 1990). These toxic effects may involve structural degeneration of occipital cortex and cerebellum, and ataxia, weakness, convulsions etc. (Scheuhammer et al., 2015). Additionally, experimental studies suggest that Hg intoxication may cause weight loss, toxic hepatitis, renal failure and death in MMs (Vos et al., 2003). Additionally, high Hg levels have been associated with parasitic infection and pneumonia (Siebert et al., 1999), resulting in a lower resistance to infectious diseases (Bennett et al., 2001). Other reports associated chronic Hg accumulation with liver abnormalities observed in stranded BNDs from the Atlantic Ocean (Rawson et al., 1993).

By comparison, an inverse association between PCB burdens and parasitism in humans has also been described (Henriquez-Hernandez et al., 2016a).

Other toxic effects are described in the literature such as porphyria induced by HCB ingestion (Peters et al., 1986).

### 2.5.3.5. Chronic exposure

Living beings are continuously exposed to complex mixtures of environmental pollutants, with different biological effects, therefore chronic effects are of growing concern. There are a wide range of effects from acute to chronic exposures, which may involve reduced growth rates of populations to killed animals, but this complex picture seems to lead to an overall effect on the immune system (de Swart et al., 1996b; Gebhard et al., 2015).

Persistent induction of biotransformation enzymes of OCs metabolism (MFO-Mixed Function Oxidase) may result in the following effects (Reijnders et al., 1999):

- Increase of oxidative stress in the corresponding cells/organs.
- Formation of bioactive metabolites.

- Greater elimination of important endogenous compounds, such as vitamins and hormones.
- Formation of mutagenic intermediates from other xenobiotics present in the mixture, such as PAHs.

In a case-control study on breast cancer, Rivero et al. (2015) analyzed the activity of different OC mixtures. They observed that slight differences in a pollutant mixture may yield anti-androgenic effects which induce cell proliferation, thus enhance the carcinogenic potential of the individual compound.

## 2.5.3.6. Mechanism of action

For this section of the thesis, the veterinary toxicology book (Gupta, 2012) and several articles were consulted and the mechanisms of action were summarized below:

There are many specific mechanisms of action. Thus, two common pathways used by xenobiotics are through oxidative damage and interference with normal enzymatic reaction thereby causing dysregulation and altered maintenance of cells in different tissues.

In addition, endocrine disruption involves many mechanisms of actions, which modulate the interactions between endogenous hormones and their receptors (“receptor-mediated endocrine disruption”) or alter hormonal functions (“endocrine disruption independent of receptor-mediated interactions”). Regarding the receptor-mediated mechanisms, the AhR-mediated endocrine disruption is one of the most used pathways by OC industrial chemicals (PCBs, PCDDs, PAHs), being the TCDD an AhR agonist prototype. The AhR regulates several genes, many of them involved in the regulation of the metabolism of xenobiotics, cell growth regulation and differentiation. Thus, the specific mode of cytochrome P-450 enzyme systems may play an important role in the OC effects, mainly coplanar PCBs. Furthermore, non-coplanar PCBs have been suggested to modulate phagocytosis through an AhR-independent pathway, thus interfering with the first immune barrier against infections (Levin et al., 2005).

Regarding OCPs, there are two main pathways of action. DDT-type insecticides affect the brain and peripheral nerves by altering the flux of sodium and potassium, resulting in excess intraneuronal potassium. In the other hand, cyclodiene insecticides may inhibit the binding of GABA neurotransmitter with its receptor. Thus, there is no synaptic down-regulation releasing other neurotransmitters in excess, resulting on neuronal overstimulation. This explains the cholinergic effects produced by dieldrin and lindane.

It is also thought that OCs are involved in gene transcription using direct or compensatory mechanisms entailing an impact on the hypothalamic-pituitary-thyroid (HPT) axis (Techer et al., 2016).

Additionally, there is also an epigenetic mechanism of action of endocrine disrupters. Thus, alterations in DNA methylation in the germline may be produced after xenobiotic exposures in mice, inducing transgenerational effects such as infertility and tumor susceptibility (Anway and Skinner, 2006; Newbold et al., 2006).

However, molecular mechanisms of different mixture profiles still remain unknown and studies have shown a wide variety of cellular processes impacted by different pollutants leading to contradictory conclusions (Krivoshiev et al., 2015).

#### 2.5.3.7. Tolerance to pollutant exposure

As said before in this thesis, MMs accumulate great amounts of pollutants which implies a high risk to the animal health status (Tanabe, 1988). In addition, toothed cetaceans have poor capacity for OC chemicals degradation because of their type of cytochrome P-450 enzyme system (Fair and Becker, 2000; Tanabe, 2002). Thus, MMs are thought to be more susceptible to dioxin-like PCBs than terrestrial animals (Fair and Becker, 2000; Kannan et al., 1989).

For that reason, due to the high levels of pollutants encountered in MMs with no toxic problem revealed, suggests a remarkable tolerance and detoxification mechanisms to endure the environmental chemical exposure (Caurant et al., 1996).

In this way, although the organic form of Hg (MeHg) appears to be the most toxic form of Hg to animals, an adaptation acquired by dolphins to counteract the toxic effects of MeHg was suggested (Betti and Nigro, 1996). The detoxification mechanism involves a demethylation of MeHg and subsequent sequestration of inorganic Hg with Se (Bjorkman et al., 1995; Caurant et al., 1996; Palmisano et al., 1995). Formation of complex Hg-Se, as insoluble tiemannite granules, provides the ability to endure high Hg exposures to odontocetes (Caurant et al., 1996; Nigro et al., 2002). Thus, Hg and Se levels above 2000 µg $g^{-1}$ dw were reported in animals with no signs of poisoning because of the protection provided by the combined presence of both trace elements (Wagemann and Muir, 1984). However, detoxification is limited in several animals such as lactating females and injured individuals and also the energy cost of the tolerance mechanisms is difficult to assess.

#### 2.5.3.8. Thresholds

Threshold levels of xenobiotics linked to detrimental health effects in MMs are difficult to assess and not well established. In fact, many of the PCB thresholds for cetaceans and seals are based on studies in mink, otter and laboratory species, which may overestimate the risk in MMs (AMAP, 2002; Vos et al., 2003). However, despite of the limitation of threshold used (Krey et al., 2015), several studies have attempted to find suitable thresholds for toxic effects thanks to surrogate species or to case-control studies or associations between pathological findings and burden chemicals observed in MMs. These thresholds reported in the literature consulted are indicated in the table below:

Table 3. Thresholds of POPs for adverse health effects in MM, from the literature consulted.

| Pollutant | Specie | Tissue/Source | Threshold | Toxic effect | Reference |
|---|---|---|---|---|---|
| PCB | Aquatic mammals | Liver | 8.7 ppm (lw) | Hepatic vitamin A, thyroid hormone concentration, suppression of NK cell activity and proliferative response of lymphocyte | Kannan et al., 2000 |
| PCB | MMs | Blubber | 12 ppm (lw) | Reproduction and immune effects | Kannan et al., 2000; Jepson et al., 2005 |
| PCB | MMs | Blubber | 17 ppm (lw) | Toxic effects | Wilson et al., 2014 |
| PCB | MMs | Field and laboratory data | 0.6-1.4 ppm | Suppression of phagocytosis | Desforges et al., 2016 |
| PCB | MMs | Field and laboratory data | 0.001–10 ppm | Suppression of lymphocyte proliferation | Desforges et al., 2016 |
| PCB | Cetaceans | Blubber | 50 ppm (lw) | Risk | Wageman and Muir, 1984 |
| PCB | Cetaceans | Blubber | 50 ppm (lw) | Immunosuppression, endocrine disruption, neuronal alteration | Lahvis et al., 1995; Tanabe, 2002 |
| PCB | BNDs | Blubber | 70 ppm (lw) | Immunosuppression and reduce thyroid hormone levels | Schwacke et al., 2012 |
| PCB | Harbor porpoises | Blubber | Each 1 ppm (lw) below 45 ppm (lw) | Risk of infectious disease mortality increase by 2% | Hall et al., 2006a |
| PCB | Harbor porpoises | Blubber | Each 1 ppm (lw) over 45 ppm (lw) | Risk of infectious disease mortality increase by 50% | Hall et al., 2006a |
| PCB | Harbor porpoises | Blubber | Each 1 ppm (lw) over 80 ppm (lw) | Risk of infectious disease mortality increase by 100% | Hall et al., 2006a |
| Hg | MMs | Field and laboratory data | 0.08-1.9 ppm | Suppression of phagocytosis | Desforges et al., 2016 |
| Hg | MMs | Field and laboratory data | 0.002–1.3 ppm | Suppression of lymphocyte proliferation | Desforges et al., 2016 |
| MeHg | MMs | Field and laboratory data | 0.009–0.06 ppm | Suppression of lymphocyte proliferation | Desforges et al., 2016 |
| THg | BNDs | Liver | 60 ppm (ww) | Large deposits of hepatic pigments | Rawson et al., 1993 |
| THg | Seals | Liver | 500 ppm (dw) | Death | Ronald et al., 1977 |
| THg | Waterbirds | Liver | 8.5 ppm (dw) | Activation of demethylation | Eagles-Smith et al., 2009 |
| THg | Fish-eating mammals | Brain | 3-5 ppm (dw) | Neurochemical effects | Dietz et al., 2013 |
| THg | Wild mink and otter | Brain | 10 ppm (ww) (40 ppm dw) | Severe poisoning and mortality | Wiener et al., 2003 |
| Cd | MMs | Field and laboratory data | 0.1-2.4 ppm | Suppression of lymphocyte proliferation | Desforges et al., 2016 |
| TEQ-PCBs | Aquatic mammals | Liver | 520 pg/g (lw) | Hepatic vitamin A, thyroid hormone concentration, suppression of NK cell activity and proliferative response of lymphocyte | Kannan et al., 2000 |
| TEQ-PCBs | Harbor seals | Blubber | 200 pg/g (lw) | Immunosuppression | Wilson et al., 2014 |
| TEQs-PCBs | Otter | Fish | 11 pg/g (lw) | hepatic vitamin A reduction in otter liver | Murk et al., 1998 |

# 3. JUSTIFICATION AND OBJECTIVES / JUSTIFICACIÓN Y OBJETIVOS

## JUSTIFICATION AND OBJECTIVES:

There is a growing awareness of the impact of anthropogenic pollution in worldwide marine ecosystems. Particularly, the potential hazard to wildlife such as resident cetacean populations is on the top public environmental concerns, because increases in anthropogenic or natural toxic compounds in coastal habitats are of concern not only for MMs but also for humans. Besides infectious diseases, these animals faced multiple anthropogenic stress factors including the exposure to xenobiotics together with climate change, habitat degradation, noise pollution, food depletion, fishery interaction, collision and by-catch, among others.

Background information on pollutant levels have been reported in humans (Luzardo et al., 2009, 2012; Zumbado et al., 2005) and in marine wildlife from Canary Islands, such as marine turtles (Camacho et al., 2012; Monagas et al., 2008; Oros et al., 2009); but very few data were available about OC residues in cetaceans from this part of the Eastern Atlantic Ocean (Carballo et al., 2008) which requires greater research effort to assess the impact of pollutants in this area.

BND is been proposed as a bioindicator of the contamination in the Canary archipelago. This species is particularly susceptible to human impacts due to its coastal and local behaviour, also important as it is considered “vulnerable” specie in the Spanish National Catalogue of Threatened Species and in the “Special Protection” category for the 2010 Canary Catalogue.

Analysis of exposure to anthropogenic pollutants in this sentinel species may have implications for public health in this region. Additionally, it is important to establish robust baseline information of contaminant exposure in order to evaluate the impact of future environmental changes on cetacean populations (Fair and Becker, 2000).

Since 1992, an assessment of the health status of cetaceans stranded on the Canary coasts has been carried out by the Cetacean Research Unit of the Institute of animal health (IUSA) at the Veterinary School of the University of Las Palmas de Gran Canaria (ULPGC). Since then, a cetacean tissue bank from necropsied cetaceans has been available for research of pathologies

(Arbelo et al., 2013; Diaz-Delgado et al., 2016; Sierra et al., 2015) and also for toxicological studies (Carballo et al., 2008).

The foregoing justifies the completion of the present doctoral thesis whose main objective is to gain a greater understanding of the toxicological status of common BND in the Canary Islands, extending the knowledge of anthropogenic pollutants in cetaceans of this archipelago as part of the international Macaronesia Cetacean Health Network with conservation aims. Additionally, this thesis could contribute to the assessment of the environmental status of the marine waters required for the Marine Strategy Framework Directive (MSFD) which recognised the importance of food webs to the ecosystem dynamics (EEA, 2015).

To address this main objective, assessment of the levels of 57 POPs (23 OCPs, 18 PCBs and 16 PAHs) and 12 trace elements in blubber and liver of BNDs stranded from 1997 to 2013 and also POPs in biopsies from live individuals collected during the period of 2003-2011 has been conducted by the Society for the Study of Cetaceans in the Canary Archipelago (SECAC).

Samples from both stranded and live individuals have been analysed, as stranded animals provide access to internal tissues combined with pathological information and biopsies allow assessment of “healthy” free ranging dolphins. In addition, it must be considered that certain chemicals degrade following death, so biopsy samples provide valuable and complementary information (Krahn et al., 2003).

The present dissertation includes three publications with the corresponding data and discussion of results:

1. Assessment of the levels of polycyclic aromatic hydrocarbons and OC contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean
2. Levels and profiles of POPs (OCPs, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain
3. Mercury and selenium status of bottlenose dolphins (*Tursiops truncatus*): A study in stranded animals on the Canary Islands

There is also a section with non-published data (section 4) which are considered of interest for this thesis and the section 2.3.4 which updates the data of stranded BND to 2016.

# JUSTIFICACIÓN Y OBJETIVOS:

Existe una creciente concienciación sobre el impacto que la contaminación global de origen antrópico pueda ejercer sobre los ecosistemas marinos. Especialmente el daño potencial sobre la fauna salvaje, como son las poblaciones de cetáceos residentes, representa una de las preocupaciones medioambientales de mayor relevancia, ya que el aumento de compuestos tóxicos de origen natural o antrópico en los hábitats costeros afectan no sólo a los mamíferos marinos sino también al ser humano. Además de a enfermedades infecciosas, estos animales están expuestos a múltiples factores de estrés originados por el hombre, incluyendo la exposición a xenobióticos, el cambio climático, degradación del hábitat, contaminación acústica, pérdida de recursos alimentarios, interacción pesquera, colisión y captura accidental, entre otros.

Se han publicado numerosos estudios sobre niveles de contaminantes en humanos (Luzardo et al., 2009, 2012; Zumbado et al., 2005) y en animales marinos de las Islas Canarias, como es el caso de las tortugas marinas (Camacho et al., 2012; Monagas et al., 2008; Oros et al., 2009); sin embargo, hay escasos datos disponibles de residuos organoclorados en cetáceos que habitan el océano Atlántico Este (Carballo et al., 2008) lo que requiere un mayor esfuerzo en investigación para evaluar el impacto de los contaminantes en este área.

El delfín mular se ha propuesto como bioindicador del nivel de contaminación del archipiélago canario. Esta especie es particularmente sensible al impacto de la actividad humana debido a su comportamiento local y costero, también importante por ser considerada como especie “vulnerable” en el Catálogo Nacional Español de Especies Amenazadas y en la categoría de “Especial Protección” en el Catálogo Canario de 2010.

El análisis de la exposición que sufre esta especie centinela a los contaminantes de origen antrópico puede tener implicaciones en la Salud Pública de esta región. Además, es importante establecer unos niveles de referencia de exposición a contaminantes para poder evaluar el impacto de posibles cambios medioambientales futuros sobre las poblaciones de cetáceos (Fair and Becker, 2000).

Desde 1992, se está realizando una evaluación del estado sanitario de los cetáceos varados en las costas canarias, llevado a cabo por la Unidad de Investigación de Cetáceos del Instituto de Sanidad Animal y Seguridad Alimentaria (IUSA) en la Facultad de Veterinaria de la Universidad de Las Palmas de Gran Canaria (ULPGC). Desde entonces, está disponible un banco de tejidos de cetáceos, gracias a las necropsias realizadas en los animales varados, lo que permite llevar a cabo investigación de patologías (Arbelo et al., 2013; Diaz-Delgado et al., 2016; Sierra et al., 2015) y estudios toxicológicos (Carballo et al., 2008).

Lo anteriormente expuesto justifica la realización de la presente tesis doctoral, cuyo principal objetivo es el de adquirir un mayor conocimiento del estado toxicológico del delfín mular común de las Islas Canarias, ampliando la información sobre contaminantes antrópicos en cetáceos del archipiélago como parte de la Red Internacional de Salud de Cetáceos de la Macaronesia con fines de conservación. Además, esta tesis puede contribuir a la evaluación del estado medioambiental de las aguas requerido por la Directiva Marco de Estrategias Marinas (MSFD) que reconoce la importancia de las cadenas tróficas para las dinámicas del ecosistema (EEA, 2015).

Para alcanzar este objetivo principal, se ha realizado el análisis de 57 contaminantes orgánicos persistentes (COPs), 23 pesticidas organoclorados (POCs), 18 bifenilos policlorados (PCBs) y 16 hidrocarburos aromáticos policíclicos (PAHs), y 12 elementos tóxicos en blubber e hígado de delfines mulares varados de 1997 a 2013; además se han analizado estos 57 COPs en blubber de biopsias muestreadas en delfines vivos en libertad durante el periodo de 2003-2011 por la Sociedad para el Estudio de los Cetáceos en el Archipiélago Canario (SECAC).

Se utilizaron muestras de animales varados y vivos, ya que los varados pueden aportan datos de patología y las biopsias permiten evaluar los contaminantes en delfines vivos en libertad que podrían considerarse "sanos". Además, se debe tener en cuenta que se puede producir cierta degradación post-mortem de algunos compuestos químicos, con lo que las biopsias podrían aportar datos complementarios valiosos (Krahn et al., 2003).

La presente tesis doctoral incluye tres publicaciones con los correspondientes resultados y discusión:

1. Assessment of the levels of polycyclic aromatic hydrocarbons and OC contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean.
2. Levels and profiles of POPs (OCPs, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain.
3. Mercury and selenium status of bottlenose dolphins (*Tursiops truncatus*): A study in stranded animals on the Canary Islands.

También se ha incluido una sección de datos no publicados (sección 4) considerada de interés para incluir en esta tesis, y otra sección donde se actualizan los datos de varamientos de delfines mulares en las Islas Canarias hasta el año 2016 (sección 2.3.4).

# 4. NON PUBLISHED RESULTS

In addition to our published work, we have included this section of the thesis with non-published data obtained in the course of the present PhD work.

1- Regarding pollutant level obtained in samples from stranded BND, an analysis of the possible confounding factors is described:

- Sex and age:

Results of Kruskal-Wallis (K-W) analysis performed with SPSS Statistics (v 23.0) did not show significant differences in PCB or OCPs burdens (lw) between different sex or age categories (analysis updated with the corrected age groups, see erratum of this thesis). However, the concentration of pollutants followed a specific pattern as described in the first publication of the present thesis (graphs below).

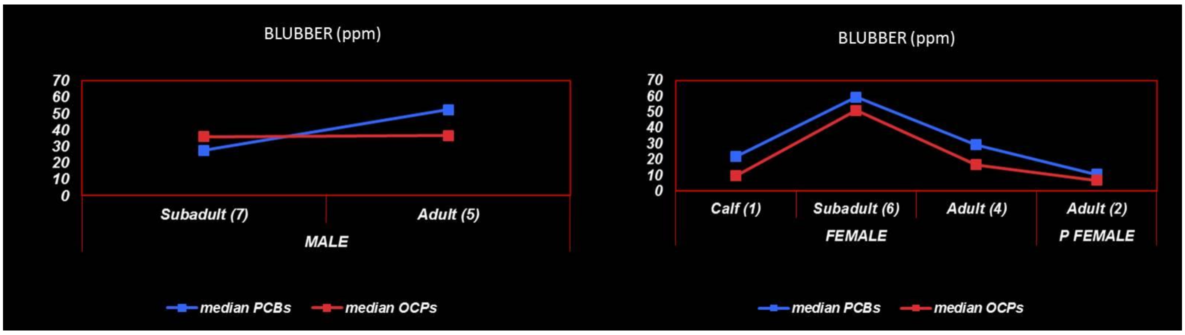



Figure 11. Levels of OCs in the blubber of males and females of stranded BND (median values; ppm lw).

The liver seems to follow the same pattern for PCBs and OCPs depending on age categories, but the trends are less apparent.

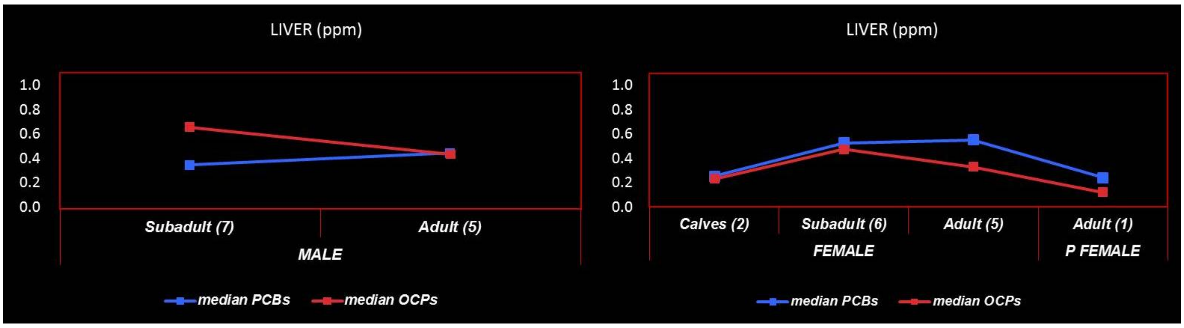



Figure 12. Levels of OCs in the liver of males and females of stranded BND (median values; ppm, lw).

Regarding toxic elements, Cd seems to increase with age but with no statistical significance and As was found significantly different in blubber between age categories ($p$=0.04), increasing the levels (dw) with the age of the animal, both in blubber and liver samples (Fig. 13):

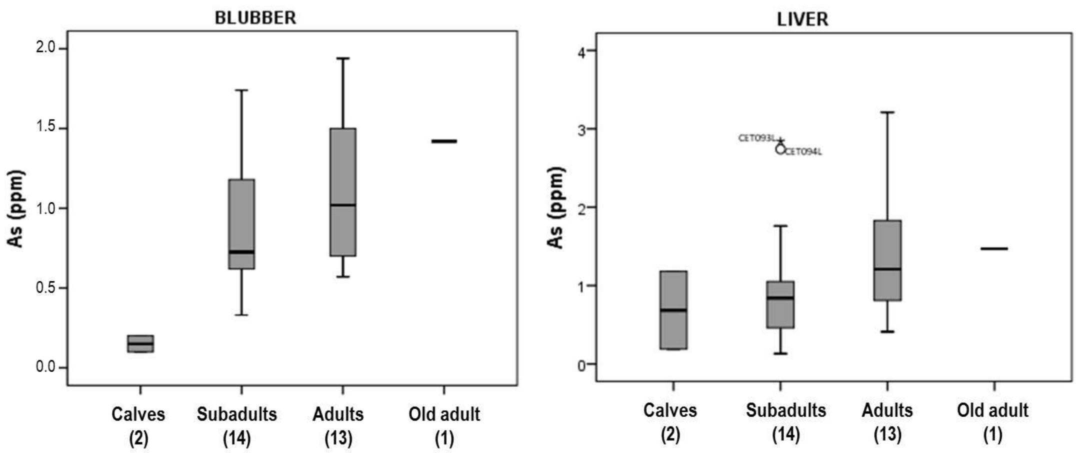



Figure 13. Levels of As (ppm, dw) in the blubber and liver of stranded BND, age segmentation.

See third publication included in this thesis for details of the correlation of Hg and Se with total length of the specimens.

- <u>Stranding location</u>:

A significant difference in PCB ($p$=0.029) and OCP (0.046) levels (lw) in the blubber was found between islands, being Fuerteventura the one where the stranded dolphins exhibited higher OCP burdens (graph below). The tendency of the OCs levels seems to decrease from Eastern islands to the western; this pollutant profile was found independently of other confounding variables like sex or age. Nevertheless conclusions should be made by caution due to the small sample size, particularly because there were only 2 animals available from Fuerteventura, and one of them is an outlier value (CET 311).

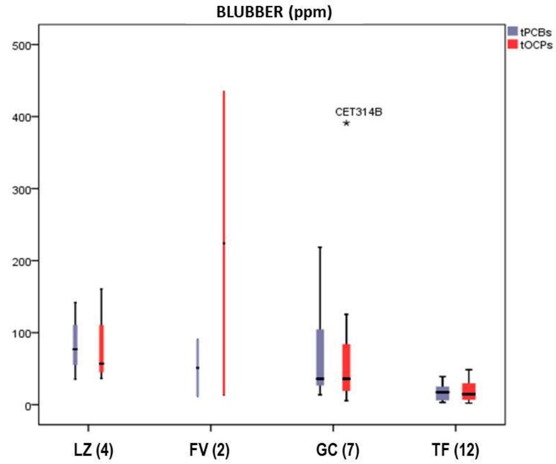



Figure 14. Levels of PCBs and OCPs (ppm, lw) in the blubber of stranded BND, by stranding locations.

Regarding inorganic pollutants, results of K-W analysis showed significant differences in Se, Cd and As hepatic burdens (dw) between different location of carcasses (Fig. 15). Most of the samples came from BND stranded in TF (14 samples), followed by GC (8), LZ (5), FV (2) and LG (1), which make difficult to reach any conclusion.

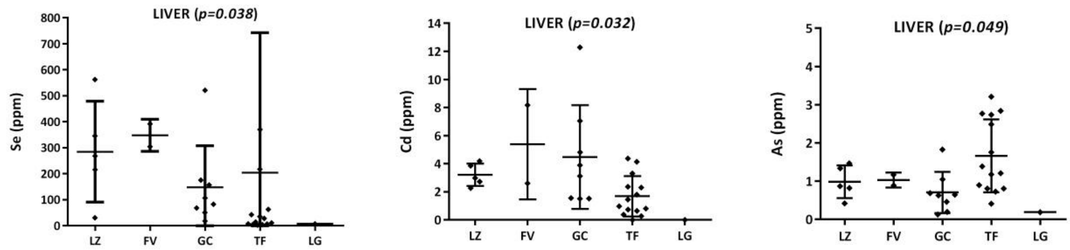



Figure 15. Levels of Se, Cd and As (ppm, dw) in the liver of stranded BND, by stranding locations.

Table 19 presents the concentration (ppm, dw and ww) of HMs and other toxic elements in the blubber and liver of BND from the present study and other marine areas worldwide. The release of contaminants in the environment is highly variable due to differences in the production and use in each specific area, which would complicate the comparison studies. It can be observed that hepatic Cd appeared to be higher in this part of the ocean compared to NW Atlantic, which may be explained by diet as suggested by other authors before (Stein et al., 2003). They stated that Atlantic BND may feed on squid, which could be a significant source of Cd.

Pb also appeared to be higher in this marine area compared to BNDs from other oceans and seas. This toxic element has been used in gasoline, paints, batteries and others resulting in a potent environmental contaminant worldwide (Thompson, 2012). In addition, Hg and Se are discussed in the third article included in this thesis.

- <u>Year of stranding</u>:

Results of analysis through K-W non-parametric test indicate that there is no statistical difference between target pollutants and the years of the study period. Even so, the Hg in the blubber almost got the limit for statistical significance between year groups, with $p$=0.058. The increasing temporal trend of this HM could be seen in the third article of this thesis.

The following box plot diagrams represent the temporal trend of Hg (dw) in the blubber counting on all samples (on the left) or only samples from males (on the right) all of them subadult and adult individuals. The same upward trend was observed discarding the specimens in poor conservation status (see table 7 with the detailed variables of the stranded specimens to see the number of samples per year).

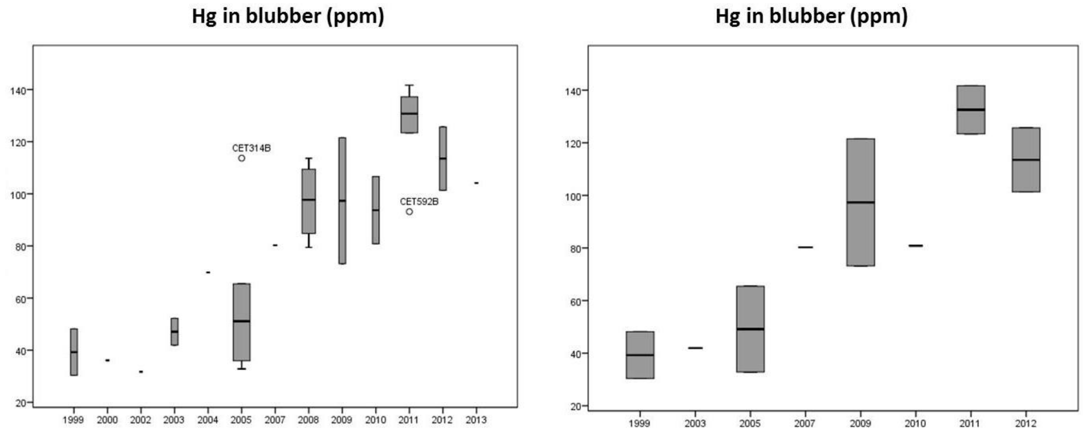



Figure 16. Levels of Hg (ppm, dw) in the blubber of stranded BND, year segmentation. All samples (box plot graph on the left), only samples from males (box plot graph on the right).

Although there is no clear upward temporal trend for pollutant levels in samples from stranded dolphins, POP burdens seem not to be declining in BND inhabiting Canary waters, as said in the first publication included in this thesis (see graphs below, units in lw):

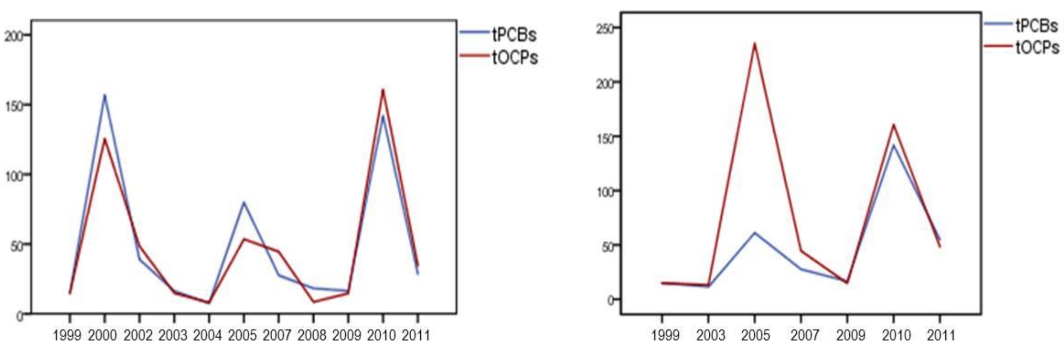



Figure 17. Levels of OCs (median values, ppm, lw) in the blubber of stranded BND, by year of stranding. All samples (on the left), only samples from males (on the right).

The temporal representation of POP median levels in the blubber showed an irregular pattern; increasing in 2000, 2005 and 2010 with all values (on the left) or 2005 and 2010 with sex segmentation, only with male values (on the right). This peaks don’t correspond with the stranding peaks of BND occurred in the study period (see section 2.3.4); thus, it likely due to other biological or pathological factors of the stranded specimens which will be deeply studied. Again the limitation is the number of samples per year (see table 7 of variables).

- Body condition:

As chronic illness may deplete blubber reservoirs, mobilizing lipids together with pollutants, it might result in a fluctuation of contaminant concentrations difficult to assess. Thus, we analysed the levels of pollutants in both blubber and liver samples related to the different categories established for body condition (from “good” to “very poor”). These categories were determined with morphological references such as the development of the dorso-axial muscular mass, the prominence of vertebral processes, the quantity of subcutaneous fat and the thickness of the blubber considering the species and age of the specimen (being this later issue under investigation).

Although the limited sample size per group conditioned any conclusion, we observed that in general the concentration of PCBs, OCPs and PAHs decrease in the blubber from good to moderate category and then increase to poor condition. Figure 18 represents these values (lw) from male individuals within good to moderate conservation state categories to avoid any interference.

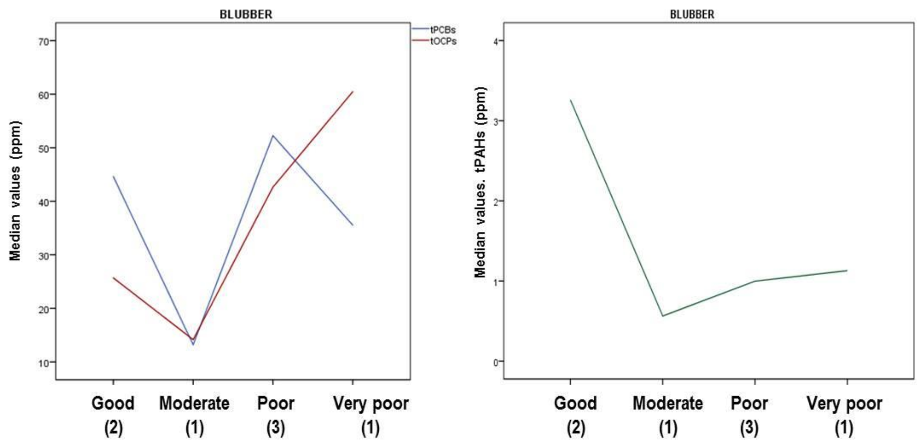



Figure 18. Levels (median values; ppm, lw) of OCs (on the left) and PAHs (on the right) in the blubber of stranded BND, by body condition. Samples from male individuals, with good to moderate conservation state.

Although little is known about the mobilization of pollutants from the blubber, a published article (Louis et al., 2014) also found a similar rise of contaminant levels in blubber during the post-weaning fast period of seal pups, stating that this result may be due to a more efficient mobilisation of triglycerides and/or a reuptake by adipocytes of some compounds previously released into the blood.

They also found that at late fast the mobilisation of pollutants from blubber increased, as we found regarding PCBs, which decrease again from poor to very poor category. In contrast, OCPs continue to rise.

Regarding the liver samples (Fig. 19) there was not an initial significant decrease, as fatty reservoir may be the main tissue for mobilization in periods of requirements. From moderate to poor state the hepatic concentration of POPs gets higher, as it happens in the blubber, maybe for the reuptake of those compounds initially released to the circulation. PCBs significantly increase to the very poor condition, contrary trend to the corresponding in the blubber, which may indicate a dynamic flow between blubber, general circulation and liver. OCPs remain almost at the same level at poor conditions and PAHs again decline to the last category group.

Concerning PAHs, there is a significant decrease in both tissues, at different category points but at very poor body condition these animals have lost almost all compounds from their tissues. However, PAHs results must be taken with caution in samples from stranded specimens as explained in the comparison with samples from live free ranging dolphins.

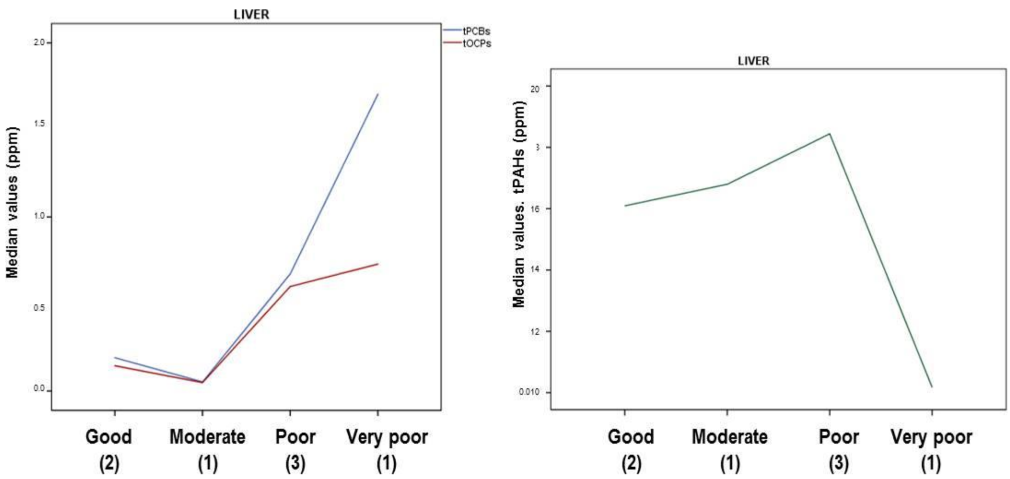



Figure 19. Levels (median values; ppm, lw) of OCs (on the left) and PAHs (on the right) in the liver of stranded BND, by body condition. Samples from male individuals, with good to moderate conservation state.

Regarding trace elements, Zn, Al and Hg levels against body condition variable behaved in a similar way that was observed with PCBs.

- <u>Conservation status</u>:

Regarding death animals, because they are samples from stranded dolphins, pollutants must be analysed versus conservation status variable. The post mortem time is important because the carcasses are exposed to sun or wind, thus lipid stores can be modified and volatile compounds can be evaporated.

Figure 20 represents the principal POP groups (total PCBs, total OCPs and total PAHs) from very fresh to very advanced autolysis states. A general decrease was observed with the decomposition state in the blubber, on the contrary, these pollutants in the liver increase from advance to very advance autolysis. These figures graphically described the behaviour of POP main groups with the autolysis but there were no significant differences and small sample size limits any interpretation; very fresh (4), fresh (7), moderate autolysis (2), advanced autolysis (9), very advanced autolysis (3).

Additionally, the results of the present thesis strongly suggest that studying PAHs in samples from stranded are not useful because they may readily volatilize from the carcasses (see comparison with biopsies).

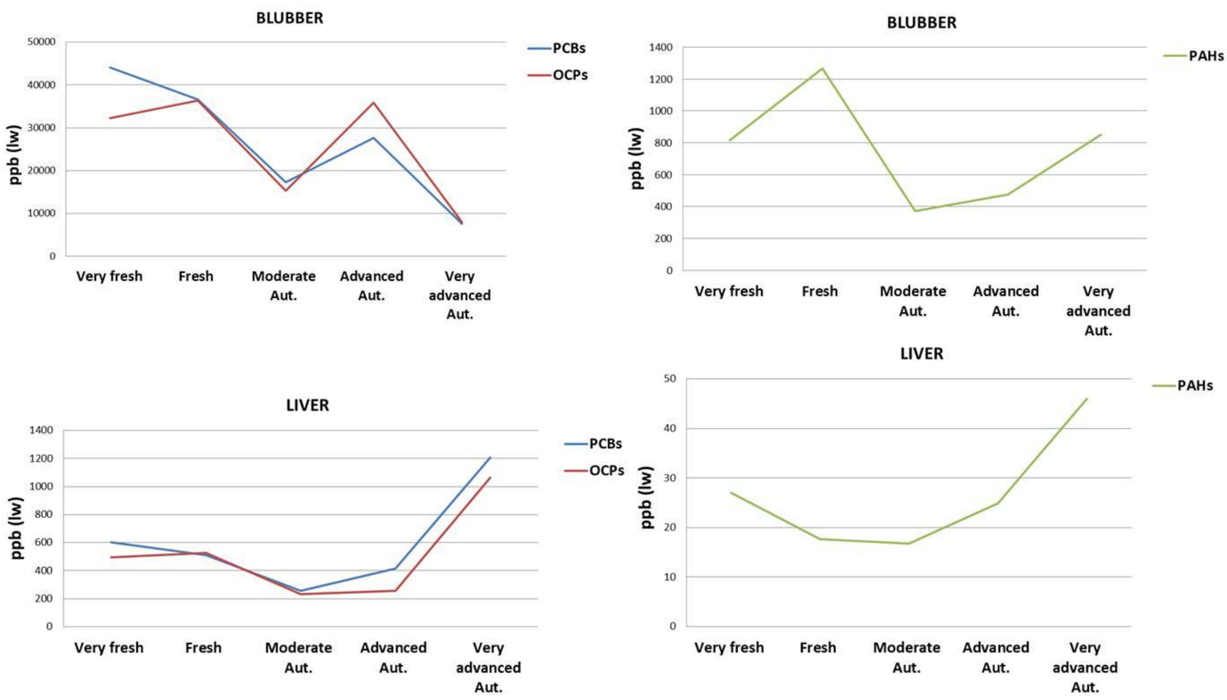



Figure 20. Levels (median values; ppb, lw) of OCs (on the left) and PAHs (on the right) in the blubber (above) and liver (below) of stranded BND, by conservation state categories.

In relation to trace elements, the K-W test did not reveal any relevant data except for Fe, which displays significant higher levels (dw) in autolytic blubber samples, may be due to the release of some of the iron of hematic compounds by autolysis, but this would not explain why there is no difference in the hepatic tissue.

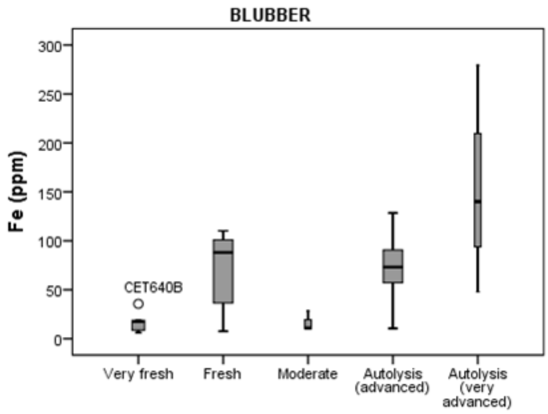



Figure 21. Levels of Fe (ppm, dw) in the blubber of stranded BND, by conservation state categories.

2- Regarding HMs and toxic trace elements analysed in samples from stranded, several positive correlations have been observed and the most relevant are represented in the following graphs:

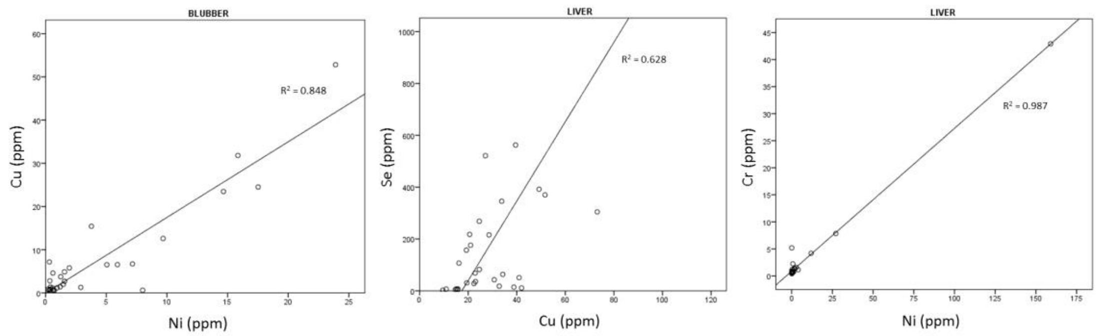



Figure 22. Correlations between Cu and Ni in the blubber (on the left), Se and Cu in the liver (middle) and Cr and Ni in the liver (on the right) of stranded BND. Units in ppm, dw.

Statistical analysis was performed using a Spearman correlation, thus Ni and Cu showed a significant positive relationship in blubber samples ($p$=0.000; rs=648; $R^2$=0.848). Concerning liver samples, Se and Hg exhibited the strongest linear association among all toxic elements (information detailed in the third article included in this thesis).

Additionally, Cr and Ni also showed a strong correlation ($p$=0.001; rs=0.568; $R^2$=0.987) together with Se and Cu ($p$=0.000; rs=0.626; $R^2$=0.628) both in liver tissue.

OCPs and PCBs concentrations in blubber highly correlated between them, being $R^2$=0.862 when outliers are discarded (CET 311 and CET 314). This could be related to a same potential and pattern of bioaccumulation and biomagnification of all OC compounds in these marine animals.

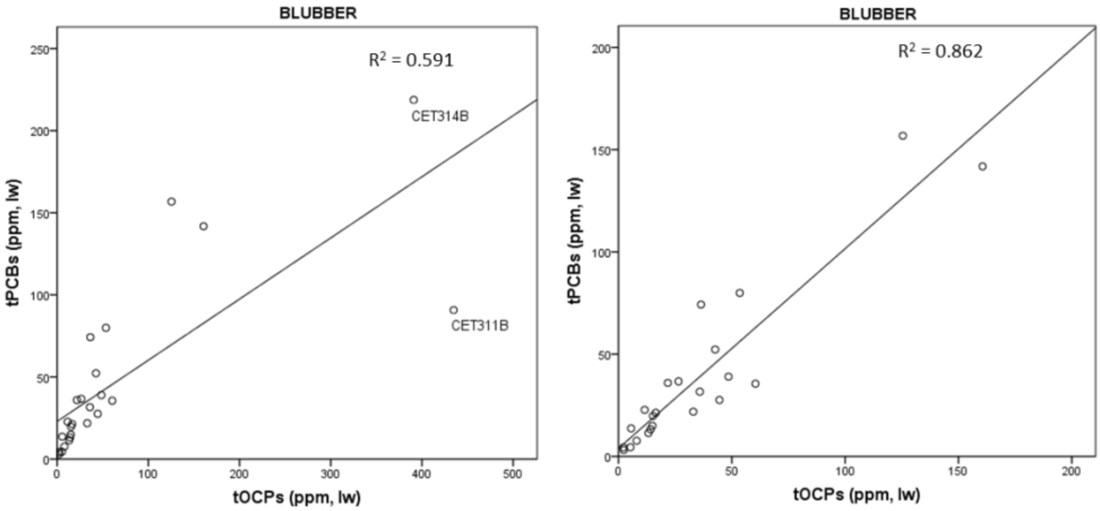



Figure 23. Correlations between PCBs and OCPs in the blubber of stranded BND, all samples (on the left), outliers discarded (on the right). Units in ppm, lw.

3- Pathology is an important possible influencing factor in pollutant concentrations, thus the available data has been partially analysed, pending completion of all pathological findings obtained by the pathologists at the Institute of the Animal Health (IUSA). After the necropsies of the specimens, a pathological study is conducted in the animals to finally assign a “pathology entity” to each of them (see table 7). These categories have been previously described (Arbelo et al., 2013) and adapted to this thesis as NPL, natural pathology associated with a significant loss of body condition; NPNL, natural pathology non-associated with loss of body condition; IF, interaction with fishing activities; IIT, intra- or interspecific physical trauma and ND, data not determined.

The evaluation of possible association between toxic residues and pathology data was based on other researches such as the one carried out by Jepson et al. (2005). They compared two groups of animals depending on the cause of death: acute physical trauma against infectious disease. The first group was considered as healthy individuals. They found significantly higher levels of PCBs in the infectious disease group. Although this assessment may lead to a risky interpretation, as many of the considered healthy individuals could be actually pathologically undervalued, also provides a useful approach to future deeper studies on this field.

In our research, the pathology entities have been split into two categories, the NPL and NPNL considered as the disease group (DG), and the IF and IIT entities as the healthy group of animals (HG). The following figures show the most interesting associations found between pollutants and pathology variables: In the blubber, Cu, Fe and Ni were higher and Zn was significantly higher ($p$=0.019) in the animals which died by fishing interaction (see Fig. 24, sample size in brackets). This finding may be due to the fact that these metals are used in the manufacture of fishing gears.

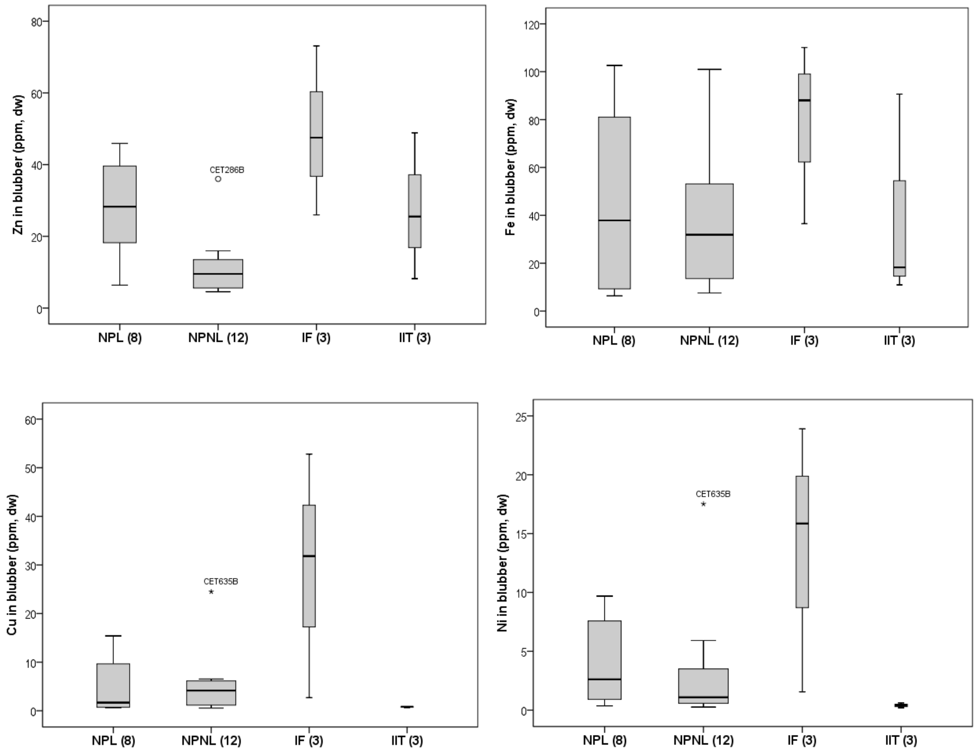



Figure 24. Levels of Zn, Fe, Cu and Ni (ppm, dw) in the blubber of stranded BND by pathology entities segmentation.

In the liver, distribution of Se levels was significantly different ($p$=0.045) between pathology categories and Hg and Se between DG and HG ($p$ = 0.019 and 0.017, respectively), see figure below:

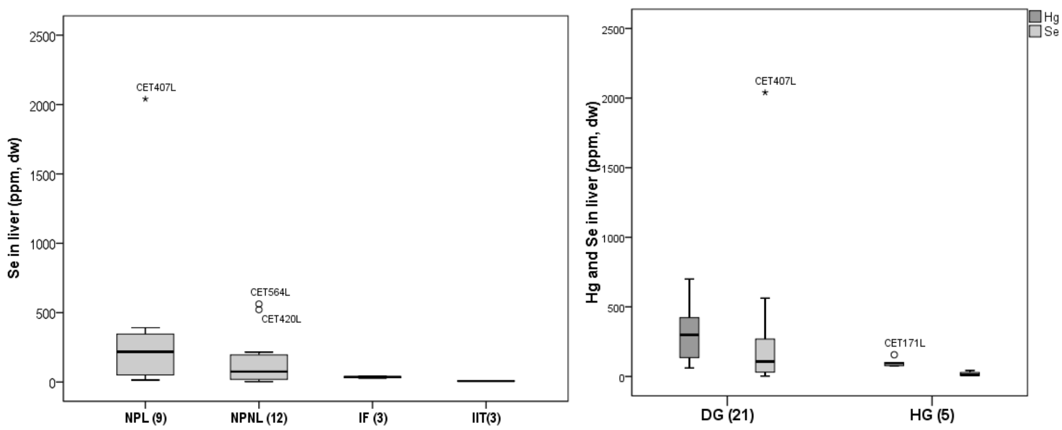



Figure 25. Levels of Se in the liver of stranded BND by pathology entities (on the left), levels of Hg and Se in the liver of stranded BND divided into DG and HG (on the right). Units in ppm, dw.

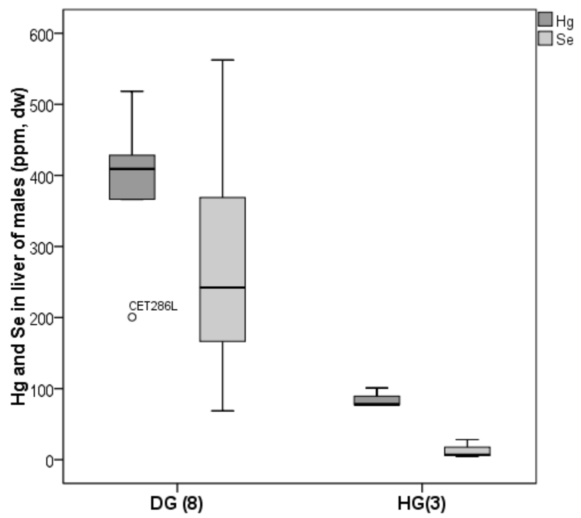



Figure 26. Levels of Hg and Se in the liver, split into DG and HG; samples only from males of stranded BND. Units in ppm, dw.

These differences were even more evident when only samples from males BNDs were analysed (*p*= 0.014 for Hg and *p* = 0.007 for Se). Both toxic elements showed greater burdens in individuals within the disease group.

In this respect, all animals exceeding a described Hg threshold for hepatic damage (Wagemann and Muir, 1984) except CET 203 (Fig. 27) are within NPL and NPNL categories.

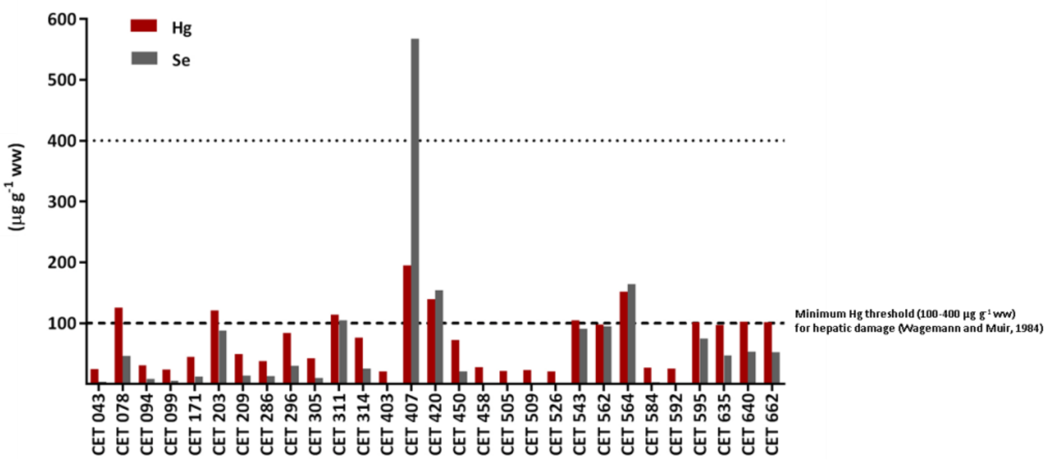



Figure 27. Levels of Hg and Se (ppm, ww) in the liver of stranded BND.

Regarding PCBs (table 4), also significant differences were found in totalPCBs and TEQ-PCBs in blubber samples between DG and HG with *p*-values of 0.029 and 0.010 respectively (Fig. 28. Left); these differences were even more relevant when only males were analysed with *p*=0.011 for tPCB and 0.019 for TEQ-PCBs (Fig. 28. Right).

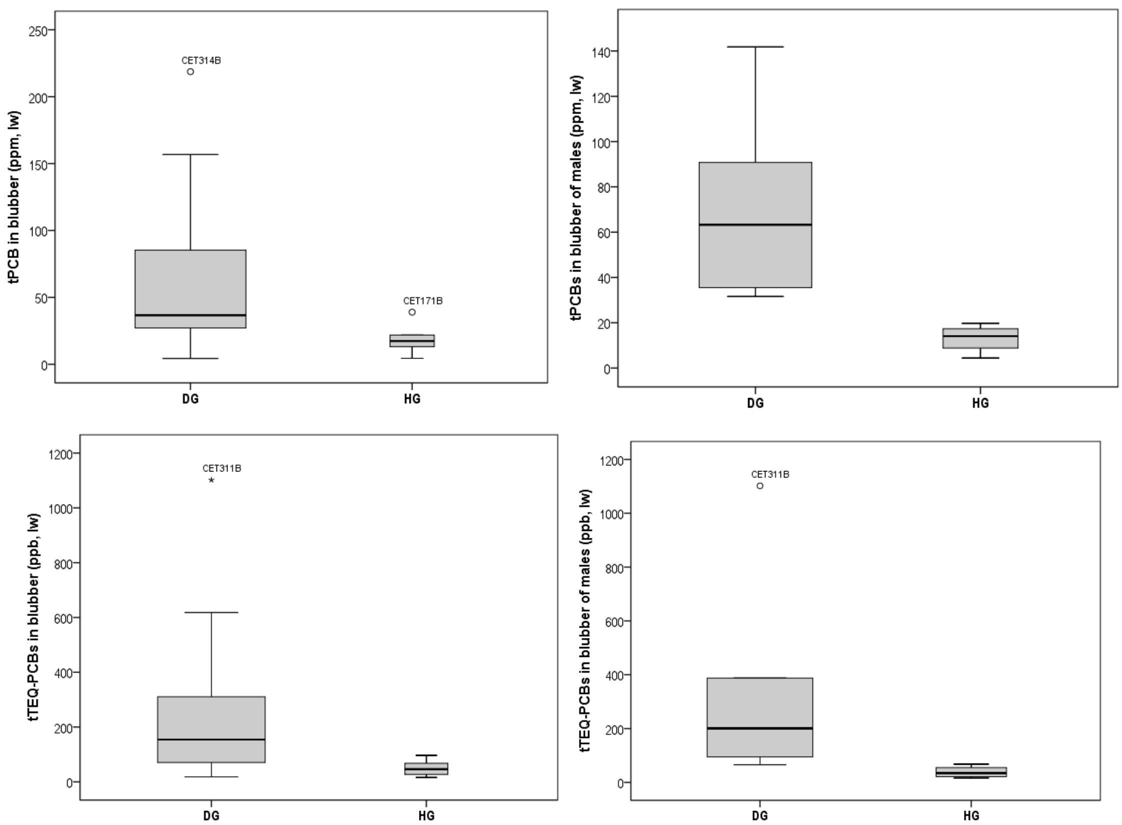



Figure 28. Levels of PCBs (above) and TEQ-PCBs (below) in the blubber of stranded BND, split in DG and HG. All samples (left) and only samples from males (right). Units on ppm and ppb, dw.

The statistical descriptions are presented in the following table:

Table 4. Statistical descriptions of PCBs in the blubber of stranded BND divided by two groups, disease and "healthy" animals. Mean, median, minimum and maximum are indicated in ppm, lw for PCBs and ppb, lw for TEQ-PCBs.

| PCBs IN BLUBBER | | | |
|---|---|---|---|
| **ND pathology entity discarded (n=21)** | | **DG (n=15)** | **HG (n=6)** |
| **TotalPCBs (ppm, lw) *p*-value=0.029** | Mean | 67.75 | 18.86 |
| | Median | 36.66 | 17.36 |
| | Minimum | 4.34 | 4.43 |
| | Maximum | 218.69 | 38.97 |
| **TotalTEQ-PCBs (ppb, lw) *p*-value=0.010** | Mean | 257.91 | 49.96 |
| | Median | 154.21 | 46.05 |
| | Minimum | 18.01 | 16.18 |
| | Maximum | 1101.86 | 96.79 |
| **Samples from males. ND pathology entity discarded (n=10)** | | **DG (n=6)** | **HG (n=4)** |
| **TotalPCBs (ppm, lw) *p*-value=0.011** | Mean | 71.03 | 13.09 |
| | Median | 63.25 | 14.08 |
| | Minimum | 31.62 | 4.43 |
| | Maximum | 141.81 | 19.75 |
| **TotalTEQ-PCBs (ppb, lw) *p*-value=0.019** | Mean | 341.92 | 38.26 |
| | Median | 200.88 | 34.53 |
| | Minimum | 65.76 | 16.18 |
| | Maximum | 1101.86 | 67.79 |

The group considered as HG showed values just above the proposed threshold for adverse health effects in MMs of 17 ppm lipid basis, and below this limit when only males were analysed. On the contrary average levels of DG were found to be over three times this threshold. These preliminary findings are consistent with the known adverse health effects produced by PCB exposure in marine animals (see section 2.5.3 of this thesis). Despite these concerning data, more long term studies with greater number of samples (n) are needed before any conclusion can be drawn in order to evaluate the toxicological risk.

Regarding OCPs, no statistical significant differences or associations with pathology entities of disease were found among all pesticides studied.

4- Biopsy samples: as explained in section 3.3 of the second publication included in this thesis, higher values of OCs were found in the biopsies collected in the last years of the study.

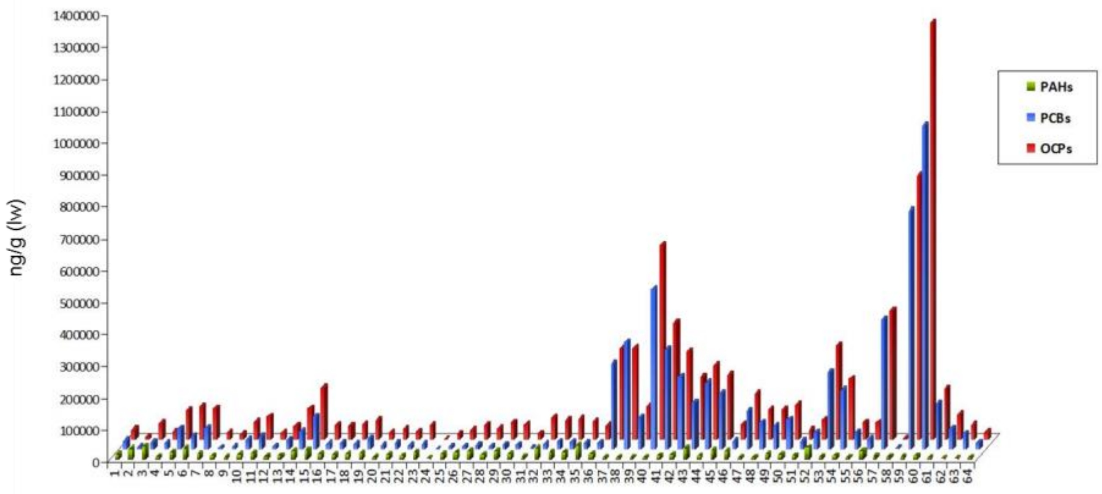



Figure 29. POP individual levels in biopsy blubber samples from live free-ranging BNDs. Units in ppb, lw.

It must be considered the small simple size for each year and also that temporal collection depended on field researches, being the first years mainly focused on the island La Gomera and the last years on the most Eastern islands. Thus, the figure 30 presented the temporal trend of pollutant levels by island segmentation.

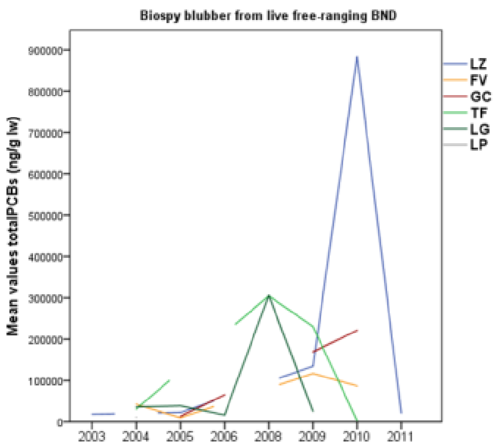



Figure 30. Mean values of PCBs (ppb, lw) in biopsy samples by island and year of collection.

No significant differences were found between islands but the graph provides more precise information of temporal tendency. As it can be seen, in general, the levels of OCs increased in the last years of sampling.

5- Comparison between blubber samples from stranded animals and biopsies from live BND. Results of K-W analysis performed between pollutant levels in blubber from stranded and live free-ranging BNDs are presented in the following table:

Table 5. K-W analysis performed between POP levels (ppb, lw) in blubber from stranded and live free-ranging BNDs.

| | | PERSISTENT ORGANIC POLLUTANTS (POPs) IN *T.truncatus* (ng $g^{-1}$ lw) | |
|---|---|---|---|
| | | LIVE FREE-RANGING (blubber biopsies) n=64 | STRANDED (blubber) n=25 |
| **TotalMPCBs** (*p*-value = 0.320) | Mean ± SD | 94386.07 ± 161173.68 | 44023.56 ± 50751.00 |
| | Median | 28197.71 (P5=3856.23 – P95=435904.26) | 25909.24 (P5=3208.80 – P95=189141.34) |
| **TotalDLPCBs** (*p-value* = 0.006) | Mean ± SD | 13486.11 ± 21777.68 | 4424.37 ± 4644.61 |
| | Median | 4596.50 (P5=789.97 – P95=60907.31) | 2933.43 (P5=364.03 – P95=17050.97) |
| **TotalPCBs** (*p*-value = 0.254) | Mean ± SD | 103822.33 ± 176960.44 | 47168.21 ± 53849.21 |
| | Median | 30783.27 (P5=4502.48 – P95=479080.99) | 27592.26 (P5=3472.10 - P95=200115.66) |
| **TotalTEQPCBs** (*p*-value = 0.756) | Mean ± SD | 266872.56 ± 491811.30 | 171974.44 ± 246552.86 |
| | Median | 67488.96 (P5=1896.23 – P95=1245087.96) | 70636.05 (P5=13220.10 – P95=956670.65) |
| **HCB** (*p*-value = 0.559) | Mean ± SD | 332.25 ± 533.93 | 244.97 ± 253.09 |
| | Median | 47.83 (P5=0.00 – P95=1158.31) | 138.34 (P5=0.96 – P95=780.71) |
| **TotalHCHs** (*p*-value = 0.000) | Mean ± SD | 206.85 ± 183.94 | 53.34 ± 70.11 |
| | Median | 147.45 (P5=11.10 – P95=591.70) | 24.44 (P5=1.95 – P95=269.71) |
| **TotalDDTs** (*p*-value = 0.499) | Mean ± SD | 104739.28 ± 202926.45 | 60959.98. ± 107050.21 |
| | Median | 24235.86 (P5=1723.10 – P95=531000.88) | 23154.59 (P5=1540.01– P95=402110.20) |
| **TotalCyclodienes** (*p*-value = 0.000) | Mean ± SD | 20413.77 ± 14736.55 | 1907.09 ± 3131.15 |
| | Median | 15928.77 (P5=2377.34 – P95=52856.72) | 1174.86 (P5=158.37 – P95=12645.37) |
| **TotalChlordanes** (*p*-value = 0.000) | Mean ± SD | 3016.88 ± 1988.48 | 445.77 ± 464.73 |
| | Median | 3017.44 (P5=356.44 – P95=7187.73) | 270.85 (P5=18.44 – P95=1421.81) |
| **Mirex** (*p*-value = 0.277) | Mean ± SD | 2947.36 ± 5434.64 | 1327.36 ± 1763.45 |
| | Median | 746.07 (P5=11.88 – P95=13118.70) | 456.18 (P5=70.03 – P95=6049.50) |
| **TotalOCPs** (*p*-value = 0.001) | Mean ± SD | 131825.71 ± 207020.17 | 64961.03 ± 111220.77 |
| | Median | 57104.12 (P5=11004.12 – P95=560385.21) | 26496.61 (P5=2200.61 – P95=421646.14) |
| **TotalPAHs** (*p*-value = 0.000) | Mean ± SD | 15932.08 ± 10232.84 | 1167.94 ± 1409.07 |
| | Median | 13598.29 (P5=2526.09 – P95=35863.94) | 788.79 (P5=106.27 – P95=5608.69) |
| **TotalTEQPAHs** (*p*-value = 0.000) | Mean ± SD | 717.79 ± 513.05 | 30.86 ± 41.63 |
| | Median | 619.81 (P5=83.50 – P95=1537.76) | 10.62 (P5=0.20 – P95=144.82) |
| **DDE/tDDT ratio** (*p*-value = 0.001) | Mean ± SD | 0.76 ± 0.17 | 0.87 ± 0.09 |
| | Median | 0.82 (P5=0.32 – P95=0.94) | 0.89 (P5=0.66 – P95=0.96) |

Significant differences were found in total DL-PCBs, total HCHs, total cyclodienes, total chlordanes, thus, in total OCPs, but the most evident difference found was in PAHs levels (see figure 31):

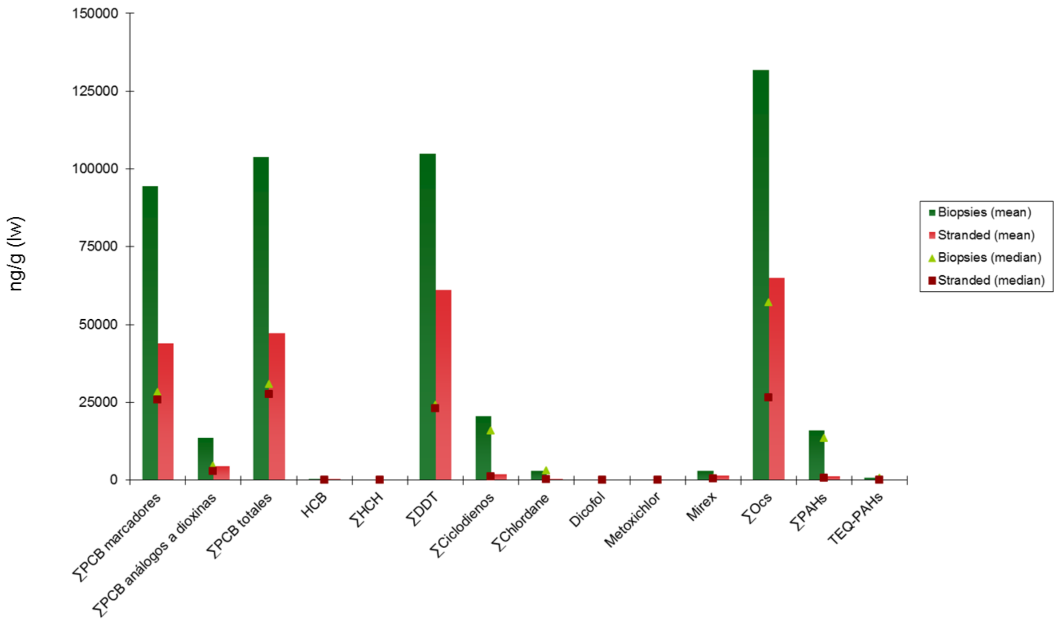



Figure 31. Levels of POP groups (ppb, lw) in biopsies from live free-ranging BND (green) and blubber from stranded BNDs (red).

Figures below graphically display these differences presented by the main groups of POPs:

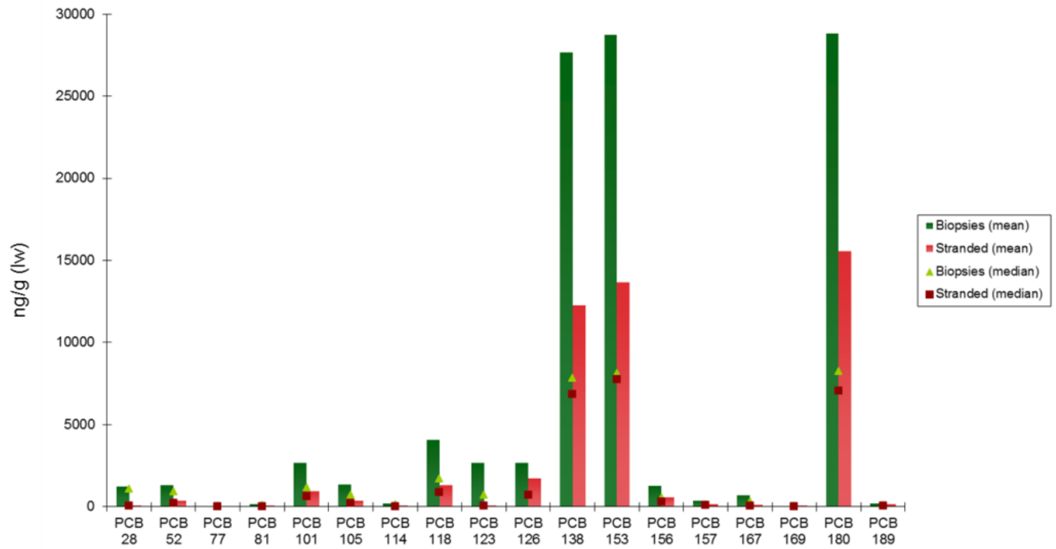



Figure 32. Levels of PCB congeners (ppb, lw) in biopsies from live free-ranging BND (green) and blubber from stranded BNDs (red).

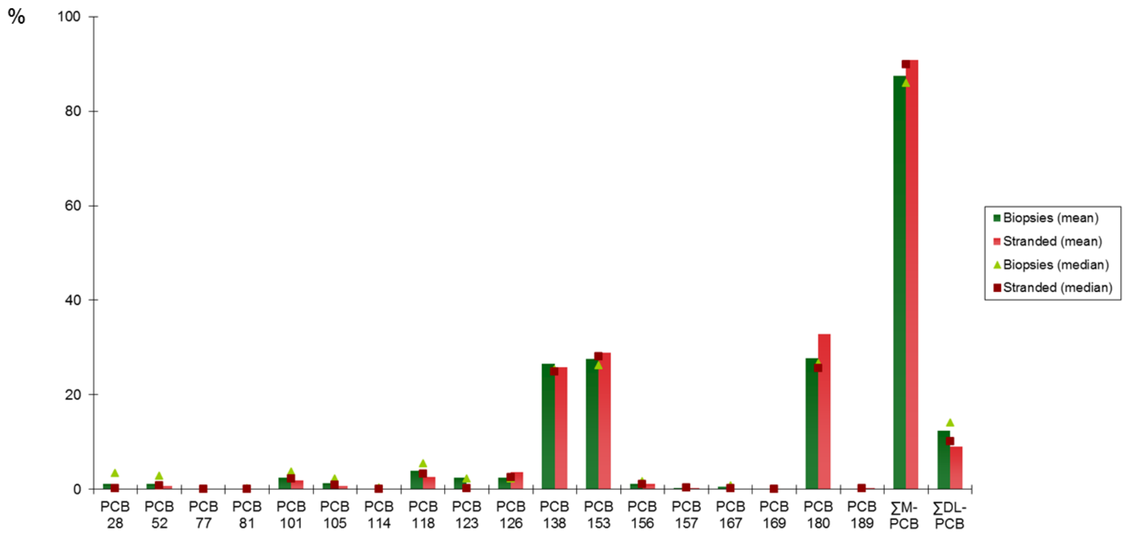



Figure 33. PCB congener profile (%) in biopsies from live free-ranging BND (green) and blubber from stranded BNDs (red).

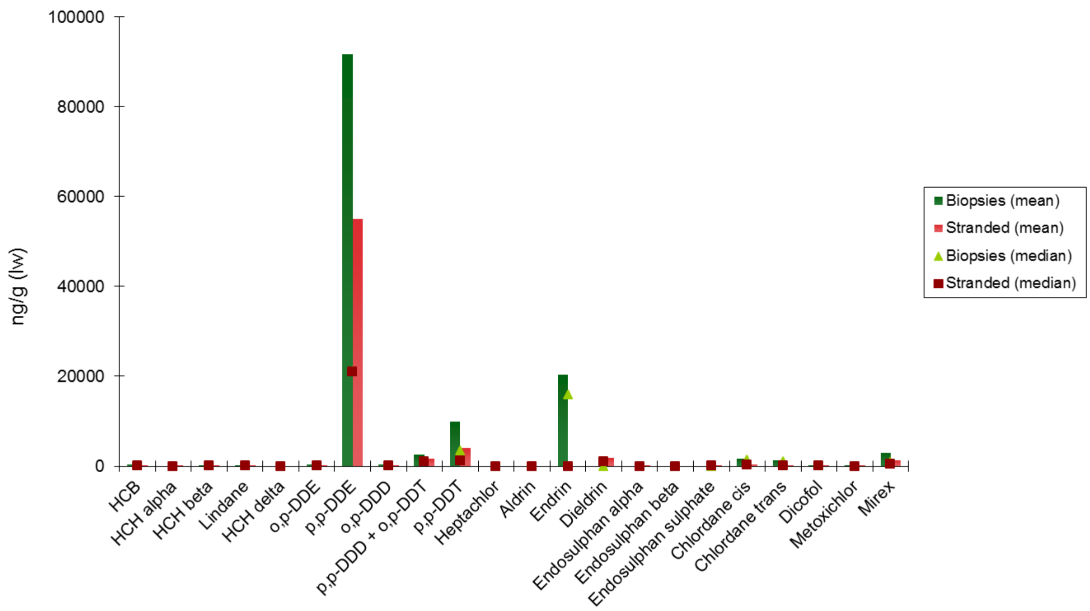



Figure 34. Levels of OCPs (ppb, lw) in biopsies from live free-ranging BND (green) and blubber from stranded BNDs (red).

PCBs and into pesticides the DDTs were predominant in both type of samples. The highly chlorinated PCB138, 153 and 180 were the prominent congeners within the 18 PCBs analysed. The pattern of PCB contaminant loads we observed was very similar in both types of samples.

The pp-DDE was the compound with the highest concentration among the 57 pollutants tested in all samples. Taking it out of the graph, the next remarkable compounds were the rest of DDTs metabolites, dieldrin and mirex for stranded samples, however, live free-ranging BND did not showed dieldrin, being remarkable to note the presence of endrin with high level (20 ppm), though not found in blubber from stranded, which could lead to a significant statistical difference in OCPs. Endrin may be broken down by exposure to high temperatures or light to form endrin ketone and endrin aldehyde what could explain the lack of this contaminant in the blubber of the BND's carcasses.

Additionally, it must be taken into account that biopsies were collected through remote biopsy darting, thus, neither age nor sex were possible to determined; however, an attempt was made to obtain them from subadults and adult individuals and assuming that sampling occurred randomly according to the sex variable; thus, this could explain the variability between both types of samples.

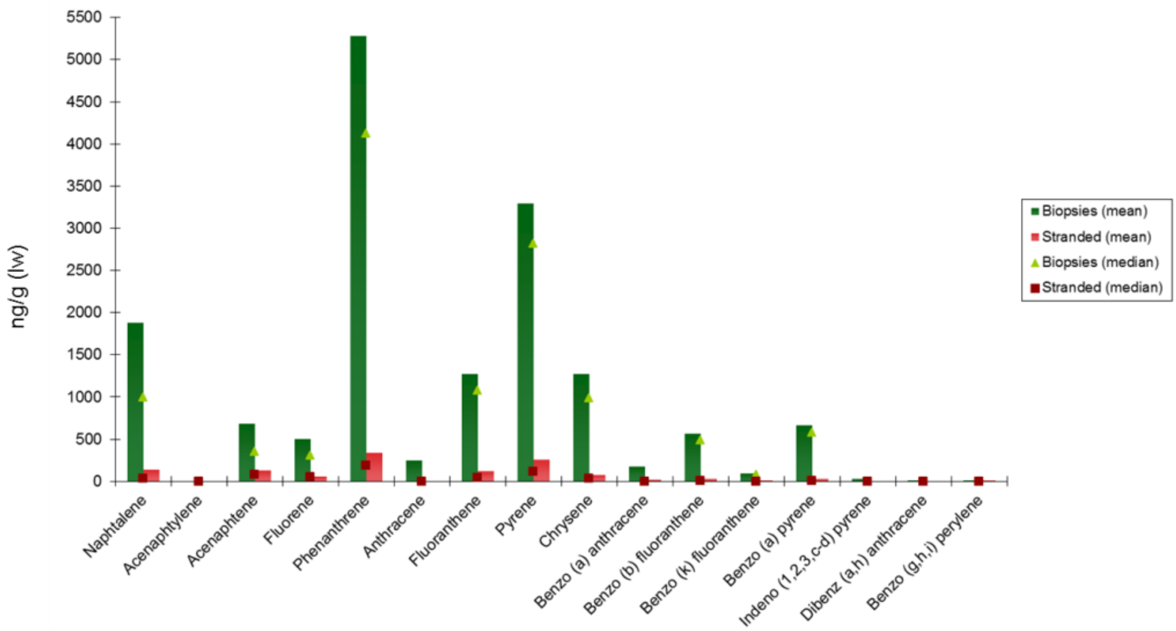



Figure 35. Levels of PAHs (ppb, lw) in biopsies from live free-ranging BND (green) and blubber from stranded BNDs (red).

Regarding PAHs, all samples showed detectable values for any of the 16 studied. Phenanthrene was the most frequently detected and at the highest levels as it was indicated in a previous research of contaminants in sea turtles of the Canary Islands (Camacho et al., 2012), followed by pyrene.

It is remarkable that biopsy samples showed PAHs concentrations more than 10 times higher compared to stranded animals (Fig. 35), suggesting that analysing PAHs in stranded animals may not be as useful that previously thought.

In a year segmentation study, differences can be seen between results from stranded and live free-ranging BND. In biopsy blubber, mean values of POPs increased in 2008 and 2010 (Fig. 36, above), and in the case of blubber from stranded animals the higher levels were found in 2005 and 2010 (Fig. 36, below). Difference found in 2008 may be because most of biopsies were collected in La Gomera, island where there is not blubber from stranded BND available for this study (Fig. 37).

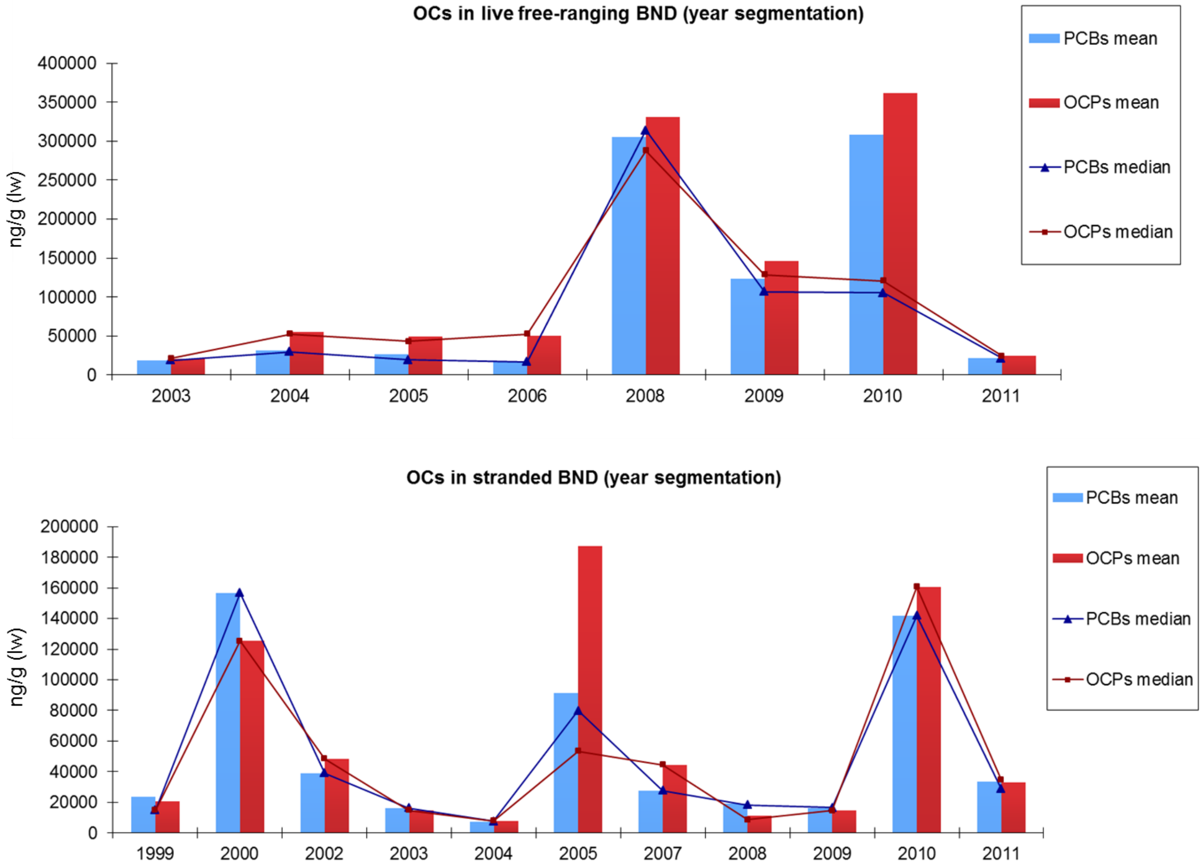



Figure 36. OC levels (ppb, lw) in biopsies from live free-ranging BND by year of collection (above); in blubber from stranded BND by year of stranding (below).

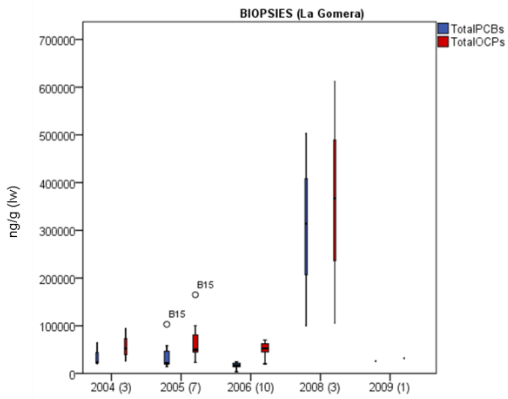
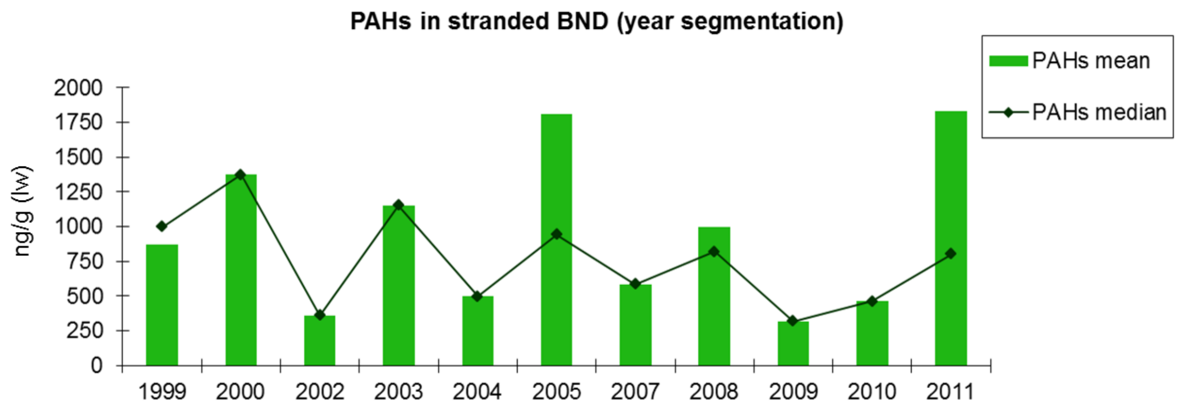



Figure 37. Levels of OCs (ppb, lw) from biopsies collected in La Gomera, year segmentation.

The differences between biopsies samples and blubber from carcasses of BND could be also attributed to the stratification of lipids and OCs in the blubber in layers, because biopsies are not as deep as the blubber samples taken from stranded dolphins (Hobbs et al., 2003). This leads to careful comparison of biopsy results and other data. The knowledge of the structure and dynamic of the blubber is important for the toxicology of MM to understand the mobilization of pollutants from the blubber and investigate possible adverse health effects (Wolkers et al., 2004).

Regarding PAHs a decreasing temporal trend is seen in the biopsy samples but not in the blubber form stranded (Fig. 38). Additionally, as said before, live BND showed 10 times greater levels of PAHs what seems that these compounds in carcasses of stranded BND volatilize and do not provide useful data for PAHs study in this species.



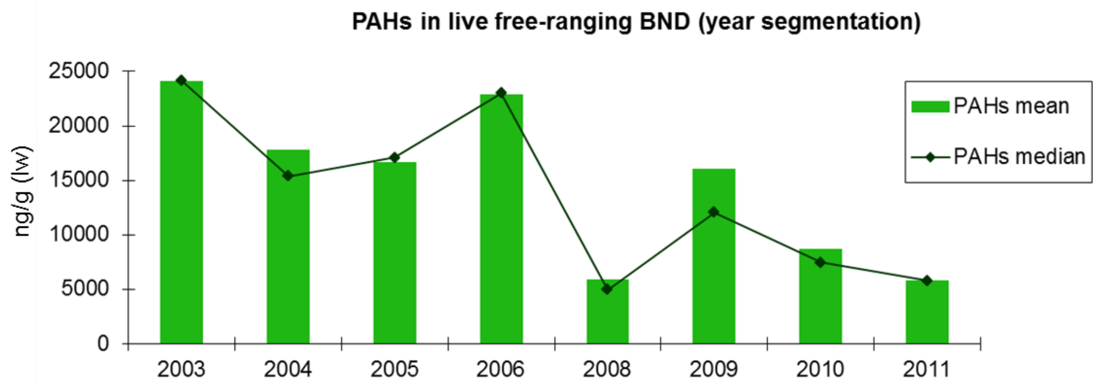




Figure 38. Levels of PAHs (ppb, lw) in blubber from stranded BND (above) and biopsies of live free-ranging BND (below), year segmentation.

# 5. PUBLICATIONS

## 5.1. Assessment of the levels of polycyclic aromatic hydrocarbons and organochlorine contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean

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# Assessment of the levels of polycyclic aromatic hydrocarbons and organochlorine contaminants in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean

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ABSTRACT

The concentrations of 18 polychlorinated biphenyls (PCBs), 23 organochlorine pesticides (OCPs), and 16 polycyclic aromatic hydrocarbons (PAHs) were determined in the blubber and liver of 27 bottlenose dolphins (*Tursiops truncatus*) stranded along the Canary Islands coasts from 1997 to 2011. DDTs (mean of 60,960 and 445 ng/g lw., respectively) and PCBs (mean of 47,168 and 628 ng/g lw., respectively) were the predominant compounds in both tissues. Among PCBs the highly chlorinated PCB 180, 153 and 138 were the predominant congeners. We found a *p,p′*-DDE/∑DDTs ratio of 0.87 in blubber and 0.88 in liver, which is indicative of DDT ageing. All the samples showed detectable values of any of the 16 PAH studied. Phenanthrene was the most frequently detected and at the highest concentration. According to our results, concentrations of OCPs, and especially PCBs, are still at toxicologically relevant levels in blubber of bottlenose dolphins of this geographical area.



## 1. Introduction

Persistent organic pollutants (POPs) are toxic chemicals that are resistant to degradation in the environment and biota. It is a well-known fact that these anthropogenic pollutants easily reach aquatic environments and that contaminants in water may dissipate. Thus, marine life serves as a living record of the pollutants discharged into the environment. Due to their fat solubility and resistance to chemical and biological degradation, ingestion of certain classes of POPs by animals leads to bioaccumulation throughout their lives, generally in the fatty tissues, and to biomagnification in the food chain (Safe, 1994). Among the POPs, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are highly prevalent in marine vertebrates. Because of their efficient metabolization, strictly speaking, polycyclic aromatic hydrocarbons (PAHs) cannot be considered as POPs, but due to their high prevalence in the environment and their lipophilicity, PAHs are usually considered as POPs. The majority of POPs, such as PCBs and OCPs, are currently banned from use and are no longer produced or used around the world; therefore, their levels have been constantly declining through the years. Nevertheless, relevant amounts of these pollutants still persist in the environment, and especially in those areas that are close to highly industrialized regions, where the anthropogenic pressure could contribute to explain the elevated concentration of pollutants found in cetaceans of these areas (Borrell and Aguilar, 2007). In Fig. 1 we show the most relevant areas of contamination for marine mammals that have been reported in literature (Esperón Fajardo, 2005).

Bottlenose dolphins (*Tursiops truncatus*) are distributed worldwide through tropical and temperate inshore, coastal, shelf, and oceanic waters, and the presence of resident populations of bottlenose dolphins in the Canary Islands is well recognized (Aguilar et al., 2000). This species has been included in the Annex II (animal and plant species of community interest whose conservation requires the designation of special areas of conservation) of the European Union's Habitats Directive (EC, 1992). Extensive research on the levels of chemical contamination of this species has been

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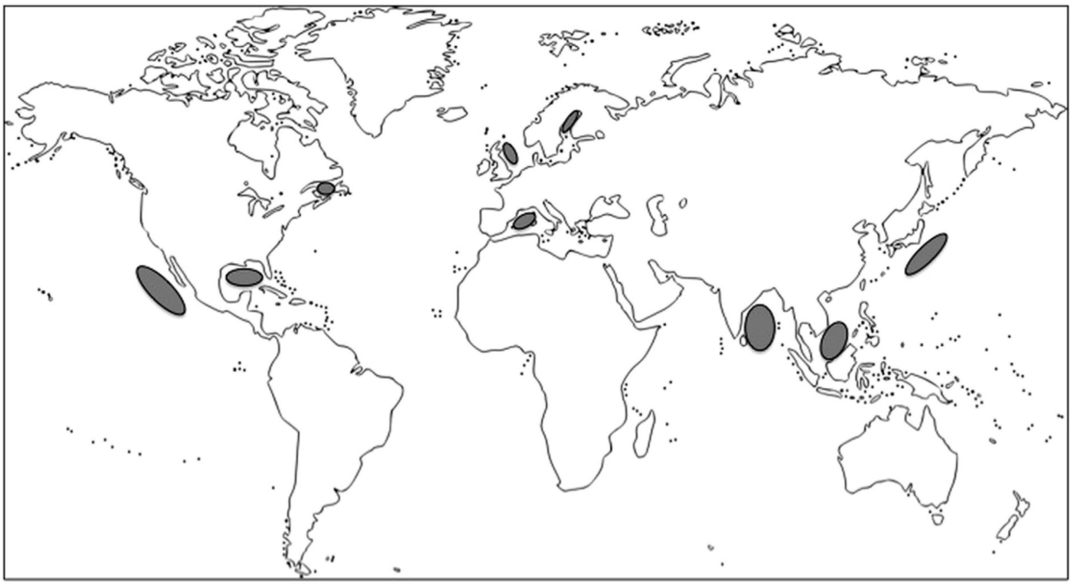

**Fig. 1.** World map indicating the most relevant polluted areas for marine mammals showed in literature (Esperón Fajardo, 2005).

done all around the world (Kucklick et al., 2011; Lailson-Brito et al., 2012; Law et al., 2012; Weijs et al., 2013; Wu et al., 2013; Yordy et al., 2010a), but very few data are available about organochlorine residues in cetaceans from this part of the Eastern Atlantic Ocean (Carballo et al., 2008).

After a morbillivirus epizooty occurred in Mediterranean sea from 1990 to 1992 that affected striped dolphins (Domingo et al., 1995), it was reported that PCBs were implicated in the development of this disease (Aguilar and Borrell, 1994). After this episode many articles have been published about contaminant levels in cetaceans and its role in facilitating infectious diseases (Ross, 2002). Many other adverse health effects have been related to the chemical load in cetaceans, such as immune suppression, endocrine disruption, reproductive impairment or carcinogenic effects, among others (Lahvis et al., 1995; Martineau et al., 1994; Schwacke et al., 2002, 2012; Wells et al., 2005; Yap et al., 2012). Several adverse health effects of pollutants have been described also for bottlenose dolphins. Thus, Lahvis et al. (1995) correlated concentrations of PCBs and DDT in the blood of inshore bottlenose dolphins with a decline in their immune system function, and also data that indicate a toxic potential of their chemical load have been reported in the literature (Schwacke et al., 2002; Wells et al., 2005).

Since 1992, the assessment of the health status of the stranded cetaceans in the Canary Islands has been carried out in the context of a long-term research project of the Cetacean Research Unit, University of Las Palmas de Gran Canaria (ULPGC). Each bottlenose dolphin that is found stranded in this region is submitted for a systematic necropsy and sampling. Samples for toxicological analyses are systematically stored in the Cetacean Tissue Bank (CTB) of this research unit. One of the aims of this project is to evaluate the levels of contaminants in tissues from the resident cetacean species. Thus, we have evaluated the concentrations of 57 organic pollutants, including 23 OCPs, 18 PCBs and 16 PAHs, in tissues from 27 bottlenose dolphins stranded along the coasts of the Canary Islands during the period 1997–2011. This study was designed to obtain an overall picture of the chemical contamination levels in the resident population of bottlenose dolphins from the Canary Islands. To our knowledge this is the first study that provides data of the contamination by PAHs in animals from this species that live in the Eastern Atlantic Ocean.

## 2. Materials and methods

### 2.1. Study area

The Canary Islands are located 1600 km away from southwest Spain, in the Atlantic Ocean and just 63 miles from the nearest point on the North African coast (south west of Morocco) (Fig. 2). The oceanographic characteristics and its strategic situation make possible the presence of many species of cetaceans. Thus, more than 30 species of cetaceans have been identified in the waters of this archipelago including both, migratory species and locally resident populations of bottlenose dolphins (*T. truncatus*), short-finned pilot whales (*Globicephala macrorhynchus*), and sperm whales (*Physeter macrocephalus*). Due to the biodiversity of this archipelago, the Canary Islands have been designated as a Particularly Sensitive Sea Area for the International Maritime Organization (IMO), and therefore considered a protected marine area. Geographically, the Islands are part of the African continent, yet from a historical, economic, political and sociocultural point of view the Canaries are completely European. It is noteworthy that in this archipelago large quantities of organochlorine pesticides have been used in the past, given the important role of agriculture in the economy of the region (Diaz-Diaz and Loague, 2001).

### 2.2. Sampling

25 blubber and 26 liver samples were collected from 27 dolphins stranded in different coastal areas of Canary Islands (see Fig. 2) over the period of 1997–2011, and stored at the CTB of the ULPGC at −80 °C until chemical analysis. The choice of tissue samples was done according to the literature, since it has been described that the highest contaminant levels in bottlenose dolphins are found in blubber followed by liver (Storelli et al., 2012).

Regarding the necropsy protocol, the tissue sampling and state of decomposition of stranded specimens were determined

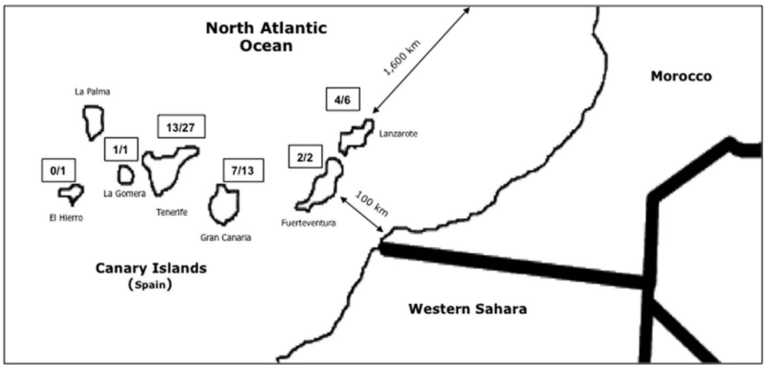


**Fig. 2.** Location of the studied samples versus the total stranded bottlenose dolphins from 1997 to 2011 (27 samples/51 stranded animals).

following Geraci and Lounsbury (2005). 12 males and 15 females (2 pregnant) were included in this study. The animals were classified as adults (9), subadults (10), juveniles (6), calves (1), and newborns (1). As regards the conservation status of the animals, these were classified as very fresh, fresh, moderately autolytic, autolytic, and very autolytic. More than 55% of the samples were included in the first three categories. According to morphological characteristics, such as the development of the dorsal muscle and bone prominence, the body condition of the animals was established as good, moderate, poor and very poor, being more than 60% of the animals classified into the two first groups. In Table 3 we show the distribution of the animals into these categories.

### 2.3. Analytes of interest

A total of 57 analytes belonging to three relevant groups of POPs were selected for this study. The 23 OCPs and metabolites included were the diphenyl-aliphatics (methoxychlor, *o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, and dicofol); the persistent and bioaccumulative contaminant hexachlorobenzene (HCB); the four isomers of hexachlorocyclohexane (α-, β-, δ-, and γ-HCH); the cyclodienes heptachlor, dieldrin, aldrin and endrin, chlordane (*cis-* and *trans*-isomers) and mirex; endosulfan (α- and β-isomers) and endosulfan sulfate. With respect to the PCBs we decided to include a total of 18 congeners: the dioxin-like congeners (DL-PCBs, IUPAC numbers# 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189), and the seven PCBs that are considered markers of environmental exposure (M-PCBs, IUPAC numbers# 28, 52, 101, 118, 138, 153 and 180). Finally, we also included in the suite of analytes the list of the 16 EPA priority PAHs that is often targeted for measurement in environmental samples (naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene).

### 2.4. Sample preparation

Because of the known fact that the contaminants included in this study are totally lipid-soluble and therefore found in the lipid fraction of tissues, we firstly extracted the fat from blubber and liver tissues. 1 g of blubber or 5 g of liver were finely chopped with scissors and homogenized in 5 ml ultrapure water with a disperser (Ultra-turrax, IKA, China). This homogenate was spiked with the 10 ppm-surrogates mix in acetone to yield a final concentration of 100 ppb and mixed with 30 g of diatomaceous earth to absorb all the humidity. The method of extraction and clean up followed that recommended by the European Standard for the determination of pesticides and PCBs in fatty foods (EN, 1996a,b), and whose validity has been previously proven in our laboratory for different fatty samples of animal origin (Almeida-Gonzalez et al., 2012; Luzardo et al., 2012, 2013a, 2014). This method combines an automated Soxhlet extraction method (FOSS Soxtec Avanti 2055) with a purification step using gel permeation chromatography (GPC), and gives acceptable recoveries that ranged between 74.5% and 104.7%. Briefly, the Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed, Denmark) apparatus consisted of an extraction unit, a control unit and a drive unit. The blubber or liver tissue, prepared as described above, was inserted into the extraction unit, 20 ml of solvent (dichloromethane) were added to the extraction cups in a closed system, and the cups were heated with an electric heating plate. The three-step extraction consisted of boiling, rinsing, and solvent recovery. The solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40 °C to prevent analyte loses. The results were calculated as total amount of fat (g) per 100 g tissue. The percentages of fat of blubber tissue ranged from 10.95 to 40.63% (mean 21.29%), and the percentages of fat of liver tissue ranged from 10.85 to 19.42% (mean 19.42%). As the status of conservation of the stranded dolphins was inhomogeneous, to more accurately compare the levels between individuals we used 100 mg of fat of each of the Soxhlet extracted samples, which were carefully weighted using a precision balance into a zeroed glass tube. The weighted fat was dissolved in 2 ml of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (BioBeads SX-3), using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 ml/min as the eluent. The first 23 min of elution, containing the great majority of the lipids (>98%), were discarded. The 23–85 min elution volume (124 ml), containing all the analytes that were co-extracted with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally the solvent was evaporated to dryness under a gentle nitrogen stream. The analytes were re-dissolved in 1 ml of cyclohexane, and used for the chromatographic analysis, without any further purification. Thus, the quantification directly represented the amount of pollutants contained in 100 mg (1/10 g), and by multiplying the results for a 10 we obtained the amount of pollutants per gram.

### 2.5. Procedure of chemical analysis

Gas chromatography analyses of 57 contaminants, 4 surrogates (PCB 12, PCB 202, *p,p′*-DDE-d8, acenaphtylene D8), and 3 Internal standards (ISs, tetrachloro-m-xylene, heptachloro epoxide trans, and benzo[a]pyrene D12) were performed in a single run on a Thermo Trace GC Ultra equipped with a TriPlus Autosampler and coupled to a Triple Quadrupole Mass Spectrometer Quantum XLS (Thermo Fisher Scientific Inc., Waltham, MA, USA), as previously described and validated in our laboratory (Camacho et al., 2012, 2013; Luzardo et al., 2013b). We added 20 µl of the ISs mixture prepared at 1 ppm in cyclohexane, just before the GC–MS/MS analysis. Since no matrix effect has been observed with this method, all quantifications were performed against a calibration curve in cyclohexane of 10 points (0.05–40 µg $L^{-1}$).

### 2.6. Statistical analysis

Database management and statistical analysis were performed with IBM SPSS Statistics v 19.0. Because POPs levels did not follow a normal distribution, non-parametric Mann–Whitney *U*-test and Kruskal Wallis test were used for analysis.

### 2.7. Quality control

The recoveries of the 57 analytes and surrogates were acceptable with this method since in all the cases were above of 74%. All the individual measurements were corrected by the recovery efficiency for each analyte. All the measurements were done in triplicate and the values used for calculations were the mean of the three data. In each batch of samples two controls were included every 12 samples: a reagent blank consisting on a vial containing only cyclohexane; and an internal laboratory quality control (QC) consisting on melted butter spiked at 20 ng $g^{-1}$ of each of the analytes that was processed with the same method than the samples. The batch analyses were considered valid when the values of the analytes in the QC were within a 10% of deviation of the theoretical value.

## 3. Results and discussion

Our results showed that pollutants were present at very high levels in many animals. Dichlorodiphenyltrichloroethanes (DDTs) and PCBs were the predominant compounds in both tissues but presented higher concentrations in blubber (mean of 60.960 and 47.168 ng $g^{-1}$ lw respectively; 31.771 and 37.816 ng $g^{-1}$ lw without outlier values) than in liver samples (mean of 445 and 628 ng $g^{-1}$ lw respectively). A summary of the levels of PCBs, OCPs and PAHs found in both tissues (blubber and liver) are shown in Tables 1 and 2, and the individual values have been graphically represented in Figs. 3–5. All concentrations are expressed in ng $g^{-1}$ lipid weight (lw).

As it usually occurs in this type of studies, in our set of samples we have found large intra-population variability in tissue pollutant levels. Thus, we have found individuals, such as CET 311 and 314, which showed extremely high values of all the pollutants included in this study. On the contrary in other individuals, such as CET 93, CET 458 or CET 562, the levels found could be considered as relatively low. As expected, higher levels were found in blubber, ∑PCBs (3100–218,691 ng $g^{-1}$ lw, mean 47,168 ng $g^{-1}$ lw), ∑OCPs (2158–434,780 ng $g^{-1}$ lw, mean 64,961 ng $g^{-1}$ lw), and ∑PAHs (75–5761 ng $g^{-1}$ lw, mean 1168 ng $g^{-1}$ lw) than in liver, ∑PCBs (52–2740 ng $g^{-1}$ lw, mean 628 ng $g^{-1}$ lw), ∑OCPs (32–2208 ng $g^{-1}$ lw, mean 503 ng $g^{-1}$ lw), ∑PAHs (10–163 ng $g^{-1}$ lw, mean 23 ng $g^{-1}$ lw). According to our results *p,p′*-DDE (that was the compound detected at the highest concentrations among the 57 pollutants studied), *p,p′*-DDT, PCB 180, PCB 153, PCB 138, and phenanthrene were the predominant compounds in both tissues, as they were detectable in 100% of the samples, and in all the cases their concentrations were higher in blubber than in liver samples.

Regarding the levels of OCPs (Fig. 3), apart from the above-mentioned high level of *p,p′*-DDE (54,959 and 408 ng $g^{-1}$ lw, in blubber and liver respectively), it was also relevant in our series the presence of *p,p′*-DDT (4062 and 4 ng $g^{-1}$ lw), dieldrin (1842 and 23 ng $g^{-1}$ lw) and mirex (1327 and 21 ng $g^{-1}$ lw). When we compare our results with those published in a review on the geographical and temporal variations of pollutant levels in bottlenose dolphins worldwide (Aguilar et al., 2002), the concentration of ∑DDTs of the bottlenose dolphins of the Canaries could be considered as relatively high as ranged between the levels found in the US Atlantic coast (<50 ppm) (Kucklick et al., 2011) and those from the Mediterranean sea (100–500 ppm) (Corsolini et al., 1995). Our group has previously described high levels of ∑DDTs in the environment of the Canary Islands that affect to human beings (Luzardo et al., 2012, 2009; Zumbado et al., 2005), and several reasons have been postulated to explain these levels, such as the intensive use of this pesticide in the past in these islands, or the vicinity to North African countries (<60 miles to Moroccan coast) where the use of this pesticide is still allowed. It is interesting to note that we found *p,p′*-DDE/∑DDTs ratios of 0.87 and 0.88 in blubber and liver respectively, which would be indicative of DDT ageing. On the other hand the ∑DDTs/PCBs ratio that we found is 0.87. Carballo et al. (2008) had previously reported a value of 0.63 for this ratio, which was interpreted by the authors as indicative of a predominance of industrial inputs over agricultural sources. Our study extends both, the sample size and the range of years that are

**Table 1**
Levels of polycyclic aromatic hydrocarbons and organochlorine pollutants (ng $g^{-1}$ lipid weight) in blubber and liver of bottlenose dolphins.

| *Tursiops truncatus* (*n* = 27) | | Tissue samples | |
|---|---|---|---|
| | | Blubber (*n* = 25) | Liver (*n* = 26) |
| Total PCBs | Mean ± SD | 47,168.21 ± 53,849.21 | 628.42 ± 664.80 |
| | Median | 27,592.26 (P5 = 3472.10–P95 = 20,011.66) | 415.61 (P5 = 55.80–P95 = 2553.81) |
| | Minimum | 3100.41 | 52.10 |
| | Maximum | 218,691.47 | 2739.85 |
| Total OCPs | Mean ± SD | 64,961.03 ± 111,220.77 | 503.23 ± 526.94 |
| | Median | 26,496.61 (P5 = 2200.61–P95 = 421,646.14) | 353.66 (P5 = 37.26–P95 = 2117.95) |
| | Minimum | 2158.41 | 32.24 |
| | Maximum | 434,779.74 | 2208.26 |
| Total PAHs | Mean ± SD | 1167.94 ± 1409.07 | 37.69 ± 36.21 |
| | Median | 788.79 (P5 = 106.27–P95 = 5608.69) | 22.78 (P5 = 10.86–P95 = 146.34) |
| | Minimum | 75.30 | 10.17 |
| | Maximum | 5761.33 | 162.83 |

SD (Standard deviation).

**Table 2**
Organic pollutant levels (ng/g lipid weight) in blubber and liver of 27 bottlenose dolphins.

| Cetacean | Sex | Age | Year | Blubber (ng/g lipid weight) | | | | Liver (ng/g lipid weight) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | tPCBs (B) | tOCPs (B) | tPAHs (B) | tPOPs (B) | tPCB (L) | tOCPs (L) | tPAHs (L) | tPOPs (L) |
| CET 43 | F | Adult | 1997 | | | | | 351.80 | 303.15 | 17.76 | 672.71 |
| CET 78 | M | Subadult | 1999 | 52,263.89 | 42,647.70 | 352.69 | 95,264.28 | 932.46 | 531.00 | 18.44 | 1481.89 |
| CET 93 | M | Juvenile | 1999 | 4426.07 | 5279.70 | 998.45 | 10,704.22 | 672.19 | 600.46 | 12.14 | 1284.79 |
| CET 94 | M | Juvenile | 1999 | 14,963.10 | 14,959.94 | 1267.39 | 31,190.42 | 320.36 | 258.69 | 14.73 | 593.78 |
| CET 99 | F | Juvenile | 2000 | 156,772.11 | 125,460.95 | 1375.25 | 283,608.31 | 523.21 | 413.70 | 24.85 | 961.75 |
| CET 171 | F | Adult | 2002 | 38,970.85 | 48,543.67 | 361.73 | 87,876.25 | 946.12 | 453.74 | 22.56 | 1422.42 |
| CET 203 | M | Subadult | 2003 | 11,434.86 | 13,224.03 | 2129.65 | 26,788.54 | 2208.31 | 1950.23 | 33.95 | 4192.49 |
| CET 209 | F | Juvenile | 2003 | 21,305.55 | 16,436.38 | 178.51 | 37,920.45 | 416.37 | 232.84 | 29.72 | 678.93 |
| CET 231 | PF | Adult | 2004 | 7629.47 | 8016.76 | 496.33 | 16,142.56 | | | | |
| CET 286 | M | Juvenile | 2005 | 31,622.28 | 35,886.22 | 941.24 | 68,449.74 | 269.02 | 257.54 | 23.00 | 549.55 |
| CET 296 | F | Subadult | 2005 | 35,937.56 | 21,770.69 | 948.66 | 58,656.91 | 62.91 | 46.58 | 35.64 | 145.13 |
| CET 305 | F | Subadult | 2005 | 79,919.29 | 53,522.45 | 839.32 | 134,281.06 | 584.40 | 601.63 | 16.92 | 1202.95 |
| CET 311 | M | Subadult | 2005 | 90,743.87 | 434,779.74 | 5761.33 | 531,284.94 | 237.57 | 2208.26 | 36.77 | 2482.60 |
| CET 314 | F | Subadult | 2005 | 218,691.47 | 391,001.07 | 548.27 | 610,240.81 | 443.99 | 553.91 | 19.89 | 1017.78 |
| CET 403 | M | Subadult | 2007 | 27,592.26 | 44,539.20 | 586.16 | 72,717.62 | 207.30 | 257.50 | 70.98 | 535.78 |
| CET 407 | F | Adult | 2008 | 36,655.52 | 26,496.61 | 2153.44 | 65,305.57 | 2739.85 | 1117.58 | 40.42 | 3897.86 |
| CET 420 | PF | Adult | 2008 | 13,699.27 | 5587.92 | 202.11 | 19,489.30 | 216.97 | 107.04 | 42.51 | 366.51 |
| CET 450 | F | Subadult | 2008 | 22,754.66 | 11,537.06 | 788.79 | 35,080.51 | 620.08 | 459.81 | 92.24 | 1172.13 |
| CET 458 | F | Subadult | 2008 | 4339.38 | 2158.41 | 850.11 | 7347.91 | 208.09 | 175.18 | 58.13 | 441.40 |
| CET 505 | M | Juvenile | 2009 | 19,754.82 | 15,226.89 | 75.30 | 35,057.02 | 1189.63 | 674.10 | 162.83 | 2026.56 |
| CET 509 | M | Adult | 2009 | 13,198.99 | 14,157.98 | 563.57 | 27,920.54 | 52.10 | 48.71 | 16.80 | 117.60 |
| CET 543 | M | Adult | 2010 | 141,796.36 | 160,620.32 | 462.79 | 302,879.48 | 414.86 | 404.16 | 19.15 | 838.17 |
| CET 562 | F | Adult | 2011 | 3100.40 | 2299.07 | 461.20 | 5860.67 | 498.43 | 240.26 | 115.70 | 854.39 |
| CET 564 | M | Adult | 2011 | 74,238.80 | 36,413.68 | 5252.53 | 115,905.02 | 62.69 | 32.24 | 17.46 | 112.38 |
| CET 584 | F | Calf | 2011 | 21,855.07 | 33,003.01 | 474.39 | 55,332.47 | 199.94 | 159.48 | 12.34 | 371.76 |
| CET 592 | F | Newborn | 2011 | | | | | 255.40 | 266.91 | 14.95 | 537.25 |
| CET 595 | M | Subadult | 2011 | 35,539.33 | 60,456.24 | 1129.24 | 97,124.81 | 1704.81 | 729.25 | 10.17 | 2444.23 |
| Mean | | | | 47,168.21 | 64,961.03 | 1167.94 | 66,128.97 | 628.42 | 503.23 | 37.69 | 1169.34 |
| Standard deviation | | | | 53,849.21 | 111,220.77 | 1409.07 | 111,842.82 | 664.80 | 526.94 | 36.21 | 1062.20 |
| Median | | | | 27,592.26 | 26,496.61 | 788.79 | 28,650.05 | 415.61 | 353.66 | 22.78 | 846.28 |

CET 311 and 314 are outlier values considering blubber concentrations. Neither blubber samples of CET 43 and CET 592 nor liver sample of CET 231 were available.

**Table 3**
Specimen details for bottlenose dolphins stranded in Canary Islands waters between 1997 and 2011.

| Reference | Year | Place[a] | Stranding | Sex[b] | Age | Conservation State[c] | Body condition[d] |
|---|---|---|---|---|---|---|---|
| CET 43 | 1997 | TF | Passive | F | Adult | 2 | Very poor |
| CET 78 | 1999 | GC | Active | M | Subadult | 1 | Poor |
| CET 93 | 1999 | TF | Passive | M | Juvenile | 2 | Poor |
| CET 94 | 1999 | TF | Passive | M | Juvenile | 2 | Good |
| CET 99 | 2000 | GC | Passive | F | Juvenile | 4 | Poor |
| CET 171 | 2002 | TF | Passive | F | Adult | 2 | Good |
| CET 203 | 2003 | FV | Passive | M | Subadult | 5 | n.a. |
| CET 209 | 2003 | GC | Passive | F | Juvenile | 3 | Poor |
| CET 231 | 2004 | TF | Passive | F (P) | Adult | 5 | n.a. |
| CET 286 | 2005 | GC | Passive | M | Juvenile | 4 | Good |
| CET 296 | 2005 | GC | Active | F | Subadult | 1 | Good |
| CET 305 | 2005 | LZ | Active | F | Subadult | 1 | Moderate |
| CET 311 | 2005 | FV | Active | M | Subadult | 2 | Poor |
| CET 314 | 2005 | GC | Passive | F | Subadult | 4 | Good |
| CET 403 | 2007 | TF | Passive | M | Subadult | 4 | Moderate |
| CET 407 | 2008 | TF | Passive | F | Adult | 2 | Poor |
| CET 420 | 2008 | GC | Passive | F (P) | Adult | 4 | Moderate |
| CET 450 | 2008 | TF | Passive | F | Subadult | 1 | Moderate |
| CET 458 | 2008 | TF | Passive | F | Subadult | 5 | Good |
| CET 505 | 2009 | TF | Passive | M | Juvenile | 4 | n.a. |
| CET 509 | 2009 | TF | Passive | M | Adult | 3 | Moderate |
| CET 543 | 2010 | LZ | Passive | M | Adult | 4 | n.a. |
| CET 562 | 2011 | TF | Passive | F | Adult | 4 | n.a. |
| CET 564 | 2011 | LZ | Passive | M | Adult | 2 | Good |
| CET 584 | 2011 | TF | Passive | F | Calf | 4 | n.a. |
| CET 592 | 2011 | LG | Passive | F | Newborn | 3 | Good |
| CET 595 | 2011 | LZ | Passive | M | Subadult | 2 | Very poor |

[a] LZ, Lanzarote; FV, Fuerteventura; GC, Gran Canaria; TF, Tenerife; LG, La Gomera.
[b] M, male; F, female; F (P), pregnant female.
[c] 1, very fresh; 2, fresh; 3, moderate autolysis; 4, advanced autolysis; 5, very advanced autolysis.
[d] n.a. = data not available.

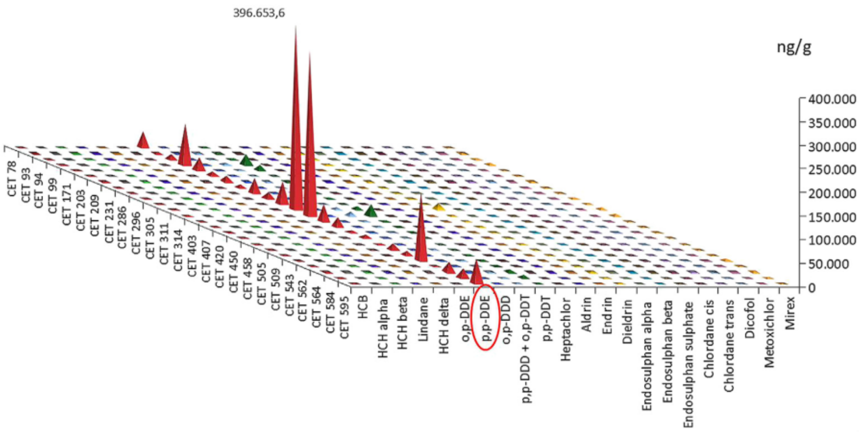


**Fig. 3.** Concentrations of OCPs in the blubber samples.

under investigation, so we consider that our calculations could be considered as more accurate than the previous report. However, this higher value in our study could be also indicating the existence of recent DDTs inputs in this area.

As regards to PCB contamination in this region, previous research of our group in humans and food items have revealed that the level is relatively low (Almeida-Gonzalez et al., 2012; Henriquez-Hernandez et al., 2011; Luzardo et al., 2012), which was expected as the Canary Islands is a relatively non-industrialized area. However, the levels that we found in bottlenose dolphins from the waters of the Canaries are higher than those of dolphins living close to industrialized regions of North Europe (10–30 ppm) (Wells et al., 1994) and similar to those found in the also industrialized Western and Eastern coasts of the United States (30–100 ppm) (Yordy et al., 2010b). It is a known fact that the levels of contamination by POPs can be enormously different among individuals depending on the geographical region in which they live, but it seems obvious that there are some other unknown factors that are influencing the levels of PCBs in these dolphins. Nevertheless, the levels in our series are much lower than those found in Mediterranean bottlenose dolphins (>500 ppm), which are well known for their extremely high pollutant burden due to the semi-enclosed nature of the sea (Aguilar and Borrell, 1994; Fossi et al., 2006; Storelli and Marcotrigiano, 2003).

As shown in Fig. 4, the most prevalent congeners in our set of samples were the highly chlorinated PCBs 138, 153 and 180, which is in agreement with previous reports in other populations of bottlenose dolphins (Marsili and Focardi, 1996; Storelli et al., 2012; Wafo et al., 2005). These three congeners accounted for 77.8–92.9% of the total PCBs concentrations in blubber, and 74.8–92.6% in liver, and in general M-PCBs represented more than 90% of the total PCBs in our series. Other PCBs that were also found with high frequency and concentration were PCBs 126, 118 and 101, showing a very similar profile in both tissues. From experimental studies of PCBs effects in seals, European otters and minks, it has been proposed a blubber threshold level for adverse health effects of 17,000 ng $g^{-1}$ lw (Kannan et al., 2000). Taking this data as a reference, the mean levels of PCBs that we have found in blubber samples almost tripled this threshold, indicating a potential risk for the health of this population of bottlenose dolphins. Moreover, it should be highlighted that the mean value of PCB 126 was 1719 ng $g^{-1}$ lw (161–31,090 ng $g^{-1}$ lw) in blubber and 23 ng $g^{-1}$ lw (1–127 ng $g^{-1}$ lw) in liver. These values can be considered extremely high and worrisome due to the high toxicity of this congener. When the Toxic

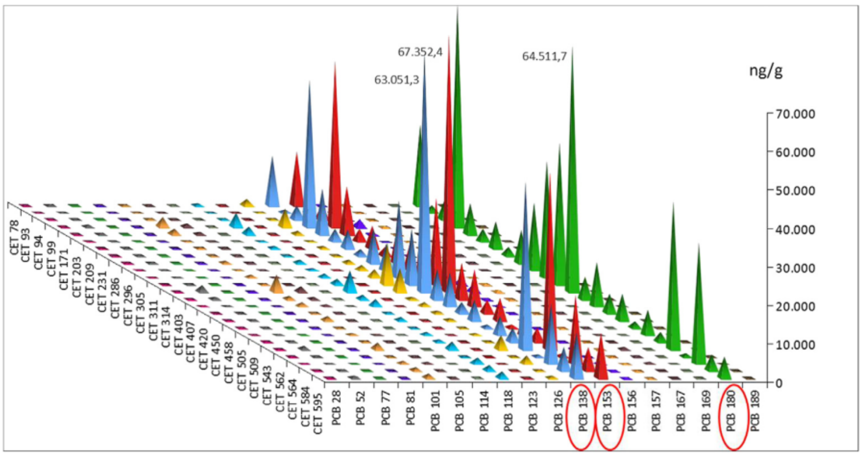


**Fig. 4.** Concentrations of PCBs congeners in the blubber samples.

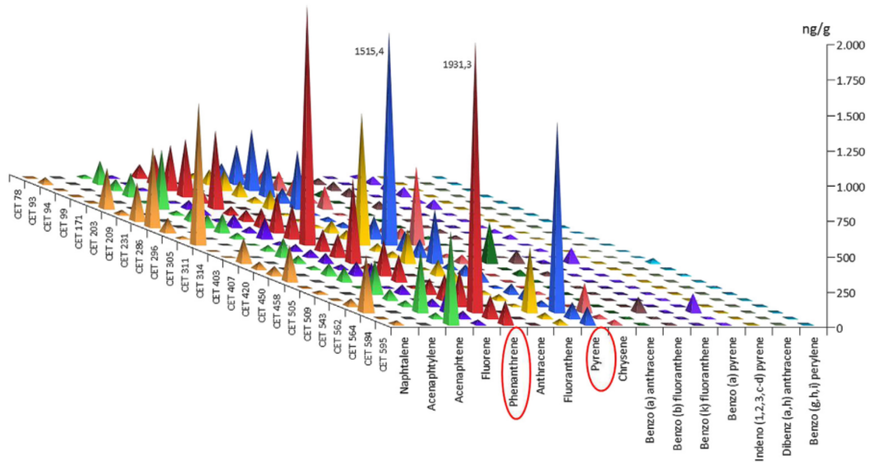


**Fig. 5.** Concentrations of PAHs in the blubber samples.

Equivalency to Dioxins (TEQ) approach is used, applying the 2005 toxic equivalence factors (TEF) (Van den Berg et al., 2006), the presence of this congener accounted for the greatest proportion of the $TEQ_{DL\text{-}PCBs}$, raising the levels from 116.7 pg $g^{-1}$ lw (without considering PCB 126) to more than 10,000 pg $g^{-1}$ lw when PCB 126 is included in the calculations (data not shown). The source of the high contamination by this PCB is unknown but such values represent a very relevant toxic load of dioxins in these animals and a considerable risk for their health status.

When considering the body burden of a population it is essential to take into consideration important variables such as the sex, the age or the body condition, among others, since it has been described that some of them can play an important role on the impact of pollutants on the health of the animals. Thus, it has been described that the concentrations of organochlorine compounds increase with the age in the males and decrease in mature females, most likely due to the pollutant transfer to offspring (Borrell and Aguilar, 2005; Tanabe et al., 1982). In concordance with the literature, we have found higher levels of both, OCPs and PCBs in adult males than in adult females, and also higher values in older animals than in the younger ones (Table 2, and Figs. 3 and 4), although, due to the low number of individuals in each group (adult males, adult females, subadult males, subadult females, pregnant females), these differences did not reach statistical significance ($p > 0.05$). Focussing on the abovementioned toxic threshold for PCBs, we found that the mean value for $\sum$PCBs in adult males exceeded it more than four times (76,411 ng $g^{-1}$ lw). In adult females this value was also surpassed but to a much lesser extent (26,242 ng $g^{-1}$ lw). The values found in subadult males and females (31,708 ng $g^{-1}$ and 35,738 ng $g^{-1}$ lw, respectively) were quite similar and also above the limit of toxicity, while the mean concentration in the pregnant females (10,664 ng $g^{-1}$ lw) was below the toxicity threshold. Therefore, all the individuals studied, except the two pregnant females, were exposed to toxic levels of PCBs, according to the threshold proposed by Kannan et al. (2000). However, we should take into consideration that this value was established for other marine mammal species and might not be applicable to dolphins.

Finally, as regard to the levels of PAHs in these animals, we found that all the samples showed detectable values of at least 6 of the 16 PAH studied (range 6–12) (Fig. 5). Acenaphthylene, anthracene, indeno[1,2,3-cd]pyrene, and dibenzo[a,h]anthracene were not detected in any sample. Phenanthrene was the most frequently detected compound of this group, and also the one detected at the highest levels in both tissues (mean of 338 and 14 ng $g^{-1}$ lw respectively). This result coincides with previous reports in other marine vertebrates from the Canary Islands (Camacho et al., 2012). The phenanthrene is a compound of crude oil, and considered less toxic than benzo[a]pyrene (Nisbet and LaGoy, 1992), although its high accumulation and ubiquity could lead in harmful effects. According to our results, the bottlenose dolphins were mainly exposed to the lower molecular weight PAHs, tri and tetra-cyclic, (520 and 455 ng $g^{-1}$ lw in blubber respectively). Lower levels were found for di-cyclic (136 ng $g^{-1}$ lw) and even lower for group of higher molecular weight, this is penta and hexa-aromatics (55 and 2 ng $g^{-1}$ lw in blubber respectively). No accidental oil spills have been reported near the Canary coasts in the last years, but it must be taken into consideration the intensive traffic of large oil tankers through the canary waters every year. This study represents the first approach of monitoring petroleum-derivate chemicals in cetaceans from Canary Islands.

## 4. Conclusions

The findings of this study reveal that the population of bottlenose dolphins of the Canary Islands is exposed to relevant levels of organic pollutants. When compared with previous studies, and also observing the levels of pollutants of bottlenose dolphins stranded along 15 years from our study, the concentrations of organochlorine compounds (OCPs and PCBs) seem not be declining in this area, and still are found at toxicologically relevant levels in these animals. Unlike organochlorines, for which some reports are available in literature, the presence of petroleum-derivate compounds such as PAHs in dolphins has been scarcely reported worldwide, and this study represents the first report of these contaminants in cetaceans of the Eastern Atlantic Ocean. Further studies are needed to investigate the possible role played for the pollutants in the survival of this specie as it is considered “vulnerable” specie in the Spanish National Catalogue of Threatened Species.

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## 5.2. Levels and profiles of POPs (organochlorine pesticides, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain

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# Levels and profiles of POPs (organochlorine pesticides, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain

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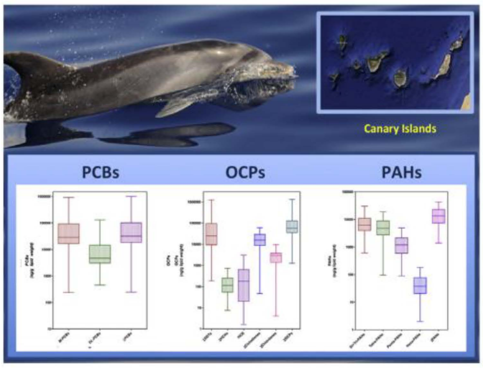
## HIGHLIGHTS

- Baseline levels of POPs in free-ranging bottlenose dolphins of the Canary Islands
- Bottlenose dolphins of this area are facing a high exposure to organic pollutants.
- Median concentrations of PCBs and TEQs widely exceeded the toxicity thresholds.
- Surprisingly the levels of contamination by OCPs and PCBs seem to be increasing.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The effect of anthropogenic pollution in marine mammals worldwide has become an important issue due to the high concentrations found in many areas. The present study represents the first report of pollutants in free-ranging cetaceans from the Canary Islands, where there are 12 marine Special Areas of Conservation (SACs), because of the presence of bottlenose dolphins (*Tursiops truncatus*). We selected this resident population of dolphins as a bioindicator to gain knowledge concerning the toxicological status of the cetaceans of this protected area. In 64 biopsy samples of live free-ranging animals sampled from 2003 to 2011, we determined the concentrations of 18 polychlorinated biphenyls (PCBs), 23 organochlorine pesticides (OCPs) and 16 polycyclic aromatic hydrocarbons (PAHs). We found high levels of many of these pollutants, and some of them were detectable in 100% of the samples. The median value for $\sum$ OCPs was 57,104 ng g$^{-1}$ lipid weight (lw), and the dichlorodiphenyldichloroethylene (p,p′-DDE) accounted for 70% of this amount. Among PCBs, congeners 180, 153 and 138 were predominant (82% of $\sum$ PCBs; median = 30,783 ng g$^{-1}$ lw). Concerning the analyzed PAHs, the total median burden was 13,598 ng g$^{-1}$ lw, and phenanthrene was the compound measured at the highest concentration followed by pyrene and by naphthalene. Surprisingly, we have found that organohalogen pollutants exhibit an upward trend in recent years of sampling. Thus, according to the

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guidelines outlined in the EU's Marine Strategy Framework Directive, further monitoring studies in Canary Islands are required to contribute to the conservation of the resident populations of marine mammals in this region.



## 1. Introduction

Currently, marine mammals face a great variety of threats, many of which are of human origin. Among all anthropogenic threats (e.g., habitat loss and degradation, maritime traffic, accidental capture, overfishing, commercial whaling, tourist human interactions, whale watching activities and acoustic pollution), the exposure to pollutants and to debris occupies a prominent place (Tanabe, 2002). Thereby, the role of persistent organic pollutants (POPs) in the habitat of marine mammals, as causative factor of poor survival and the continued decline of their populations, is an area of ongoing research (Aguilar et al., 1999; Aguilar and Borrell, 1994; Balmer et al., 2011; Kakuschke et al., 2010; Kuehl et al., 1991).

Although the adverse health effects of POPs are difficult to assess, some studies have demonstrated that POPs adversely affect the endocrine and immune system, or cause reproductive impairment in these animals (Letcher et al., 2010; Miyazaki et al., 2004; Schwacke et al., 2012; Tanabe, 2002). Moreover, some massive die-off and stranding episodes of marine mammals have been related to chemical pollutants, which have been proposed as contributors of the emergence and pathogenicity of infectious disease epidemics (Aguilar and Borrell, 1994; Hall et al., 2006; Van Bressem et al., 2009). Various authors have established thresholds for toxicity in different tissues and endpoints in marine mammals (AMAP, 2002; Kannan et al., 2000; Letcher et al., 2010), and numerous studies worldwide have shown that these thresholds are commonly exceeded in these animals. However, the majority of these studies have been performed on samples taken from the remains of stranded animals. It is well known that pollutant concentrations found in stranded animals may not be indicative of levels of live individuals, because disease is often the cause of death in strandings, and the possibility exists that these animals may carry abnormal pollutant loads (Aguilar et al., 1999; Camacho et al., 2014). Thus far, relatively few investigations have been performed on healthy live captive or free-ranging marine mammals primarily due to the complexity and cost of sampling (Balmer et al., 2011; Berrow et al., 2002; Fair et al., 2010; Formigaro et al., 2014; Hansen et al., 2004; Kucklick et al., 2011; Yordy et al., 2010). To date, none of these investigations on live animals has been performed in cetaceans from the Canary Islands, which have been declared a “Particularly Sensitive Sea Area” by the International Maritime Organization (IMO) and are considered a protected marine area. This archipelago is an area of great diversity of cetaceans, and its geographical location and oceanographic conditions have caused the establishment of year-round resident populations of cetaceans, such as the common bottlenose dolphin (*Tursiops truncatus*), among others (Arbelo et al., 2013).

Bottlenose dolphin is a species of high interest for the study of pollutants for various reasons: a) its worldwide distribution (MMC, 1999), b) the great amount of available studies concerning different aspects of its biology and physiopathology, and c) the fact that there are many coastal populations throughout the planet with a potential to reflect the contamination because their proximity to urban and industrial sources increases their POP exposure (Kucklick et al., 2011). For all these reasons, this species has been proposed as a good bioindicator of marine pollution, and many of the above-mentioned studies have been performed on these dolphins.

The primary objective of the present monitoring study was to obtain a baseline of many relevant anthropogenic pollutant loads in bottlenose dolphins from the Canary Islands to assess the potential toxic impact of these pollutants in cetacean species of this area with conservation aims. Although life history data of wild animals are difficult to obtain, blubber biopsy samples collected from these 64 free-ranging bottlenose dolphins can be representative of this population and are preferred over samples collected from necropsies (Aguilar and Borrell, 1994).

## 2. Materials and methods

### 2.1. Study area

The Canary Islands are located 1600 km away from southwest Spain, in the Atlantic Ocean, and 100 km away from the nearest point on the North African coast (southwest of Morocco) (Fig. 1). Thus, although geographically part of the African continent, from political and socioeconomic points of view, the Canary Islands belong to the European Union. As above-mentioned, this region is a protected marine area. However, notably large quantities of organochlorine pesticides (OCPs) have been used in the past in this archipelago because of the important role of agriculture in the economy of the region. In fact, high loads of OCPs and other anthropogenic pollutants have been described in the human population (Luzardo et al., 2012, 2009) and in biota of this archipelago, including marine wildlife in nearby areas (Camacho et al., 2012, 2013a, 2013b, 2013c; Luzardo et al., 2014; Zumbado et al., 2005).

There are 12 marine Special Areas of Conservation (SACs, Natura 2000 network) in the Canary Islands due to the presence of bottlenose dolphins, which are listed on Annex II and IV in the European Habitats Directive. By means of individual identification (individual photo-ID) it has been shown that this population is year-round resident with inter-island movements of animals, at least for the last 15 years. The populations of bottlenose dolphin in at least three of these SACs are under the same synergistic threats (e.g. maritime traffic, whale-watching, professional fishing, high-speed ferries and coastal degradation).

### 2.2. Sample collection and ethics statement

The biopsy samples were collected by the Society for the Study of Cetaceans in the Canary Archipelago (SECAC), from 2003 to 2011, through monitoring surveys in the SACs of the Canary Islands and in the eastern waters of the islands of Lanzarote and Fuerteventura. The use of crossbows and the Ceta-Dart® bolt and cutting head (Ceta-Dart, Copenhagen, Denmark) allows efficient, minimally invasive, and humane sampling of cetaceans in the wild (Wenzel et al., 2010). Biopsy sampling on individual cetaceans, if performed responsibly, is likely to cause only low-level and short-term reactions and is not likely to produce any long-term deleterious effects. Therefore, this sampling is deemed as relatively irrelevant for the animals' welfare. In this study, we have used this methodology to collect 64 blubber samples from bottlenose dolphins during the period 2003–2011 (Table 1). In Fig. 1, we depict the exact location of each sampling. Samples were responsibly collected by trained personnel using a modified arrow that was fired with a stainless hollow cylindrical head of 0.8 cm in internal diameter and 2.5 cm in length. After removing the tissue from the tip, the skin was separated from the blubber using solvent-rinsed scalpel and forceps. The skin was used for both trophic studies (stored at −20 °C) and genetic studies (stored in a saturated salt solution of 20% DMSO). The blubber samples were stored in pre-solvent-cleaned Eppendorf® vials and frozen at −20 °C (ship) and at −80 °C (laboratory) until chemical analyses. All biopsies were collected from the dorsal and mid-lateral regions near the dorsal fin. A limitation of this type of remote sampling is that reliable data concerning the age and sex of individuals cannot be recorded. However, all animals were photographed and videotaped for later stock identification. Besides, the individuals were classified as

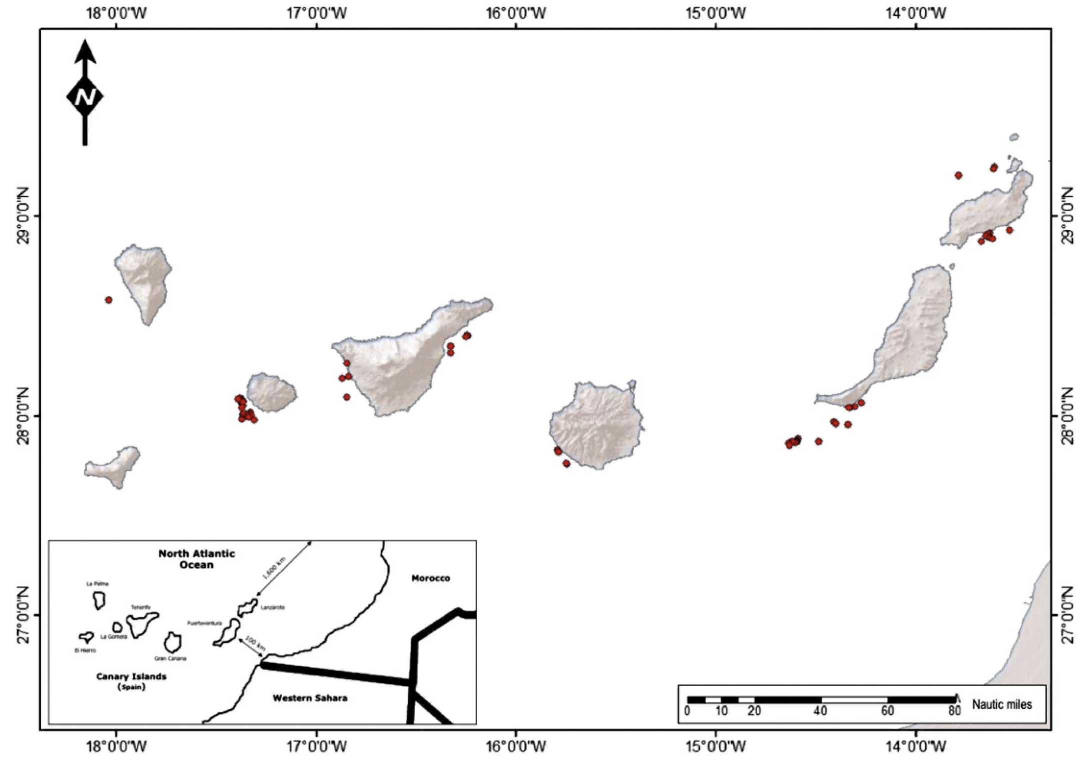


**Fig. 1.** Map of the Canary Islands archipelago in the northwestern African coast, showing the exact location of each sampling (inset: minimal distances from Spanish and Moroccan coasts).

undetermined (n = 47) adults (n = 9) or sub-adults (n = 8) based on morphometry and other morphological characteristics following the expert criteria of the SECAC researchers.

### *2.3. Analytes of interest*

In total, 57 analytes belonging to three relevant groups of POPs were determined for this study. The 23 OCPs and metabolites selected were the diphenyl-aliphatics (methoxychlor, dichlorodiphenyltrichloroethanes (o,p′-DDT, p,p′-DDT, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD), and dicofol), the hexachlorobenzene (HCB), the four isomers of hexachlorocyclohexane (α-, β-, δ-, and γ-HCH), and the cyclodienes (heptachlor, dieldrin, aldrin and endrin, chlordane (cis- and trans-isomers), mirex, endosulfan (α- and β-isomers) and endosulfan sulfate). Concerning polychlorinated biphenyls (PCBs), 18 congeners were included in the present study: 12 dioxin-like congeners (DL-PCBs, congener numbers 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) and 7 marker PCBs (M-PCBs, congener numbers 28, 52, 101, 118, 138, 153 and 180). Besides organochlorine contaminants, the 16 EPA priority polycyclic aromatic hydrocarbons (PAHs) were also measured (naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz [a, h]anthracene, and benzo[ghi]perylene) in the suite of analytes.

### *2.4. Sample preparation*

Because of the known fact that the contaminants included in this study are completely lipid-soluble and, therefore, found in the lipid fraction of tissues, we first extracted the fat from blubber. The blubber sample (0.03–0.31 g) was finely chopped with scissors and homogenized in 5 ml ultrapure water with a disperser (Ultra-turrax, IKA, China). This homogenate was spiked with a 10 ppm-surrogate mix (PCB 12, PCB 202, p, p′-DDE-D8, acenaphtylene D8) in acetone to yield a final concentration of 100 ppb and was mixed with 30 g of diatomaceous earth to absorb all the humidity. The extraction and clean up method followed method recommended by the European Standard for the determination of pesticides and PCBs in fatty foods (EN, 1996a; EN, 1996b) and whose validity has been previously proven in our laboratory for different fatty samples of animal origin, including blubber samples (García-Álvarez et al., 2014; Luzardo et al., 2014). This method combines an automated Soxhlet extraction method (FOSS Soxtec Avanti 2055) with a purification step using gel permeation chromatography (GPC) and gives acceptable recoveries that ranged between 74.5% and 104.7%. Briefly, the Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed, Denmark) apparatus consisted of an extraction unit, a control unit and a drive unit. The blubber, which was prepared as described above, was inserted into the extraction unit, 20 ml of solvent (dichloromethane) was added to each of the extraction cups in a closed system, and the cups were heated using an electric heating plate. The three-step extraction consisted of boiling, rinsing, and solvent recovery. The solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40 °C to prevent analyte losses. The results were calculated as the total amount of fat (g) per 100 g tissue. Using a precision balance, the fat obtained from blubber samples was carefully weighted into a zeroed glass tube. The weighted fat was dissolved in 2 ml of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (BioBeads SX-3) using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 ml/min as the eluent. The first 25 minute elution volume, which contained the great majority of the lipids (>98%), was discarded. The 25–85 minute elution volume (120 ml), which contained all the analytes that were co-extracted

**Table 1**
Biopsy details collected from bottlenose dolphins in Canary Islands waters from 2003 to 2011, and the concentrations of the main groups of persistent organic pollutants (POPs).

| Biopsy | Year | Location | Age[a] | PCBs[b] | DDTs[c] | OCPs[d] | PAHs[e] |
|---|---|---|---|---|---|---|---|
| 1 | 2003 | Lanzarote | Undet. | 29,035 | 22,199 | 33,270 | 15,527 |
| 2 | 2003 | Lanzarote | Undet. | 8167 | 3545 | 9032 | 32,749 |
| 3 | 2004 | La Gomera | Undet. | 22,994 | 30,124 | 52,229 | 40,964 |
| 4 | 2004 | La Gomera | Undet. | 20,505 | 20,396 | 26,126 | 9344 |
| 5 | 2004 | La Gomera | Undet. | 64,430 | 58,831 | 93,997 | 21,568 |
| 6 | 2004 | Fuerteventura | Undet. | 42,468 | 53,021 | 103,913 | 33,420 |
| 7 | 2004 | Tenerife | Undet. | 67,742 | 55,840 | 98,838 | 16,374 |
| 8 | 2004 | Tenerife | Undet. | 7735 | 3838 | 23,555 | 10,596 |
| 9 | 2004 | Tenerife | Undet. | 9036 | 4094 | 16,920 | 6582 |
| 10 | 2004 | Tenerife | Undet. | 31,930 | 22,760 | 56,843 | 15,392 |
| 11 | 2004 | Tenerife | Undet. | 40,596 | 42,831 | 74,005 | 20,145 |
| 12 | 2004 | La Palma | Undet. | 10,919 | 5748 | 22,930 | 9642 |
| 13 | 2004 | Tenerife | Undet. | 29,636 | 15,324 | 44,119 | 11,670 |
| 14 | 2005 | La Gomera | Undet. | 57,949 | 37,263 | 100,343 | 25,491 |
| 15 | 2005 | La Gomera | Undet. | 102,965 | 93,374 | 164,909 | 28,143 |
| 16 | 2005 | La Gomera | Undet. | 20,263 | 7670 | 47,059 | 17,078 |
| 17 | 2005 | La Gomera | Undet. | 21,104 | 13,359 | 43,019 | 13,290 |
| 18 | 2005 | La Gomera | Undet. | 19,445 | 13,780 | 49,685 | 18,005 |
| 19 | 2005 | La Gomera | Undet. | 35,490 | 32,551 | 60,993 | 19,957 |
| 20 | 2005 | La Gomera | Undet. | 14,290 | 10,571 | 22,996 | 6508 |
| 21 | 2005 | Lanzarote | Undet. | 22,497 | 19,777 | 35,212 | 13,468 |
| 22 | 2005 | Gran Canaria | Undet. | 13,381 | 7663 | 25,533 | 11,088 |
| 23 | 2005 | Fuerteventura | Undet. | 18,978 | 10,370 | 46,380 | 23,656 |
| 24 | 2005 | Fuerteventura | Undet. | 245 | 189 | 1270 | 1856 |
| 25 | 2005 | Fuerteventura | Undet. | 10,939 | 4463 | 20,297 | 16,461 |
| 26 | 2005 | Fuerteventura | Undet. | 9913 | 2746 | 30,727 | 21,942 |
| 27 | 2006 | La Gomera | Undet. | 13,821 | 5716 | 45,689 | 25,600 |
| 28 | 2006 | La Gomera | Undet. | 9010 | 3940 | 36,191 | 13,729 |
| 29 | 2006 | La Gomera | Undet. | 15,255 | 4960 | 54,333 | 26,435 |
| 30 | 2006 | La Gomera | Undet. | 15,967 | 5407 | 50,406 | 17,958 |
| 31 | 2006 | La Gomera | Undet. | 3425 | 1019 | 20,254 | 6579 |
| 32 | 2006 | La Gomera | Undet. | 18,207 | 8487 | 69,889 | 33,955 |
| 33 | 2006 | La Gomera | Undet. | 23,773 | 25,054 | 62,275 | 22,058 |
| 34 | 2006 | La Gomera | Undet. | 24,315 | 13,370 | 67,117 | 23,957 |
| 35 | 2006 | La Gomera | Undet. | 20,682 | 11,084 | 57,365 | 42,577 |
| 36 | 2006 | La Gomera | Undet. | 21,471 | 11,634 | 45,107 | 16,314 |
| 37 | 2008 | Tenerife | Undet. | 271,069 | 260,156 | 285,446 | 5431 |
| 38 | 2008 | Tenerife | Undet. | 338,013 | 266,855 | 287,726 | 4099 |
| 39 | 2008 | La Gomera | Adult | 99,816 | 93,037 | 105,545 | 4208 |
| 40 | 2008 | La Gomera | Sub-adult | 503,011 | 582,954 | 611,895 | 4932 |
| 41 | 2008 | La Gomera | Sub-adult | 313,895 | 337,409 | 367,017 | 10,926 |
| 42 | 2009 | Tenerife | Adult | 230,300 | 249,806 | 277,355 | 12,940 |
| 43 | 2009 | Fuerteventura | Undet. | 146,307 | 152,187 | 197,678 | 33,661 |
| 44 | 2009 | Fuerteventura | Undet. | 209,884 | 209,079 | 233,295 | 11,124 |
| 45 | 2009 | Fuerteventura | Undet. | 175,628 | 184,356 | 203,412 | 29,761 |
| 46 | 2009 | Fuerteventura | Undet. | 27,946 | 19,745 | 49,253 | 25,211 |
| 47 | 2009 | Fuerteventura | Undet. | 119,808 | 133,552 | 145,581 | 5195 |
| 48 | 2009 | Fuerteventura | Undet. | 85,844 | 80,971 | 96,241 | 5798 |
| 49 | 2009 | Fuerteventura | Sub-adult | 75,318 | 72,506 | 96,182 | 16,135 |
| 50 | 2009 | Fuerteventura | Adult | 94,270 | 98,545 | 111,248 | 14,075 |
| 51 | 2009 | La Gomera | Undet. | 25,794 | 22,748 | 31,863 | 10,712 |
| 52 | 2009 | Lanzarote | Sub-adult | 53,773 | 32,389 | 64,590 | 36,500 |
| 53 | 2009 | Lanzarote | Undet. | 243,951 | 275,683 | 294,777 | 7925 |
| 54 | 2009 | Lanzarote | Sub-adult | 186,791 | 174,709 | 192,578 | 11,113 |
| 55 | 2009 | Lanzarote | Sub-adult | 53,094 | 47,635 | 54,772 | 4496 |
| 56 | 2010 | Gran Canaria | Adult | 34,620 | 23,418 | 54,577 | 25,943 |
| 57 | 2010 | Gran Canaria | Adult | 407,291 | 375,143 | 405,854 | 9410 |
| 58 | 2010 | Tenerife | Adult | 2761 | 1382 | 6077 | 7501 |
| 59 | 2010 | Lanzarote | Sub-adult | 748,072 | 783,831 | 829,106 | 7397 |
| 60 | 2010 | Lanzarote | Undet. | 1,016,851 | 1,252,210 | 1,308,240 | 11,537 |
| 61 | 2010 | Fuerteventura | Adult | 145,155 | 150,243 | 160,655 | 4384 |
| 62 | 2010 | Fuerteventura | Adult | 65,598 | 75,639 | 80,421 | 1394 |
| 63 | 2010 | Fuerteventura | Adult | 49,775 | 44,839 | 48,180 | 2002 |
| 64 | 2011 | Lanzarote | Sub-adult | 21,414 | 19,289 | 24,455 | 5767 |

[a] Undet., undetermined age.
[b] PCBs, polychlorinated biphenyls.
[c] DDTs, dichlorodiphenyltrichloroethanes.
[d] OCPs, organochlorine pesticides.
[e] PAHs, polycyclic aromatic hydrocarbons.

with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally, the solvent was evaporated to dryness under a gentle nitrogen stream. The residue was then reconstituted in 1 ml cyclohexane, 10 μl of the solution of internal standards (ISs, tetrachloro-m-xylene, heptachloro epoxide trans, and benzo[a]pyrene D12) at 1 μg $ml^{-1}$ was added, and the mixture was transferred to a GC vial that was used for the chromatographic analysis The amount of pollutants per gram of fat was obtained multiplying by the correspondent correction factor.

### 2.5. Procedure of chemical analysis

Gas chromatography (GC) analyses of 57 contaminants plus surrogates and ISs were performed in a single run on a Thermo Trace GC Ultra equipped with a TriPlus Autosampler and coupled to a Triple Quadrupole Mass Spectrometer Quantum XLS (Thermo Fisher Scientific Inc., Waltham, MA, USA), as previously described and validated in our laboratory (García-Álvarez et al., 2014; Luzardo et al., 2013). Because no matrix effect has been observed with this method, all quantifications were performed against a 10-point calibration curve prepared in cyclohexane (0.05 to 40 μg/l).

### 2.6. Statistical analysis

The database management and statistical analysis were performed using PASW Statistics v 19.0 software (SPSS Inc., Chicago, IL, USA). Non-parametric statistics were used because the data lacked normality and homoscedasticity, even after log-transformation based on the Kolmogórov–Smirnov test. Significant differences in contaminant concentrations among dolphin groups were evaluated using the non-parametric Mann–Whitney U-test and Kruskal–Wallis test. *P* values of less than 0.05 (two tailed) were considered statistically significant.

### 2.7. Quality control

The recoveries of the 57 analytes and surrogates were acceptable with this method because the recoveries were within the 79–107% range. All the individual measurements were corrected by the recovery efficiency for each analyte. All extracts were injected in triplicate in the GC, and the values used for calculations were the mean of the three data sets. To ensure reliability of the data obtained for the analytes targeted in our study, various QA/QC measures were conducted during sample preparation and analysis. We used one procedural blank (melted butter spiked at 20 ng $g^{-1}$ with each of the analytes processed by the same method used for the samples) per batch of 12 samples. Additionally we also injected blind sample duplicates and random injection of standards and solvent blanks. The batch analyses were considered valid when the values of the analytes in the QC were within 10% of the deviation of the theoretical value.

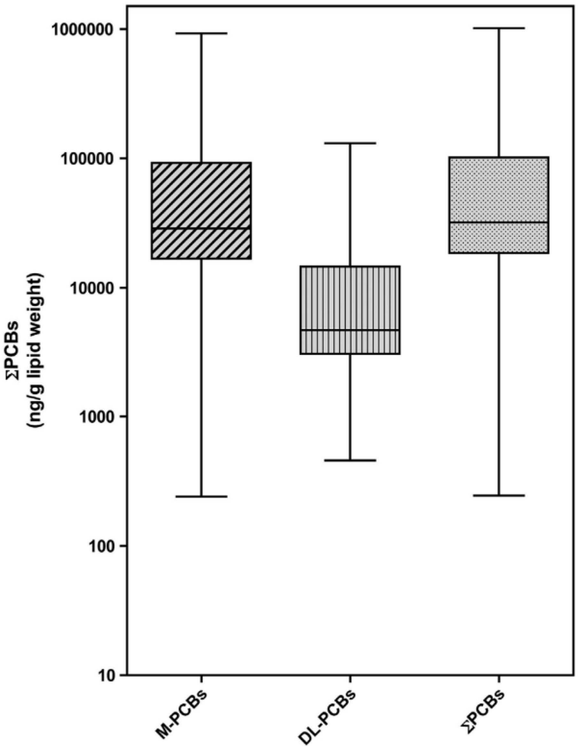


**Fig. 2.** Box plot indicating the levels of ΣPCBs. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, and the lines extending vertically from the boxes indicate the variability outside the upper and lower quartiles.

## 3. Results and discussion

### 3.1. Assessment of concentrations of organochlorinated pollutants and polycyclic aromatic hydrocarbons in bottlenose dolphins

As expected, within a population sample, individual variability in pollutant concentrations was extremely high because biological factors, such as age, sex, diet, habitat, metabolism, parturition and lactation, affect the POP profiles in cetaceans (Aguilar et al., 1999; McKenzie et al., 1997). To facilitate comparisons among individuals and with

**Table 2**
Persistent organic pollutants (POPs) in free-ranging bottlenose dolphin blubber biopsies (n = 64). Results are expressed in ng $g^{-1}$ lipid weight.

| | Mean ± SD[a] | Median (perc. 5th–95th) | Range |
|---|---|---|---|
| Polychlorinated biphenyls (PCBs)[b] | | | |
| ∑M-PCBs | 94,386 ± 161,174 | 28,198 (3856–435,904) | (240–925,446) |
| ∑DL-PCBs | 13,486 ± 21,778 | 4597 (790–60,907) | (6–130,861) |
| ∑PCBs | 103,822 ± 176,960 | 30,783 (4502–479,081) | (245–1,016,851) |
| $\sum TEQ_{DL\text{-}PCBs}$ | 267 ± 492 | 67 (2–1245) | (0–2764) |
| Organochlorine pesticides (OCPs)[c] | | | |
| HCB | 332 ± 534 | 48 (0–1158) | (0–3064) |
| ∑HCH | 207 ± 184 | 147 (11–592) | (0–855) |
| ∑DDT | 104,739 ± 202,926 | 24,236 (1723–531,001) | (189–1,252,210) |
| Endrin | 20,414 ± 14,737 | 15,929 (2377–52,857) | (47–60,699) |
| ∑Chlordanes | 3017 ± 1988 | 3017 (356–7188) | (4–9689) |
| Mirex | 2947 ± 5435 | 746 (12–13,119) | (1–29,541) |
| ∑OCPs | 131,826 ± 207,020 | 57,104 (11,004–560,385) | (1270–1,308,240) |
| Polycyclic aromatic hydrocarbons (PAHs) | | | |
| ∑PAHs | 15,932 ± 10,233 | 13,598 (2526–35,864) | (1394–42,577) |

[a] SD, standard deviation.
[b] M-PCB, marker-PCB; DL-PCB, dioxin-like PCB, TEQ, toxic equivalency to dioxins.
[c] HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; DDT, dichlorodiphenyltrichloroethane.

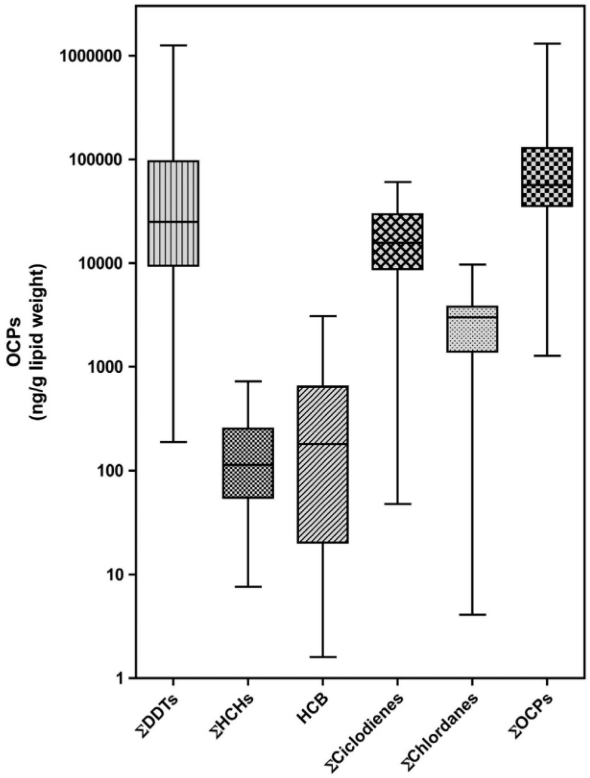


**Fig. 3.** Box plot indicating the levels of ΣOCPs. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, and the lines extending vertically from the boxes indicate the variability outside the upper and lower quartiles.

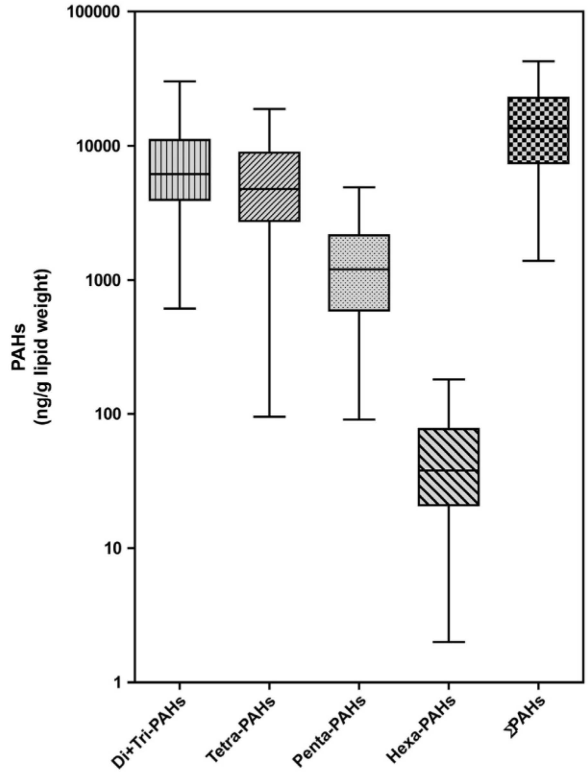


**Fig. 4.** Box plot indicating the levels of ΣPAHs. The line inside the box represents the median, the bottom and top of the box are the first and third quartiles of the distribution, and the lines extending vertically from the boxes indicate the variability outside the upper and lower quartiles.

other studies, we have expressed the results in a lipid weight basis (ng $g^{-1}$ lw). The results showed that $\sum$PCBs, with a mean and a median concentration of 103,822 and 30,783 ng $g^{-1}$ lw ($P_5$–$P_{95}$ = 4502–479,081 ng $g^{-1}$ lw), and $\sum$DDTs, with a mean and median of 104,739 and 24,236 ng $g^{-1}$ lw ($P_5$–$P_{95}$ = 1723–531,001 ng $g^{-1}$ lw), were predominant. Because the values were not normally distributed, the median concentrations were far apart from the mean levels. For this reason, we preferred using the median because this measurement is a better representation of the entire population. Concerning PAH concentrations, the mean and median levels were quite similar; therefore, both are considered representative of the levels of these samples (15,932 and 13,598 ng $g^{-1}$ lw, respectively). We present the obtained results in Table 2 and in Figs. 2 to 4. Concerning compounds, notably, dichlorodiphenyldichloroethylene (p,p′-DDE), PCB 180, PCB 153, PCB 138, endrin, p,p′-DDT, and phenanthrene were the outstanding compounds because they were detectable in 100% of the samples. In the following sections, we thoroughly present and discuss our results, which are grouped by chemical class.

### 3.1.1. Polychlorinated biphenyls

As previously mentioned, there was a large difference between mean and median values for $\sum$PCBs (Fig. 2) due to the existence of four extreme values (4 high-level outliers). The most abundant congeners were the highly chlorinated M-PCBs 180, 153 and 138. In fact, the M-PCBs represented almost 86% of the $\sum$PCBs in these animals (median = 28,198 ng $g^{-1}$ lw), with the median concentration of the DL-PCBs equal to 4597 ng $g^{-1}$ lw (14%). M-PCBs are usually the most prevalent in biota that live close to urban settlements (as expected in resident bottlenose dolphins) because these congeners are associated with Aroclor PCB formulations; thereby, their presence may indicate contamination from urban PCB sources. Thus, our results are consistent with other previously published data, such as the report of Kucklick et al. (2011) in bottlenose dolphins from the western North Atlantic Ocean and from the Gulf of Mexico. A similar profile was also reported in free-ranging resident individuals of this species from Sarasota Bay, Charleston in 2004 (Houde et al., 2006). In addition, in dolphins stranded in the Canary coasts, the same pattern of contamination by PCBs was also described (García-Álvarez et al., 2014).

Notably, the levels of PCBs found in these animals can be considered quite high since PCB levels from 17,000 ng $g^{-1}$ lw put animals at risk for adverse biological effects (Jepson et al., 2005; Kannan et al., 2000). Thus, according to the median value obtained in this study, the concentrations of PCBs in these animals are two-fold greater than this threshold, even one individual exceeds as much as 50 times this value (Table 1 and Fig. 2). In fact, in the literature, several references relate high levels of PCBs in bottlenose dolphins with different adverse health effects, such as anemia, thyroid disorder or functionality of the immune response, among others (Kucklick et al., 2011; Schwacke et al., 2012). Moreover, when we calculated the blubber toxic equivalent quantity (TEQ) levels for dioxin-like PCBs using the toxic equivalency factors (TEFs) determined in 2005 (Van den Berg et al., 2006), we found an extremely high mean value for $TEQ_{DL\text{-}PCBs}$ (267 ng $g^{-1}$ lw), which reinforced the worrisome results discussed above indicating that these individuals are actually at high health risk. Our results suggest that a conservation plan is required to assess the effects of PCBs in the cetacean populations and attempt to reduce anthropogenic pollution in the studied marine area.

### 3.1.2. Organochlorine pesticides

We detected 15 of 23 OCPs in the blubber biopsies of these animals. As in the case of PCBs, there was a large difference between mean and median values for OCPs (Fig. 3) due to the existence of extreme values. The group of pesticides that contributed more to the $\sum$ OCPs was that of DDTs, and, in particular, p,p′-DDE, which accounted for a mean average of 87.6% of ΣDDTs, followed by p,p′-DDT (9.4%). Accordingly, the p,p′-DDE/ΣDDTs ratio was 0.76, which is a common indicator of DDT degradation. We also found that the presence of endrin (mean = 20,414 ng $g^{-1}$ lw; median = 15,929 ng $g^{-1}$ lw), mirex (mean = 2947 ng $g^{-1}$ lw; median = 746 ng $g^{-1}$ lw) and $\sum$ chlordanes cis and trans (mean = 3017 ng $g^{-1}$ lw; median = 3017 ng $g^{-1}$ lw) were relevant contributors to $\sum$ OCPs. We have highlighted these contaminants because of their concentration; levels of organohalogenated compounds above 1 ppm (1000 ng $g^{-1}$ wet weight) in any tissue of marine mammals can be considered a toxic concentration (Letcher et al., 2010). Due to the high percentage of lipids present in the blubber, we can say that all these pesticides exceeded this threshold of toxicity in the studied animals.

Other pesticides were also found in the blubber of these dolphins, although at much lower concentrations. This situation is the case for HCB (mean = 332 ng $g^{-1}$ lw; median = 48 ng $g^{-1}$ lw), β-HCH (mean = 138 ng $g^{-1}$ lw; median = 100 ng $g^{-1}$ lw), and γ-HCH (mean = 69 ng $g^{-1}$ lw; median = 47 ng $g^{-1}$ lw). The lower concentrations of HCB and HCH isomers compared with DDTs and PCBs may be because less volatile pollutants, such as DDT and highly chlorinated PCBs, are generally found close to their emission source and the highly volatile HCB and HCHs are more globally transported (Iwata et al., 1993; Li et al., 2002). Besides, the absence of dieldrin and the low concentration of HCB may be explained as both are subject to metabolism via cytochrome P450 enzymes (Oberholser et al., 1977; Takazawa and Strobel, 1986). However, we must take into consideration that both HCB and HCH are highly bioaccumulated by aquatic organisms. The alpha and delta isomers of HCH were not detected in any of the biopsy samples; heptachlor, aldrin, dieldrin, alpha and beta-endosulfan, and endosulfan sulfate were not detected either.

Importantly, several differences were found between this study and the results obtained in bottlenose dolphins stranded on the shores of the Canary Islands (García-Álvarez et al., 2014). However, there are some plausible reasons that may explain the differences between free-living and stranded dolphins, even in the case that all or some belonged to the same population (as supported by PCB results). First, it is remarkable that high levels of endrin (mean of 20 ppm) were found in these free-ranging bottlenose dolphins, whereas this pesticide was not found in the blubber from stranded dolphins. However, endrin may be easily broken down by exposure to high temperatures or light to form both endrin ketone and endrin aldehyde (ATSDR, 1997). Unfortunately, we did not include the determination of these two metabolites in our studies; however, we can hypothesize that the lack of endrin in the blubber of the bottlenose dolphin carcasses is due to in situ degradation. It is important to consider that endrin is highly toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment (risk phrases R50/53). In contrast, in this study, we report sevenfold higher mean concentrations of chlordanes in blubber biopsies (3017 ng $g^{-1}$ lw) than in stranded animals (446 ng $g^{-1}$ lw). It is a known fact that chlordane can readily volatilize from application places and, hence, contaminate nearby coasts (Bidleman, 1998; Bidleman et al., 2002; Hung et al., 2005). Additionally, the existence of a high percentage of food samples that contained levels above the maximum residue limit (MRL) of this pesticide has been recently described in Western Africa (extremely close to the Canary Islands), which indicates recent use in this area (Akoto et al., 2013; Bempah and Donkor, 2011).

### 3.1.3. Polycyclic aromatic hydrocarbons

Regarding the content of PAHs in the analyzed samples, we included those 16 PAHs initially considered by the Environmental Protection Agency (EPA) as priority contaminants due to their potential toxicity (mainly mutagenicity and carcinogenicity) because some of these PAHs were shown to be capable of inducing toxic effects on cetaceans and could act as procarcinogens in these animals (Wilson et al., 2005). In our study, unlike OCs and PCBs, no significant differences were found in the PAHs levels among individuals. Phenanthrene was detected in 100% of the samples and reached the highest concentrations (mean =5278 ng $g^{-1}$ lw; median = 4127 ng $g^{-1}$ lw). Pyrene (mean = 3295 ng $g^{-1}$ lw; median = 2820 ng $g^{-1}$ lw), naphthalene (mean = 1873 ng $g^{-1}$ lw; median = 997 ng $g^{-1}$ lw), and chrysene (mean = 1267 ng $g^{-1}$ lw; median = 988 ng $g^{-1}$ lw) were also found at high frequencies (100%, 95%, and 98%, respectively). All 16 compounds were detected in some of the samples. However, some of these pollutants had low detection frequencies, including anthracene (14%), dibenz[ah]anthracene (3%), and benzo[ghi]perylene (3%).

When we individually studied the profile of PAHs according to their homolog groups, we found that the lower molecular weight compounds (di-, tri- and tetra-cyclic) were the most frequent and most concentrated (Fig. 4). This profile is consistent with previous reports in marine wildlife from the same area (Camacho et al., 2012, 2013b, 2014) and suggests a petrogenic origin rather than urban sources of PAHs (Kannan and Perrotta, 2008). A similar pattern has also been reported in other species of marine mammals (Kannan and Perrotta, 2008), whereas in other reports, completely different profiles of contamination have been found, as is the case for southern sea lions (*Otaria flavescens*) from Argentina (Marsili et al., 1997). However, these differences can be easily explained because PAHs are efficiently metabolized, unlike most organohalogenated compounds, and the levels present in a given moment reflect only the recent exposure. Thereby, if we consider that all these studies usually rely on unique sampling and are not during a prolonged period, then these reports should be considered cross-sectional studies, where the data reflect only the exposure at the time of sampling.

The content of PAHs found in these animals, notably, corresponds to a low toxicity profile, at least concerning their carcinogenicity, since most of the PAHs indicated as probable carcinogens by the U.S. Environmental Protection Agency (EPA) belong to the high molecular weight compounds, less frequently detected in this study. However, we must consider that chrysene and benzo[a]anthracene, which are also listed among the most toxic compounds of this group, were detected in 98% and 48% of the samples, respectively.

### 3.2. Geographic variation of pollutant concentrations

Pollutant assessment in live animals allows making conclusions about the distribution of contaminants in the population, temporally and geographically. This knowledge provides baseline information needed to determine the magnitude of a possible risk. More recently, it has been suggested that geographic variation in the habitat of bottlenose dolphins deeply affects POP profiles (Hansen et al., 2004).

Performing geographic analyses, a Kruskal–Wallis statistical test was conducted to examine differences between geographic regions in the concentrations of pollutants.

Among the major groups or POPs analyzed, we found that levels of endrin and HCB varied between islands ($P = 0.007$ and $P = 0.021$ respectively). Endrin appeared to be higher in the island of La Gomera, and HCB amply distributed in Gran Canaria. Both pollutants are of great concern, as they are considered very toxic for aquatic organisms. However, there was not difference for HCB when we statistically compare the median values.

According to our results, levels of endrin differed significantly between islands, suggesting a certain degree of isolation of resident population.

The limitation of a lack of biological information of individuals must be considered in the absence of geographic variations to understand the pollutant profiles. Data on age, sex, reproductive status and movement

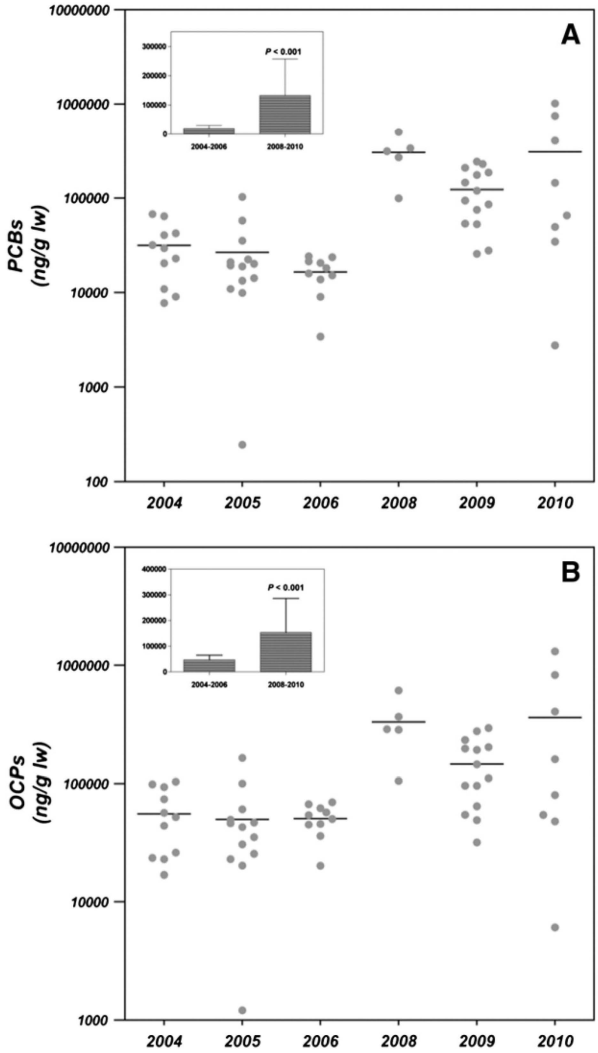


**Fig. 5.** Main figures: Scatter dot plots indicating the temporal distribution of ΣPCBs (panel A) and of ΣOCPs (panel B). The horizontal line represents the mean, and dots extending vertically from the line indicate the SEM. Insets: Comparison between median values of ΣPCBs (panel A) and ΣOCPs (panel B) of the samples from the periods 2004–2006 and 2008–2010. The lines extending vertically represent the interquartile range.

patterns are essential but difficult to assess in free-ranging populations (Yordy et al., 2010). The movement of contaminated prey and/or dolphins between areas should also be taken into consideration. Recent work reported that the effect of dolphin foraging behavior changes pollutant patterns but is less important than differences resulting from local contamination (Kucklick et al., 2011).

### 3.3. Temporal variation of contamination profiles

When we analyzed the values according to their temporal pattern, the results were surprising because the animals with higher levels of contamination by organohalogenated compounds of the entire series were those animals sampled in the most recent years (2008–2010). For PAHs, we did not observe this effect, and variations among years did not show any trend (neither increasing nor decreasing). However, as stated above, these contaminants are efficiently metabolized in mammals. Therefore, the cross-sectional determinations only reflect recent exposure, and the comparison among years provides little information. Besides, we must consider the sample size for each year to interpret the results, 2003 (n = 2); 2004 (n = 11); 2005 (n = 13); 2006 (n = 10); 2008 (n = 5); 2009 (n = 14); 2010 (n = 8); 2011 (n = 1).

In contrast, because organochlorine compounds are legacy pollutants that have been banned for several decades, the evident increase in the levels of OCPs and PCBs from 2008 is hard to explain. For this reason, the injections of all extracts in the GC were repeated in blind, random order, to rule out possible instrumental artifacts, and all possible sources of external contamination were checked. In Fig. 5, we show the obtained results for OCPs and PCBs after the segmentation of the series according to the year of sampling, excluding the samples from years 2003 (n = 2) and 2011 (n = 1) due to the low size of the groups (data of PAHs are presented in Supplementary Fig. 1). All the main POP groups analyzed varied significantly by the period of the present study ($P < 0.005$), only the chlordanes ($P = 0.089$), and tri ($P = 0.085$) and hexa-cyclic PAHs ($P = 0.0336$) did not seem to follow any temporal trend. As shown in this figure, the mean and median values of both PCBs and organochlorine pesticides significantly increased from 2008 onwards ($P < 0.001$ in both cases). Although many of studies usually show a decreasing trend in the levels of these compounds (Jepson et al., 2009), a similar pattern to our results was recently reported in the Gulf of Manila, where there has been an increase in the levels of PCBs in marine sediments in recent years (Kwan et al., 2014). In this case, the authors hypothesize that the possible leakage of PCBs from old machineries may have entered the aquatic environment in recent years. It is possible that something similar could have occurred in this area. We should consider that the Canary Islands are located only 100 km from the African coast (Fig. 1), and several studies have shown an increase in PCB sources in Africa due to leakage and wrongly disposed transformers, continuing import of e-waste from countries of the North, shipwrecks, and biomass burning (Gioia et al., 2013). Moreover, OCP and PCB contamination is higher near possible input sources, such as shipping, industrial activities and urban areas (Barakat et al., 2013), and waters of the Canary Islands experience intense maritime traffic, with the ports of the archipelago as traditional basis of scale and supplying ships on their way through the Middle Atlantic. Besides, the high DDT levels found in the studied animals, suggest that an input into the marine environment maybe from agriculture activities or long-range atmospheric transport from Africa, where this pollutant is still used for malaria control (Van Dyk et al., 2010). However, we must consider the low sample size for the interpretation of these results. Due to the fact that most of the samples were collected from SAC areas, these results have important implications for the conservation and management of bottlenose dolphins in this archipelago. Thus, further studies in this area are required to confirm this increasing trend.

## 4. Conclusions

Among the threats that may act together to negatively affect bottlenose dolphins, we believe, as other authors do, that anthropogenic pollution is a major stressor determinant in the population dynamics. We present the first evidence of the bioaccumulation of organochlorine compounds and PAHs in free-ranging cetaceans from the Canary Islands and the first reference of baseline concentrations of live bottlenose dolphins from this area. The results reveal that this species is facing a high exposure to organic pollutants, highlighting the requirement for ongoing monitoring of these contaminants on cetaceans in this marine area to evaluate if the pollutant trend continues upward. The industrial development along the coastline and future petroleum activities in the Canary Islands increase the concern and the requirement for a conservation plan to assess the health effects in the cetacean populations in the studied area.

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## 5.3. Mercury and selenium status of bottlenose dolphins (*Tursiops truncatus*): A study in stranded animals on the Canary Islands

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# Mercury and selenium status of bottlenose dolphins (*Tursiops truncatus*): A study in stranded animals on the Canary Islands

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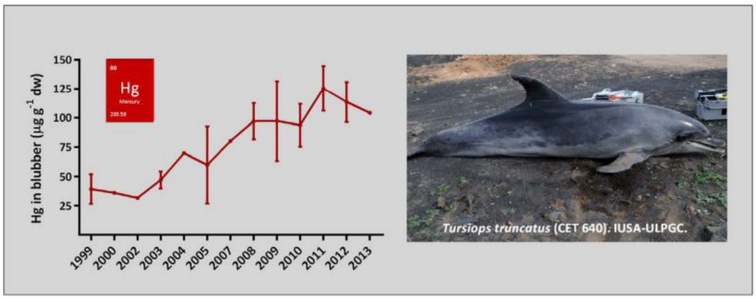
## HIGHLIGHTS

- Hg and Se in bottlenose dolphins stranded on the Canary Islands from 1997 to 2013
- Upward temporal trend of Hg concentration during the study period
- The youngest and oldest animals appear to be of greater toxicological concern
- Some dolphins have Hg levels within the threshold established for hepatic damage
- First report of Se/Hg molar ratio in cetaceans stranded along the Canary coasts

## GRAPHICAL ABSTRACT

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## ABSTRACT

The mercury (Hg) level in the marine environment has tripled in recent decades, becoming a great concern because of its high toxic potential. This study reports Hg and selenium (Se) status, and the first Se/Hg molar ratio assessment in bottlenose dolphins (*Tursiops truncatus*) inhabiting the waters of the Canary Islands. Total Hg and Se concentrations were determined in the blubber and liver collected from 30 specimens stranded along the coasts of the archipelago from 1997 to 2013. The median values for total Hg in the blubber and liver were 80.83 and 223.77 μg $g^{-1}$ dry weight (dw), and the median levels for Se in both tissues were 7.29 and 68.63 μg $g^{-1}$ dw, respectively. Hg concentrations in the liver were lower than 100 μg $g^{-1}$ wet weight (ww), comparable to those obtained in bottlenose dolphins from the North Sea, the Western Atlantic Ocean and several locations in the Pacific Ocean. The Mediterranean Sea and South of Australia are the most contaminated areas for both elements in this cetacean species. In addition, it must be stressed that the levels of Hg and Se in the liver showed an increasing trend with the age of the animals. As expected, a strong positive correlation between Hg and Se was observed ($r_s = 0.960$). Surprisingly, both younger and older specimens had a Se/Hg molar ratio different from 1, suggesting that these individuals may be at greater toxicological risk for high concentrations of both elements or a deficiency of Se without a protective action against Hg toxicity.



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## 1. Introduction

Mercury (Hg) is a natural element that is a ubiquitous environmental contaminant. It is distributed around the world by atmospheric transportation. The sources of Hg contamination can be both natural (e.g., degassing of the earth's crust, volcanic activities and forest fires) and anthropogenic (e.g., mining, chlorine industry, coal-burning power plants, cement and metallurgical industries, paper mills, agricultural pesticides, or medical waste incineration) (Van de Merwe et al., 2010). Natural inputs might be highly relevant in certain areas (Andre et al., 1991), but industrial activities might increase the exposure to this toxic element (AMAP, 2011; Magos and Clarkson, 2006), and recently published data even suggested that the amount of Hg in water has almost tripled compared to the pre-industrial period (Lamborg et al., 2014). Hg in its inorganic form is moderately toxic, but once in the aquatic environment, it is quickly transformed into methylmercury (MeHg), a highly toxic form of Hg of most concern to the health of humans and biota. MeHg is strongly neurotoxic (Clarkson and Magos, 2006), harmful to the kidneys, lungs, the thyroid gland, and the immune system (Das et al., 2008; De Guise et al., 1995); it is also teratogenic (Crespo-Lopez et al., 2009) and carcinogenic (Vos et al., 2003; Vos et al., 2000). In the marine environment, MeHg accumulates and biomagnifies along the food chain (Seixas et al., 2014) representing a serious threat, especially to top predators such as humans (Visnjevec et al., 2014) or cetaceans, which are exposed to this metal mainly via the diet (Bennett et al., 2001; Storelli et al., 2005).

It is well known that the toxic potential of Hg is suppressed in the presence of sufficient amounts of selenium (Se) (Parizek and Ostadalova, 1967). This effect has been shown in studies in a variety of species, including marine mammals (Cuvin-Aralar and Furness, 1991; Frodello et al., 2000; Gui et al., 2014; Sakamoto et al., 2013) exposed to these elements for a long time, even before the industrial period (Holsbeek et al., 1998). Thus, several mechanisms for resistance to the adverse effects of Hg have been proposed. On the one hand, Se can easily combined with various forms of Hg to yield complexes with lower toxicity, such as methylmercury selenide (MeHg-Se), methylmercury selenocysteinate (MeHg-Sec), or mercury selenide, tiemannite (HgSe), which is considered the last step in Hg detoxification (Palmisano et al., 1995). These compounds also contribute to the mobilization of mercury from the most vulnerable targets (such as kidney or nervous system tissues) to other less sensitive bodily regions, such as muscle. Furthermore, Se competes with Hg for its various biological targets, which also contributes to lowering the potential toxicity of Hg (Khan and Wang, 2009). Therefore, the Se/Hg molar ratio has been widely used (McHuron et al., 2014; Mendez-Fernandez et al., 2014; Squadrone et al., 2015; Vos et al., 2003), and many authors have established that Se, in a molar ratio of 1:1 or above with Hg, protects against the toxic effects of this metal (Ganther et al., 1972; Ralston and Raymond, 2013; Ralston et al., 2007; Ralston and Raymond, 2010; Sormo et al., 2011; Squadrone et al., 2015). However, paradoxically, this protective action can be harmful to the body because complex formation also results in the sequestration of both elements, causing them to become biologically unavailable (Martoja and Berry, 1980). Se is a well-known essential element with multiple biological functions, such as its critical participation in reproduction, the metabolism of thyroid hormones or DNA synthesis, in addition to its important antioxidant role (anticarcinogenic activity), among other functions (Schwarz and Foltz, 1957; Taylor et al., 2009; Zhang et al., 2014). Therefore, the presence of high levels of Hg could lead to Se deficiency, which could even cause the death in extreme cases (Chen, 2012; Sunde, 2006). Thus, the toxicological effects might be due to both MeHg toxicity and the induced Se deficiency (Zhang et al., 2014). However, Se levels have increased dramatically in many marine areas, presenting an environmental toxicity problem (Lavery et al., 2008). Se pollution probably occurs as a result of anthropogenic activities such as coal burning, smelting, ceramic and glass manufacturing, or copper refining (Van de Merwe et al., 2010). Therefore, to evaluate the health status of the ecosystems, the simultaneous study of Hg and Se and the relationship between them is of great interest, particularly in those species usually considered as sentinels for environmental pollution.

Because of its large size, longevity, and high position within the food chain, many authors have proposed the cetaceans as good sentinels for ocean health. Species with a worldwide distribution such as the bottlenose dolphin (*Tursiops truncatus*), are usually employed to assess global pollution and regional variations (Wilson et al., 2012). Therefore, this species has been selected for the present study because previous reports indicate that bottlenose dolphins clearly reflect the contamination of the waters of the Canary Islands (Eastern Atlantic Ocean) due to their proximity to likely anthropogenic sources (Garcia-Alvarez et al., 2014a; Garcia-Alvarez et al., 2014b). Moreover, these cetaceans have been extensively studied, allowing comparison of the results of this research with other marine areas around the world, to obtain more comprehensive approach to pollution observations.

Bottlenose dolphins inhabit the Canary Islands as local resident populations that show inter-island movements within the archipelago (Tobeña et al., 2014). This species faces a high exposure to organic pollutants (Garcia-Alvarez et al., 2014a; Garcia-Alvarez et al., 2014b) and is considered a valuable biomarker of the health status of the marine ecosystems. A high concentration of contaminants has also been reported in humans from this archipelago (Luzardo et al., 2012; Luzardo et al., 2009) and in other marine animals from the Canary Islands waters (Camacho et al., 2014) and other nearby areas (Camacho et al., 2013). Although there is a previous research concerning a few inorganic pollutants in 12 bottlenose dolphins stranded on the canary coasts (Carballo et al., 2004), there is a need of more recent and comprehensive data from this marine region.

The major goal of this study was to investigate the levels, relationship and toxicity of Hg and Se in bottlenose dolphins, through the direct measurement of these toxic elements in blubber and liver of animals stranded on the Canary Islands; thus, extending the knowledge on the contamination status of this cetacean species, which is frequently used as sentinel of the pollution of seas and oceans.

## 2. Materials and methods

### 2.1. Study area

The Canary archipelago is located in the Eastern North Atlantic Ocean near Europe and North Africa. These islands are a protected territory with 12 marine Special Areas of Conservation (SACs) because of the presence of bottlenose dolphins, species listed in Annex II and IV in the European Habitats Directive (EC., 1992).

However, as mentioned above, a lack of data exists concerning toxic metals and other inorganic pollutants from cetaceans inhabiting this marine region.

### 2.2. Sample collection

Over a period from 1997 to 2013, 29 each of blubber and liver samples were collected from 30 bottlenose dolphins stranded on the Canary Islands coasts. According to the literature, Hg and Se were found to accumulate in both tissues, reaching the highest levels in the liver (Beck et al., 1997). Besides, these tissues have been selected to be in accordance with previous studies of contaminants in stranded dolphins from this archipelago (Garcia-Alvarez et al., 2014a). The blubber is considered as a main target for pollutant assessment, in order to possible future comparisons with biopsy samples from live cetacean. On the other hand, the liver tissue was also selected because pattern distribution of metals is tissue specific, being the mercury mostly concentrated in the liver (Das et al., 2003).

Tissue sampling and the state of decomposition of the stranded specimens were determined by adapting the Geraci and Lounsbury

(2005) protocol. Thirteen males and 17 females (including 2 pregnant females) were divided into age categories i.e., newborn (1), calf (1), juvenile (5), subadult (11), adult (11) and old (1), based on body length and gonadal appearance. The bodily condition of the specimens was classified from a good to a very poor state according to morphological features. All of the characteristics of the animals studied are summarized in Table 1. Samples were stored in plastic bags at −80 °C in the Cetacean Tissue Bank of the University of Las Palmas de Gran Canaria (ULPGC) until analysis.

### 2.3. Sample preparation and analysis of trace elements

All samples were first lyophilized (freeze-dried) for a subsequent microwave digestion method using a Milestone ETHOS ONE oven. The fresh weight of each sample was recorded such that the results could be expressed both on a dry (dw) and a wet weight (ww) basis. In different vessels, 0.5 g aliquots of freeze-dried samples were mineralized with 6 ml of nitric acid plus 50 μl of Itrio (Y) as an internal standard. Each vessel was placed into the microwave oven to obtain solutions, which were then diluted to a final volume of 50 ml with distilled water. After digestion, the analysis of the elements was performed with an Inductively Coupled Plasma-Optic Emission Spectrometry method (ICP-OES) using a Perkin Elmer Optima 2100 DV instrument. Two blanks were run during each analysis to check chemical purity, and the accuracy of the method was verified with reference materials (lyophilized mussel; CRM 278, Community Bureau of Reference, BCR, Brussels). All the values of the reference materials were within certified limits. The recovery values for Hg and Se were 120 ± 8% and 115 ± 11%, respectively. The instrumental detection limits were 0.061 ng $ml^{-1}$ ww for Hg and 0.1 ng $ml^{-1}$ ww for Se.

### 2.4. Data analysis

#### 2.4.1. Calculation of the Se/Hg molar ratio

The molar ratio of Se to Hg was calculated as:

$$Se/Hg = (Se/78.96) / (Hg/200.59)$$

where 200.59 and 78.96 g $mol^{-1}$ are the atomic masses of Hg and Se, respectively.

#### 2.4.2. Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics v 22.0. Because trace elements did not follow conditions of normality (Kolmogorov–Smirnov and Shapiro-Wilk tests) or homogeneity of variances in all variable groups, non-parametric tests were used. Thus, the statistical significance between different categories was assessed using the Mann–Whitney U-test and the Kruskal–Wallis test for differences between two or more independent groups, respectively. Spearman's correlation test was performed to determine a possible relationship between both trace elements. As usual, the level of statistical significance was set at *p-value* = 0.05.

## 3. Results and discussion

Individual and descriptive statistics of Hg and Se concentrations in the blubber and liver of bottlenose dolphins (mean ± SD, median and range) are shown in Table 2. The data are expressed as μg $g^{-1}$ (ppm) on a dry weight (dw) basis. However, to allow comparison with other reports, the wet weight (ww) results were also determined using conversion factors calculated for each sample based on their respective percentages of dry residue (Table 2). The mean correction factor for blubber and liver tissue (0.48 and 0.28 respectively) are comparable with values reported in the literature (Becker et al., 1995; Mackey

**Table 1**
Identification and details of 30 bottlenose dolphins stranded along the Canary Islands coasts during the period of 1997–2013.

| Specimen | Year | Place[a] | Stranding[b] | Sex[c] | Age | Length[d] | Conservation state[e] | Bodily condition |
|---|---|---|---|---|---|---|---|---|
| CET 43 | 1997 | TF | 2 | F | Adult | NA | 2 | Very poor |
| CET 78 | 1999 | GC | 1 | M | Subadult | 294 | 1 | Poor |
| CET 94 | 1999 | TF | 2 | M | Juvenile | 260 | 2 | Good |
| CET 99 | 2000 | GC | 2 | F | Juvenile | 212 | 4 | Poor |
| CET 171 | 2002 | TF | 2 | F | Adult | 238 | 2 | Good |
| CET 203 | 2003 | FV | 2 | M | Subadult | 300 | 5 | NA |
| CET 209 | 2003 | GC | 2 | F | Juvenile | 215 | 3 | Poor |
| CET 231 | 2004 | TF | 2 | F (P) | Adult | 298 | 5 | NA |
| CET 286 | 2005 | GC | 2 | M | Juvenile | 245 | 4 | Good |
| CET 296 | 2005 | GC | 1 | F | Subadult | 296 | 1 | Good |
| CET 305 | 2005 | LZ | 1 | F | Subadult | 250 | 1 | Moderate |
| CET 311 | 2005 | FV | 1 | M | Subadult | 265 | 2 | Poor |
| CET 314 | 2005 | GC | 2 | F | Subadult | 210 | 4 | Good |
| CET 403 | 2007 | TF | 2 | M | Subadult | 258 | 4 | Moderate |
| CET 407 | 2008 | TF | 2 | F | Adult | 305 | 2 | Poor |
| CET 420 | 2008 | GC | 2 | F (P) | Adult | 279 | 4 | Moderate |
| CET 450 | 2008 | TF | 2 | F | Subadult | 260 | 1 | Moderate |
| CET 458 | 2008 | TF | 2 | F | Subadult | 205 | 5 | Good |
| CET 505 | 2009 | TF | 2 | M | Juvenile | 203 | 4 | NA |
| CET 509 | 2009 | TF | 2 | M | Adult | 260 | 3 | Moderate |
| CET 526 | 2010 | TF | 2 | F | Adult | 228 | 2 | Good |
| CET 543 | 2010 | LZ | 2 | M | Adult | 327 | 4 | NA |
| CET 562 | 2011 | TF | 2 | F | Adult | 287 | 4 | NA |
| CET 564 | 2011 | LZ | 2 | M | Adult | 306 | 2 | Good |
| CET 584 | 2011 | TF | 2 | F | Calf | 137 | 4 | NA |
| CET 592 | 2011 | LG | 2 | F | Newborn | 152 | 3 | Good |
| CET 595 | 2011 | LZ | 2 | M | Subadult | 278 | 2 | Very poor |
| CET 635 | 2012 | GC | 2 | M | Subadult | 285 | 2 | Moderate |
| CET 640 | 2012 | LZ | 1 | M | Old | 315 | 1 | Moderate |
| CET 662 | 2013 | TF | 2 | F | Adult | 260 | 1 | Very poor |

NA, data not available.
[a] LZ, Lanzarote; FV, Fuerteventura; GC, Gran Canaria; TF, Tenerife; LG, La Gomera.
[b] 1, active; 2, passive.
[c] M, male; F, female; F (P), pregnant female.
[d] Total length in cm.
[e] 1, very fresh; 2, fresh; 3, moderate autolysis; 4, advanced autolysis; 5, very advanced autolysis.

**Table 2**
Mercury and selenium levels in the blubber and liver of bottlenose dolphins collected in the Canary Islands, 1997–2013. Values expressed as μg g$^{-1}$ dry weight (dw) and wet weight (ww).

| Specimen | Blubber (n = 29) | | | | Liver (n = 29) | | | |
|---|---|---|---|---|---|---|---|---|
| | Hg | | Se | | Hg | | Se | |
| | (dw) | (ww) | (dw) | (ww) | (dw) | (ww) | (dw) | (ww) |
| CET 43 | NA | NA | NA | NA | 87.15 | 24.82 | 14.43 | 4.11 |
| CET 78 | 30.36 | 12.14 | 3.29 | 1.32 | 429.63 | 126.04 | 157.15 | 46.10 |
| CET 94 | 48.15 | 17.38 | 4.94 | 1.78 | 100.93 | 30.59 | 28.29 | 8.57 |
| CET 99 | 36.09 | 15.52 | 5.07 | 2.18 | 84.25 | 23.95 | 18.28 | 5.20 |
| CET 171 | 31.71 | 11.35 | 7.41 | 2.65 | 156.05 | 44.21 | 42.90 | 12.15 |
| CET 203 | 41.92 | 18.38 | 5.77 | 2.53 | 419.72 | 120.85 | 304.78 | 87.76 |
| CET 209 | 52.21 | 43.01 | 7.17 | 5.91 | 181.15 | 49.21 | 51.03 | 13.86 |
| CET 231 | 69.83 | 33.08 | 11.14 | 5.28 | NA | NA | NA | NA |
| CET 286 | 32.81 | 16.90 | 4.30 | 2.21 | 200.65 | 37.77 | 68.63 | 12.92 |
| CET 296 | 51.10 | 21.83 | 6.01 | 2.57 | 298.55 | 83.38 | 107.03 | 29.89 |
| CET 305 | 35.92 | 24.51 | 4.76 | 3.25 | 135.66 | 42.37 | 30.95 | 9.67 |
| CET 311 | 65.44 | 14.60 | 8.62 | 1.92 | 427.28 | 114.04 | 391.96 | 104.61 |
| CET 314 | 113.67 | 67.58 | 14.48 | 8.61 | 253.01 | 76.06 | 82.72 | 24.87 |
| CET 403 | 80.25 | 50.01 | 8.90 | 5.55 | 59.53 | 20.50 | 4.89 | 1.68 |
| CET 407 | 79.45 | 21.29 | 11.68 | 3.13 | 699.80 | 194.77 | 2040.21 | 567.83 |
| CET 420 | 90.16 | 73.71 | 6.66 | 5.44 | 470.22 | 139.15 | 521.46 | 154.31 |
| CET 450 | 105.25 | 80.79 | 13.89 | 10.66 | 223.77 | 72.79 | 63.37 | 20.61 |
| CET 458 | 113.62 | 71.74 | 7.17 | 4.53 | 96.24 | 27.82 | 7.64 | 2.21 |
| CET 505 | 121.48 | 57.99 | 7.29 | 3.48 | 77.67 | 21.49 | 6.90 | 1.91 |
| CET 509 | 73.18 | 59.97 | 2.09 | 1.72 | 77.13 | 22.69 | 4.69 | 1.38 |
| CET 526 | 106.60 | 76.29 | 3.39 | 2.43 | 61.45 | 20.63 | 2.79 | 0.94 |
| CET 543 | 80.83 | 54.16 | 4.47 | 3.00 | 398.48 | 104.40 | 345.83 | 90.61 |
| CET 562 | 137.17 | 45.33 | 21.29 | 7.04 | 381.88 | 97.66 | 370.27 | 94.69 |
| CET 564 | 141.69 | 41.74 | 16.10 | 4.74 | 518.35 | 151.41 | 562.48 | 164.30 |
| CET 584 | 130.76 | 104.46 | 19.70 | 15.73 | 96.15 | 26.35 | 10.96 | 3.01 |
| CET 592 | 93.15 | 78.59 | 6.63 | 5.59 | 75.45 | 25.42 | 7.24 | 2.44 |
| CET 595 | 123.39 | 43.17 | 9.06 | 3.17 | 366.54 | 101.54 | 268.50 | 74.38 |
| CET 635 | 125.64 | 56.54 | 13.69 | 6.16 | 366.27 | 96.95 | 176.00 | 46.59 |
| CET 640 | 101.37 | 68.23 | 9.99 | 6.73 | 419.87 | 102.39 | 215.80 | 52.62 |
| CET 662 | 104.13 | 34.38 | 14.84 | 4.90 | 422.39 | 101.66 | 217.74 | 52.41 |
| Mean ± SD | 83.36 ± 35.49 | 45.33 ± 25.50 | 8.96 ± 4.95 | 4.63 ± 3.09 | 261.56 ± 174.65 | 72.44 ± 47.88 | 211.20 ± 388.05 | 58.33 ± 108.30 |
| Median | 80.83 | 43.17 | 7.29 | 3.48 | 223.77 | 72.79 | 68.63 | 20.61 |
| Min. | 30.36 | 11.35 | 2.09 | 1.32 | 59.53 | 20.50 | 2.79 | 0.94 |
| Max. | 141.69 | 104.46 | 21.29 | 15.73 | 699.80 | 194.77 | 2040.21 | 567.83 |

SD, standard deviation. Min, minimum concentration obtained. Max, maximum concentration obtained. NA, data not available.

et al., 1995). Mean and median Hg values of 83.36 and 80.83 μg g$^{-1}$ dw were found in the blubber, which were lower than the mean and median Hg results in the liver (261.56 and 223.77 μg g$^{-1}$ dw, respectively). For Se, the mean and median levels of 8.96 and 7.29 μg g$^{-1}$ dw in the blubber were much lower than the Se concentration in the liver, in which the mean value of 211.20 μg g$^{-1}$ dw was quite far from the median of 68.63 μg g$^{-1}$ dw because of the data dispersion.

Bioaccumulation of contaminants in marine mammals has been reported to be highly dependent on both biotic and abiotic factors, such as sex, age, diet and pollution gradients in the aquatic environment (Storelli et al., 2005). Thus, an analysis of Hg and Se concentrations against different variables (Table 1) is essential to fully understand the effects of these elements on the specimens studied.

### 3.1. Influence of sex and age on mercury and selenium levels

Age is the most important biotic factor for Hg and Se accumulation. Although an increasing hepatic concentration of both trace elements was observed, the small and unequal sample sizes of age categories discouraged any statistical test assessment. This enables us to use the body length as a surrogate for age class. Testing correlations between pollutant levels and the length of the animal, the hepatic Hg was found to be positively correlated against this variable with a Spearman coefficient ($r_s$) of 0.769 (Fig. 1A). In accordance with previous authors (Bellante et al., 2012), an increasing trend throughout the life of cetaceans was observed (Fig. 1), probably due to bioaccumulation from the continuous uptake of Hg in the diet and the decreasing ability to excrete this metal and storage in stable forms such as HgSe (Aguilar et al., 1999; Mackey et al., 1995; Wagemann et al., 2000). Other authors also found an upward trend of hepatic Hg in sharks and rays with age (Gutierrez-Mejia et al., 2009; Storelli and Marcotrigiano, 2002), suggesting a higher rate of assimilation than excretion of Hg and a lower efficiency of detoxification. Moreover, the largest specimens may capture bigger prey, which are more likely to contain higher levels of Hg. Although essential trace elements are regulated via homeostasis in marine mammals (Mendez-Fernandez et al., 2014), Se levels in liver samples were notably higher in adults than in the youngest individuals (Fig. 1) and were also correlated with animal length ($r_s = 0.764$) in accordance with other studies (Woshner et al., 2001). The high level of Se may result from its accumulation with Hg during the detoxification process or from a highly concentrated diet because most ocean fish are Se-rich, as was reported in a study in the Faroe Islands (Budtz-Jorgensen et al., 2007).

Fig. 1B also shows tissue distribution of Hg and Se throughout the lifetime of the animals, highlighting the concentration of both elements mostly in the liver, with a statistically significant difference between tissues ($p = 0.000$). Therefore, the liver appeared to be the preferential tissue, as indicated in previous studies (Bellante et al., 2012; Frodello et al., 2000). However, it is also interesting to stress that the newborn and calf specimens of this research accumulated greater levels of Hg in the blubber than the liver, especially for the calf. Concerning the Se, the newborn showed equal levels in both tissues and the calf individual doubled the concentration in the blubber. These results were in contrast to the following sampling ages where the liver showed higher levels of both trace elements with an evident increasing trend. One could hypothesize that this variation on the tissue distribution is due to the different pollutant sources. Thus, the Hg and Se were initially transferred through placental barrier entering the fetal circulation to be

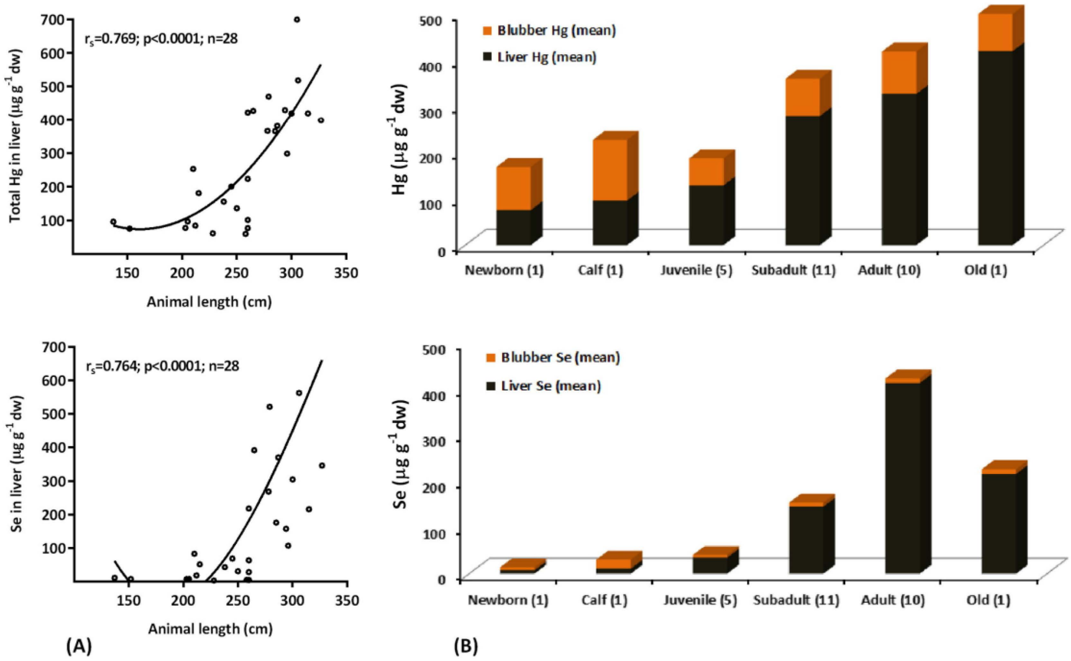


**Fig. 1.** (A) Hepatic mercury (above) and selenium (below) concentrations in bottlenose dolphins correlated with the total length of the specimens with Spearman correlations ($r_s$) of 0.769 and 0.764 respectively. (B) Mean concentrations (µg g$^{-1}$ dw) of mercury (above) and selenium (below) in the blubber and liver of bottlenose dolphins comparing age groups. Sample size (n) is in brackets. Note that there is only one animal in the newborn, calf and old age categories.

transported to the liver for metabolism, and then distributed to the blubber for accumulation. The calf showed the highest concentrations of both elements in the blubber among all the animals studied, likely as a result of exposure through lactation. On the contrary, the juvenile group had the lowest level of Hg and Se in the blubber compared to the rest of the age categories. This may be due to the release of Hg and Se from the blubber into the circulation at weaning stage, which could be considered a period of negative energy balance (Louis et al., 2015). However, little is known concerning the factors that affect mobilization of pollutants from adipose tissue (Louis et al., 2014). Therefore, this finding should be interpreted with caution also because only one newborn and one calf of bottlenose dolphins were available for this research.

In the present study no influence of sex on Hg or Se accumulation was observed, as has been found in other marine areas of the North Atlantic Ocean (Mendez-Fernandez et al., 2014).

### 3.2. Influence of stranding location on mercury and selenium levels

Clear differences for Hg and Se concentrations in the liver between the geographical areas, in which animals stranded, were found (with *p-values* of 0.060 and 0.046 respectively). The results for the eastern Canary Islands of Lanzarote (LZ), Fuerteventura (FV) and Gran Canaria (GC) showed the highest Se and Hg levels as compared to the western islands of Tenerife (TF) and La Gomera (LG). Thus, median hepatic Hg molar concentration from animals stranded in the eastern islands (1824.21 nmol g$^{-1}$ dw) was 4 times greater than that from specimens stranded in the western islands (427.97 nmol g$^{-1}$ dw). This difference between both Canary regions was even more prominent for Se, which reached a 22-fold hepatic molar concentration in the eastern region of the archipelago. In fact, there is a decreasing trend from the nearest to the furthest island from the African continent. This finding could be related to geographical differences of natural and/or anthropogenic sources, but is more likely affected by the age of the animals at the various locations. The youngest individuals were found stranded in the western Canary Islands, so these results should be cautiously considered, also due to the low and unequal number of samples (LZ, n = 5; FV, n = 2; GC, n = 8; TF, n = 13; LG, n = 1).

### 3.3. Temporal trends of mercury and selenium concentrations

Fig. 2A illustrates the total Hg and Se concentrations in the blubber of individuals grouped according to the year of stranding (between 1999 and 2013). For this context, it is preferable to analyze the blubber samples because no influence of age or length on Hg or Se levels in this tissue was obtained in the present study (see Fig. 1B). Despite a careful interpretation required by the low sample size of the groups (1999, n = 2; 2000, n = 1; 2002, n = 1; 2003, n = 2; 2004, n = 1; 2005, n = 5; 2007, n = 1; 2008, n = 4; 2009, n = 2; 2010, n = 2; 2011, n = 5; 2012, n = 2; 2013, n = 1), an increasing temporal trend of Hg in the blubber can be seen throughout the study period. In addition, the Mann–Whitney U-test revealed a significant difference ($p = 0.016$) between 2005 and 2011, each with 5 specimens available. From the individual stranded in 1999 to the last one in 2013, the Hg level has tripled in the blubber, consistent with a recently published report (Lamborg et al., 2014). Analyzing the blubber samples from adult individuals, an even more marked increase was observed (Fig. 2A, inset). Lamborg's group found that deep and intermediate North Atlantic waters are abnormally enriched in Hg, probably because of anthropogenic activities such as mining and fossil fuel combustion. Furthermore, no temporal rising tendency of the Se burden in blubber was observed (Fig. 2A) or in the hepatic levels of either trace element (Fig. 2B).

### 3.4. Study of the relationship between mercury and selenium

As previously mentioned, a common approach to assess the risk of exposure to Hg is to determine the molar ratio of Hg and Se in the body (Se/Hg). A high positive correlation between Hg and Se with an equimolar ratio in the liver as well as the protective effect of Se against Hg toxicity is well documented (Cuvin-Aralar and Furness, 1991; Geraci, 1989; Koeman et al., 1973; Yang et al., 2007).

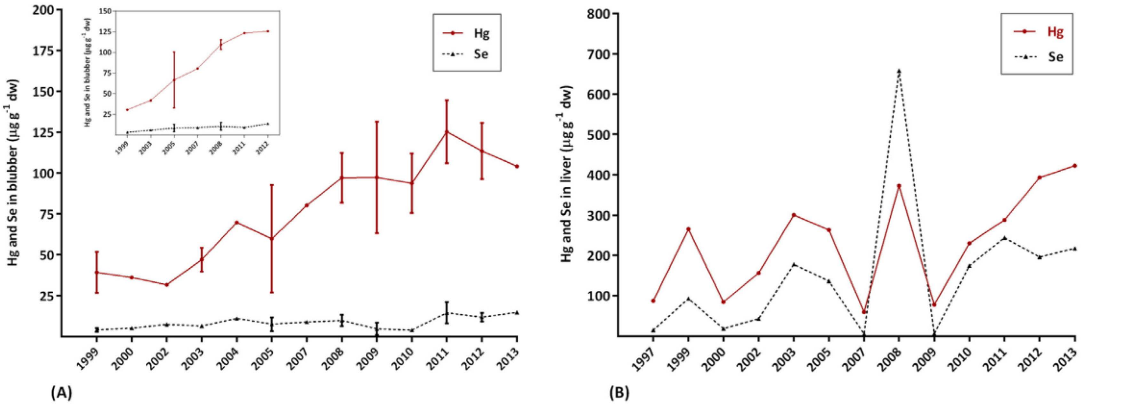


**Fig. 2.** (A) Temporal distribution of total mercury and selenium concentrations (µg $g^{-1}$ dw) in blubber samples of bottlenose dolphins stranded from 1999 to 2013 on the Canary Islands coasts. The plot represents the mean level with standard deviation (SD). Sample size in each group: 1999 (n = 2); 2000 (n = 1); 2002 (n = 1); 2003 (n = 2); 2004 (n = 1); 2005 (n = 5); 2007 (n = 1); 2008 (n = 4); 2009 (n = 2); 2010 (n = 2); 2011 (n = 5); 2012 (n = 2); 2013 (n = 1). Inset: data from adult specimens; all other age categories have been excluded. Sample size in each group: 1999 (n = 1); 2003 (n = 1); 2005 (n = 4); 2007 (n = 1); 2008 (n = 2); 2011 (n = 1); 2012 (n = 1). (B) Temporal trends of both trace elements (µg $g^{-1}$ dw, individual or mean values) in the liver of the animals studied. Sample size in each group: 1997 (n = 1); 1999 (n = 2); 2000 (n = 1); 2002 (n = 1); 2003 (n = 2); 2005 (n = 5); 2007 (n = 1); 2008 (n = 4); 2009 (n = 2); 2010 (n = 2); 2011 (n = 5); 2012 (n = 2); 2013 (n = 1).

In the present study, the results showed that increasing Hg levels were associated with increasing Se concentrations, as described for other dolphin populations (Palmisano et al., 1995). Spearman's correlation coefficient ($r_s$), calculated between molar concentrations of hepatic Hg and Se, showed a strong positive relationship (Fig. 3). Excluding the outlier data for Se (CET 407), the correlation slightly decreased from 0.960 to 0.955, although the coefficient of determination ($R^2$) for linear regression increased from 0.592 to 0.807. It is remarkable that the strongest linear association ($R^2 = 0.973$) between these two elements occurred below 1500 nmol $g^{-1}$ (300 µg $g^{-1}$ dw) of Hg in the liver, comparable to a total Hg threshold of 100 µg $g^{-1}$ ww, as obtained by other authors (Palmisano et al., 1995). Above this concentration, the level of hepatic Se significantly exceeded the Hg concentration, so the Se/Hg molar ratio was higher than 1 (Figs. 3B and 4). Se was in molar excess of Hg in 11 of 29 livers evaluated (37.9%). Other publications reported a similar levels of Se compared to Hg in both pelagic fish (Kaneko and Ralston, 2007) and cetaceans (Mendez-Fernandez et al., 2014), and the authors stated that this excess reflects the good health status of individuals or a high proportion of young animals. In the present study, individuals with a Se/Hg molar ratio above 1 were all included in older categories (Fig. 4), contrary to such statement and other results obtained for several cetaceans species (Caceres-Saez et al., 2013; Palmisano et al., 1995; Yang et al., 2007). Regarding the place of stranding, LZ and FV showed a Se/Hg molar ratio over 1; by contrast, GC, TF and LG had a median ratio below 1. However, the limited sample size per group undercut any conclusion.

### 3.5. Assessment of the health risk of mercury and selenium

Wagemann and Muir (1984), established a threshold for hepatic damage in marine mammals in a range of 100–400 µg $g^{-1}$ ww Hg burden (Wagemann and Muir, 1984). Comparing total hepatic Hg concentration to this threshold, 10 of 29 livers of stranded individuals (34.5%) exceeded the minimum Hg tolerance level, and 4 had values just below 105 µg $g^{-1}$ ww. All these samples were from subadult and adult specimens, corresponding to 45.5% of the total of subadults and adults in this study. Other authors obtained comparable results for stranded bottlenose dolphins in Australian and Floridian waters (Lavery et al., 2008; Stavros et al., 2011). These results coincided with animals with a Se/Hg molar ratio greater than 1.

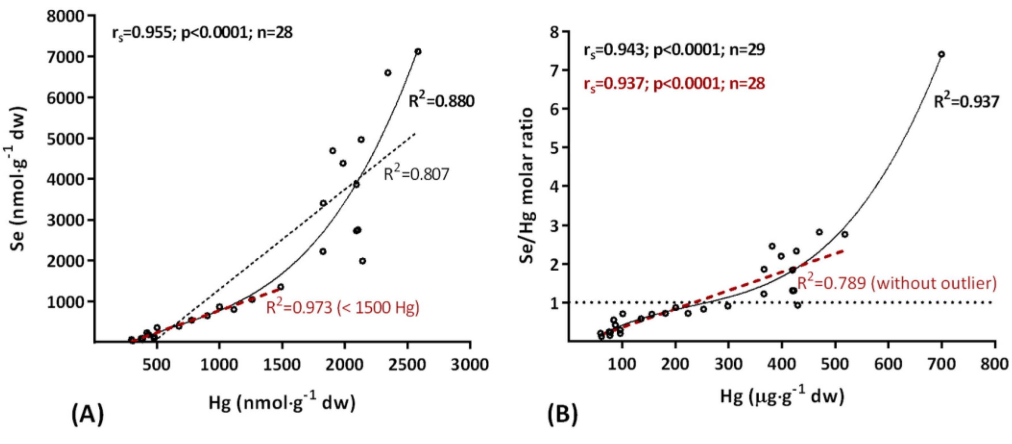


**Fig. 3.** (A) Correlation between mercury and selenium molar concentrations (nmol $g^{-1}$ dw) in the liver of bottlenose dolphins from the Canary Islands. Spearman correlation ($r_s = 0.955$) excluding the outlier data (CET 407) with a graphic representation of linear ($R^2 = 0.807$) and potential cubic regression ($R^2 = 0.880$). Linear regression of Hg molar concentration below 1500 ($R^2 = 0.973$). (B) Dependence of the Se/Hg molar ratio on the total mercury (µg $g^{-1}$ dw) in liver samples of bottlenose dolphins. Spearman correlation ($r_s = 0.943$) considering all samples (n = 29) and its potential cubic regression ($R^2 = 0.937$). Spearman correlation excluding the outlier value ($r_s = 0.937$) and its linear regression ($R^2 = 0.789$).

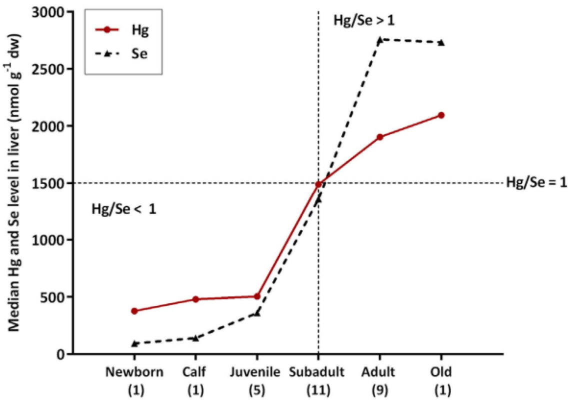


**Fig. 4.** Trends for hepatic mercury and selenium levels with the age of bottlenose dolphins (median of molar concentrations, nmol $g^{-1}$ dw); the selenium outlier data (CET 407) is excluded. Sample size (n) is in brackets.

The results discussed above indicate that the youngest and oldest bottlenose dolphins may be of greater toxicological concern (Fig. 4). Although the newborn and the calf among the animals studied had the lowest Hg content, they were deficient in Se which could lead to Hg toxicity and they also had a Se/Hg molar ratio less than 1, indicating a limited protection by Se. This result is consistent with human studies in which authors argue that prenatal and postnatal Hg exposure negatively affects central nervous system functions (Rasmussen et al., 2005). Additionally, a molar ratio of 1 or lower may indicate that all of the available Se is bound to Hg, conferring a possible oxidative stress risk (Caceres-Saez et al., 2013). However, these results must be carefully considered because there was only one specimen available from each, newborn and calf categories. By contrast, the older animals had the highest concentrations of both Hg and Se in the liver and Se/Hg ratios greater than 1 suggesting a Se molar excess which could become toxic at high levels (O'Hara et al., 2003). Nevertheless, the inter-relationships between the Hg and Se concentrations, age, nutritional status and disease are complex (Law et al., 2012) and the limits of deficiency, essentiality, and poisoning is quite difficult to assess and not well studied.

**Table 3**
Hg and Se concentrations (μg $g^{-1}$) in the blubber and liver of bottlenose dolphins from the present study and other marine areas worldwide. Data expressed as individual, mean or (median) value, when available.

| Location | Year | Tissue (n) | Hg | Se | References |
|---|---|---|---|---|---|
| NE Atlantic Ocean | | | | | |
| Canary Islands | 1999–2013 | Blubber (29) | 83.36 (80.83)[a] | 8.96 (7.29)[a] | The present study |
| | | | 45.33 (43.17)[b] | 4.63 (3.48)[b] | |
| | 1997–2013 | Liver (29) | 261.56 (223.77)[a] | 211.20 (68.63)[a] | |
| | | | 72.44 (72.79)[b] | 58.33 (20.61)[b] | |
| Canary Islands | 1997–2005 | Blubber (8) | | | Esperón (2005) |
| | | Liver (7) | 809.83 (320.67)[a] | 331.4 (104.9)[a] | |
| NW Iberian Peninsula | 2004–2008 | Liver (8) | 19.1[b] | 10.8[b] | Méndez-Fernández et al. (2014) |
| France | 1999–2004 | Liver (10) | 38[b] | | Lahaye et al. (2006) |
| France | 1978–1990 | Liver (5) | 421.2[a] | | Holsbeek et al. (1998) |
| Mediterranean Sea | | | | | |
| Croatia (Adriatic Sea) | 2000–2002 | Liver (14) | 450 (177)[b] | | Bilandzic et al. (2012) |
| Croatia (Adriatic Sea) | 1990–1999 | Liver (14) | 358 (87.7)[b] | | Pompe-Gotal et al. (2009) |
| Israel | 2004–2006 | Liver (7) | 35.6[b] | | Shoham-Frider et al. (2009) |
| Israel | 1993–2001 | Blubber (14) | 1.5 (0.58)[b] | | Roditi-Elasar et al. (2003) |
| | | Liver (14) | 97 (32)[b] | | |
| France | 1999–2004 | Liver (5) | 204[b] | | Lahaye et al. (2006) |
| Corsica | 1997 | Liver (1) | 62[a] | | Frodello et al. (2002) |
| Corsica | 1995 | Liver (1) | 4250[a] | | Frodello et al. (2000) |
| Italy (Ligurian Sea) | 1999–2002 | Liver (2) | 13.55–3737[a] | 8.73–1708[a] | Capelli et al. (2008) |
| Italy (Tyrrhenian Sea) | 1987–1989 | Liver | 12.2–13150[a] | | Leonzio et al. (1992) |
| North Sea | | | | | |
| United Kingdom – 18 marine mammal species* | 1991–2006 | Liver (492) | 25 (7.0)[b] | 13 (4.2)[b] | Law et al. (2012) |
| NW Atlantic Ocean | | | | | |
| New Jersey to Florida | 1987–1988 | Liver (59) | 22[b] | 9[b] | Geraci (1989) |
| South Carolina | < 1997 | Liver (34) | 17.8[b] | 9.54[b] | Beck et al. (1997) |
| South Carolina | 2000–2007 | Liver (8–12) | 34.3[a] | 14.5[a] | Stavros et al. (2011) |
| Florida (Indian River Lagoon) | 2004–2008 | Liver (15) | 300[a] | 109[a] | |
| Gulf of Mexico (Florida) | 1991–1992 | Liver (13) | 304[a] | 65[a] | Meador et al. (1999) |
| Gulf of Mexico (Texas) | 1991–1992 | Liver (30) | 212[a] | 124[a] | |
| Pacific Ocean | | | | | |
| Australia (east coast) | 1995–1996 | Liver (2) | 0.72–32[b] | 1.5–12[b] | Law et al. (2003) |
| Australia (south coast) | 1988–2004 | Liver (10–11) | 213.94[b] | 70.19[b] | Lavery et al. (2008) |
| Australia (south coast) – *T.aduncus** | 1988–2004 | Liver (59–63) | 475.78[b] | 178.85[b] | |
| Hong Kong | 1994–1995 | Blubber (3) | <0.8–0.9[a] | 2.69–7.67[a] | Parsons and Chan (2001) |
| | | Liver (3) | ~100.20[a] | 31.57[a] | |
| SW Atlantic Ocean | | | | | |
| Argentine – *T. gephyreus** | 1990 | Liver (1) | 86[b] | 196.2[b] | Marcovecchio et al. (1990) |
| Dolphinarium | | | | | |
| Israel | 1995 | Liver (1) | 1.7[b] | | Shlosberg et al. (1997) |

[a] Data expressed in dry weight basis;
[b] Data expressed in wet weight basis.
* indicates other species of bottlenose dolphins apart from *Tursiops truncatus*.

*3.6. Mercury and selenium in bottlenose dolphins from different marine areas*

A comparison of Hg and Se levels in bottlenose dolphins from the Canary Islands (this study) and from different marine areas worldwide was made (Table 3). All these published results, compiled as ranges of Hg concentrations in the liver, were plotted on a map (Fig. 5), as well as others previously performed using Hg content in the hair of pinnipeds (McHuron et al., 2014) and organic pollutants in cetaceans (Aguilar et al., 2002). Bottlenose dolphins from the Mediterranean Sea had greater Hg concentrations than published values elsewhere, as was previously reported for striped dolphins (Andre et al., 1991). Even within the same marine area some differences in the Hg content were observed. Thus, the Ligurian and Tyrrhenian Sea, showed the maximum measured of hepatic Hg (13,150 μg $g^{-1}$ dw) ever reported before, followed by the Adriatic Sea which appears to be significantly more polluted by Hg than the less-contaminated Eastern Mediterranean coast. The Hg levels from the North Sea and the Northeast Atlantic Ocean, including the Canary Islands (results from this study), were below 100 ppm (ww), similar to mean concentrations in bottlenose dolphins from the Western Atlantic Ocean and from several locations in the Pacific Ocean (Hong Kong and east coast of Australia). In contrast, results from the south coast of Australia showed a greater Hg contamination that nearly matched the Adriatic Sea values (see Table 3 for references). Thus, this last sea displayed 6 times higher Hg burden compared with the results from bottlenose dolphins from the Canary Islands (present research). Furthermore, the Tyrrhenian Sea showed the highest Hg value obtained in the literature, more than 50 times greater than the values obtained in this study. On the other hand, *T. truncatus* and *Tursiops aduncus* from South of Australia had 3 to 7 times higher Hg levels respectively, than the specimens from the Canary archipelago.

It has been reported that Mediterranean prey had higher Hg levels than Atlantic prey (Lahaye et al., 2006), which explains the Hg enrichment in the Mediterranean food webs, and also in the liver of bottlenose dolphins. The authors suggest that this might be due to natural Hg sources in the Mediterranean Sea (Andre et al., 1991) and high anthropogenic Hg emissions especially from France (Bellante et al., 2012).

There are not many studies on Se levels in the liver of bottlenose dolphins (Table 3). The mean hepatic concentration of Se was below 50 μg $g^{-1}$ ww in most marine areas worldwide, but two locations far exceeded this value, which also corresponded to places that had the highest Hg burdens, the Ligurian Sea (Capelli et al., 2008) and the liver of *T. aduncus* in the south of Australia (Lavery et al., 2008). The bottlenose dolphins from both regions showed 8 and 3-fold greater Se levels, respectively, than the results obtained in this study. These geographical differences are difficult to explain because Se is an essential element and many factors, such as dietary intake or natural sources, but also differences in physiologic needs or the retention of Se for detoxification processes, might influence its concentrations (McHuron et al., 2014).

## 4. Conclusions

The present study contains the first reported evidence of Hg and Se concentrations in the blubber of bottlenose dolphins stranded along the coasts of the Canary Islands and broadens the data previously available in liver tissue. In addition, it represents the first Se/Hg molar ratio assessment in cetaceans from this marine area.

There is an increasing temporal trend of Hg concentration during the period of the study (1997–2013) and is consistent with recently published results for Hg in Atlantic waters (Lamborg et al., 2014).

Hg and Se accumulate in the liver of dolphins during their lifetime and are strongly positively correlated with each other. Hg increases with body length probably because of continual dietary uptake and Se due to detoxification processes or from eating Se-rich fish. Individuals with Se/Hg molar ratios over 1 are all subadults and adults. Conversely, young animals have lower Hg burdens and are also deficient in Se. Thus, according to our results, the youngest and oldest animals seem to be of greater toxicological concern. In addition, variation on these two elements in the blubber between the earliest stages of life (newborn and calf) and the following ages, likely indicates the influence of lactation and weaning on the lypophilic pollutant accumulation. Nevertheless, this finding must be carefully discussed considering the limited data available per age group.

A comparison of the present study with literature values from other worldwide marine areas indicates that hepatic Hg results from this part of the Northeast Atlantic Ocean are comparable to those obtained in bottlenose dolphins from the North Sea, the Western Atlantic Ocean


**Fig. 5.** Relative mercury concentration in the liver of bottlenose dolphins from the Canary Islands (the present study) and other marine areas worldwide (see Table 3 for references). 1, <50 (μg $g^{-1}$ ww); 2, 50–100 (μg $g^{-1}$ ww); 3, 100–500 (μg $g^{-1}$ ww); 4, >500 (μg $g^{-1}$ ww).

and several locations in the Pacific Ocean. The Mediterranean Sea and the South of Australia are hot spot contaminated areas for both elements; by contrast, the median results of this study show that the bottlenose dolphin population from the Canary Islands is not especially threatened by Hg or Se. However, it must be emphasized that the concentrations of the elements were highly variable between specimens; some fall into the Hg threshold established for hepatic damage, and others are Se deficient. In light of these results, further work is required to assess the individual effects of high loads of Hg and either large amounts or a deficiency of Se. In addition, an evaluation of the possible toxic impact of chronic exposure is also necessary.

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# 6. SUMMARY RESULTS OF PUBLICATIONS

## 6.1. General results:

- The OCs and Hg were found at important levels in many animals, being the adult males the most contaminated group, consequently with the highest potential toxicological risk.
- The BNDs from the Canary Islands seem not to be especially threatened by Hg or Se. However, concentrations of the elements were highly variable between specimens; some fall into the Hg threshold established for hepatic damage, and others are Se deficient.
- The levels of POPs in blubber tissues were higher than in the liver from stranded dolphins. In contrast, the burden of hepatic toxic elements was much greater than the results obtained in the blubber.

### 6.1.1. Temporal trends

- An increasing temporal trend of Hg concentration in the blubber during the period of the study (1997–2013) was found, being consistent with recently published results for Hg in Atlantic waters (Lamborg et al., 2014).
- Concentrations of OCPs, PCBs, and PAHs are not declining either in this area in the study period, still being at toxicologically relevant levels in blubber of BNDs.

### 6.1.2. Comparison of POPs between stranded and live dolphins

- Comparing the results between blubber from stranded and live free-ranging dolphins (see table 5 in section 4), among the 57 POPs studied, PCBs and DDTs were predominant in both samples. PCB median of 31 ppm in biopsies and 28 ppm in stranded individuals were observed; also OCP median of 57 ppm and 26 ppm respectively, and a PAHs median of 14 ppm in biopsies against a 0.8 in stranded animals were found. Thus, significant statistical differences in median values of pesticides and PAHs, not so in PCBs, were obtained.

- See section 6.2 for differences on specific chemical compounds between stranded and live animals.

### 6.1.3. Comparison with other marine areas worldwide

- Regarding OCPs, in general higher levels of DDTs than those found in BNDs from the North Sea and quite similar to US Atlantic coast were detected. However, higher concentration is reported in Mediterranean BNDs, well known for their extremely high OCs burden (Aguilar et al., 2002), and US Pacific coast. This significant level despite been banned for decades, may be related to the proximity of the Moroccan to Canary Islands coast, where DDT is still being used for the fight against Malaria (UNEP, 2010-2015) and may put the cetacean populations at risk, but this speculation has not been proven. In fact, a recently published study suggested that OCs in Canarian people were more related to their lifestyle and historical use of pesticides in this area (Henriquez-Hernandez et al., 2016b).
- Regarding industrial residues, in general the study highlights a slightly higher levels of PCBs than North Sea and similar to PCBs found in BNDs from Western and Eastern coasts of USA, but slightly lower than United Kingdom. The Mediterranean Sea shows much greater amounts of PCBs, known as a hot spot area of contamination. It is remarkable the levels of PCBs found in MMs from this archipelago, as it is considered as a relatively low industrialized region.
- Regarding Hg burdens, results obtained in liver of stranded BNDs from Canary Islands (present study) were comparable to those reported from North Sea, Western Atlantic Ocean and several locations in the Pacific Ocean. The Mediterranean Sea and the South of Australia are hot spot contaminated areas for Hg and Se in this species.

## 6.2. Specific results related to different contaminant groups:

### 6.2.1. PCBs

- The highly chlorinated PCB138, 153 and 180 were the prominent congeners within the 18 PCBs analysed in all samples.
- Despite being banned for decades, all the individuals studied, except the two pregnant females, were exposed at toxic levels of PCBs, exceeding proposed thresholds (see table 3). However, other biology factors should be taken into consideration.
- When the Toxic Equivalency to Dioxins (TEQ) approach is used, the results represent a relevant toxic load of dioxins in these animals which constitute a considerable risk for their health status.

- Live versus stranded dolphins:
    - The pattern of PCB contaminant loads observed was very similar in the blubber from both stranded and live animals.
    - Higher mean concentration of total PCBs in biopsies in accordance with other studies (Jepson et al., 2016), although median PCB results were found to be quite similar in both tissues (31 ppm vs 28 ppm) without statistically significant difference.
    - Significant differences were found in total DL-PCBs between both types of samples ($p$=0.006), being higher in biopsies. However, median values of congeners PCB 126 and PCB 189 were the only determined values found at slightly higher levels in blubber from stranded compared to biopsies, in accordance to other studies which state that the most chlorinated congeners are greater in stranded animals (Hobbs et al., 2003). This difference makes the median value for TEQ-PCBs also higher in blubber from stranded dolphins.

### 6.2.2. OCPs

- The pp-DDE was the compound with the highest concentration among the 57 pollutants tested in all samples.
- The next remarkable compounds were the rest of DDTs metabolites, dieldrin and mirex for stranded dolphins.
- Live vs stranded dolphins:
    - All OCPs had higher values in blubber biopsies compared to blubber from stranded dolphins, but total DDTs was quite similar in both types of samples.
        - Clordanes were 10 fold greater in biopsies, but the ratio trans:cis was equal to samples from stranded animals.
        - Dicofol was also 10 times higher in biopsies.
        - Mean of HCB was higher in biopsies in contrast to median value which were found to be 5 times greater in stranded samples (Hobbs 2003).
        - Blubber biopsies from live BNDs did not show dieldrin, endosulphan-alpha or endosulphan-sulphate, which were all detected in samples from stranded.
        - A significant presence of endrin was found in biopsies in a high level (20 ppm), though not found in blubber from stranded dolphins, which could lead to a significant statistical difference in OCPs between both types of samples.

### 6.2.3. PAHs

- All samples showed detectable values for at least 6 of the 16 studied PAHs.
- Among PAHs, phenanthrene was the most frequently detected and at the highest levels in all individuals, followed by pyrene.
- The animals were mainly exposed to the lower molecular weight PAHs.
- The autolysis increases the PAHs concentration in liver with statistical significance (higher sample size is needed to confirm this assumption).
- Live vs stranded dolphins:

    - Statistically significant difference in levels of PAHs between samples from live and stranded dolphins was found. It is remarkable that biopsy samples showed 10 fold higher levels of PAHs compared to stranded animals, which could be related to the degradation and volatility of PAHs in exposed carcasses to the environment. This raises doubts about the value of PAHs determination in tissues from stranded dolphins.
    - Anthracene was not detected in any sample from stranded specimens but was found in 14.1% of the biopsies analysed.

### 6.2.4. Hg and Se

- Hg and Se accumulate in the liver of dolphins during their lifetime and are strongly positively correlated with each other.
- Individuals with Se/Hg molar ratios over 1 are all subadults and adults.
- Young animals have lower Hg burdens and are also deficient in Se.
- Thus, according to our results the youngest and oldest animals seem to be of greater toxicological concern.

# 7. CONCLUSIONS / CONCLUSIONES

## CONCLUSIONS

1. The present study represents the first determination of pollutants in live free-ranging cetaceans from the Canary Islands.
2. It also provides the first data of PAHs in bottlenose dolphins (*Tursiops truncatus*) from the Eastern Atlantic Ocean and doubles the number of OC pollutants and toxic elements previously analysed in stranded cetaceans on Canary coasts (Carballo et al., 2008), also extending the period under investigation to fifteen years.
3. This thesis contains the first reported evidence of Hg and Se burdens in the blubber of BNDs stranded along the coasts of the Canary Islands and broadens the data previously available in liver tissue. In addition, it also represents the first Se/Hg molar ratio assessment in cetaceans from this marine area.
4. According to our results, the BNDs inhabiting the canary waters is facing a significant exposure to anthropogenic pollutants, highlighting the need for ongoing monitoring of contaminant accumulation in cetaceans from this marine area to understand the overall status of these populations.
5. Concentrations of contaminants were highly variable between specimens but some of them exceed the toxic threshold proposed in the consulted literature.
6. Adult males and the youngest individuals are considered to be at higher toxicological risk due to the accumulation of xenobiotics and the critical development stage of calves, respectively.
7. An increasing temporal trend of Hg in the blubber of BNDs from the Canary Islands was observed (it has tripled over the study period, 1997-2013) and POPs were not decreasing either considering the period of strandings, which are unlikely to decline in the near future.

8. In general, xenobiotics found in the present study are slightly higher than those from the North Sea and comparable to Western Atlantic Ocean, several locations in the Pacific Ocean and the waters surrounding the UK. As expected, our results are much lower than those observed in the Mediterranean Sea where BNDs accumulate great burdens of chemical residues.
9. Further studies are needed to investigate potential associations between pollutants and health status of cetacean populations, which requires assessing both the individual effect caused by high loads of anthropogenic xenobiotics and the toxic impact of chronic exposure; thus to determine the impact of human activities on these animals.
10. Environmental pollution should be seen as a global issue, with a multidisciplinary approach to attempt to reduce the levels of these legacy pollutants in the apex predators inhabiting our seas and oceans.

# CONCLUSIONES

1. En esta tesis doctoral se presenta la primera determinación de contaminantes en cetáceos vivos en libertad de las Islas Canarias.
2. Además, proporciona los primeros datos de PAHs en delfines mulares (*Tursiops truncatus*) del océano Atlántico Este y duplica el número de compuestos organoclorados y elementos tóxicos previamente analizados en cetáceos varados en costas canarias (Carballo et al., 2008), ampliando el periodo de estudio a 15 años.
3. Esta tesis doctoral contiene la primera publicación que evidencia niveles de Hg y Se en muestras de blubber de delfines mulares varados en las costas de las Islas Canarias y aumenta los datos de niveles hepáticos disponibles. Además, este estudio representa la primera evaluación del ratio molar Se/Hg en cetáceos de este área marina.
4. Según los resultados obtenidos, los delfines mulares que habitan las aguas canarias están expuestos a niveles significativos de contaminantes antrópicos, lo que resalta la necesidad de un monitoreo continuo de la acumulación de xenobióticos en cetáceos de este área marina para entender el estado general de estas poblaciones.
5. Los niveles de contaminantes fueron altamente variables entre los especímenes, excediendo algunos de ellos los umbrales tóxicos propuestos en la bibliografía consultada.
6. Los machos adultos y los individuos más jóvenes se consideran los grupos de mayor riesgo toxicológico debido a la acumulación de xenobióticos y a la fase crítica de desarrollo de las crías, respectivamente.
7. Se observó una tendencia temporal en aumento de los niveles de Hg en el blubber de delfines mulares varados en las Islas Canarias (triplicándose en el periodo de estudio, 1997-2013) y los COPs no mostraron una tendencia a disminuir en el periodo de estudio.
8. En general, los xenobióticos analizados en este estudio son ligeramente superiores a los observados en delfines mulares del Mar del Norte y comparables a los del océano

Atlántico Oeste, varias localizaciones en el océano Pacífico y las aguas circundantes al Reino Unido. Como era de esperar, los resultados obtenidos son mucho menores a los observados en el Mar Mediterráneo donde los delfines mulares acumulan grandes cantidades de residuos químicos.

9. Son necesarios nuevos estudios para investigar asociaciones potenciales entre contaminantes y el estado sanitario de las poblaciones de cetáceos, lo que requiere una evaluación de los efectos individuales causados por altas concentraciones de xenobióticos y el impacto tóxico de la exposición crónica; para, de este modo, determinar el impacto de las actividades humanas sobre la salud de estos animales.
10. La contaminación medioambiental debe tratarse como un tema global, con una aproximación multidisciplinar para intentar reducir los niveles de estos contaminantes en las especies depredadoras que habitan nuestros mares y océanos.

# 8. ANNEXES

## 8.1. Challenges faced in the present PhD study

**Comparison between datasheets:**

The lack of long-term surveys of POPs in cetaceans enhances the need of combination of data from different studies worldwide in the attempt to gain spatial and temporal trends (Aguilar et al., 2002). However, this comparison is difficult to approach due to the heterogeneity of these surveys. The following aspects must be taken into consideration (Krahn et al., 2003):

- **Sampling methods:** for instance samples from stranded death animals versus biopsy samples taken from free ranging dolphins.
- **Analytical methodology:** comparison of results obtained with different extraction procedures or different analytical equipment.
- **Parameters or biological factors of the cetaceans sampled:** age, sex, reproductive status, body condition, health status. It is recommended to compare groups within similar age and sex categories because OCs usually increase with age in males and decrease in females after pregnancy through transfer to offspring. On the other hand disease could affect hepatic and renal function altering metabolism and excretion of pollutants, resulting in variation in OC levels (Aguilar et al., 1999). As explained in section 2.5.3 of this thesis, high OC levels may produce reproductive impairment, and this failure could also result in pollutant increase. Regarding other infectious diseases, for instance females affected by brucellosis do not seem to suffer pollutant transfer resulting in abnormally high levels of OCs. In this thesis, two stranded cetaceans, both subadult females (CET305 and CET314) were found to be affected by infectious agents, CET305 by systemic morbillivirus (Sierra et al., 2014), and CET314 being herpes positive. Both animals were found to be highly polluted compared to the individuals within the same age and sex category.

- **Post mortem time:** this may affect the contaminant concentrations in different tissues (see section of non-published results)
- **Sums of contaminants analysed:** when comparing different articles the results are usually shown as the sum of several compounds. For example summed PCBs which is quite difficult to compare between studies due to the large number of existing congeners and different sums selected for each specific research. Summed TEQs, which may include PCDDs, PCDFs and DL-PCB. Nevertheless it is known that DL-PCBs are the major contributor to total TEQs (Ross et al., 2000).
- **Concentration units:** it must be checked that the datasets use the same concentration units, or enough information to be able to convert the units for comparison results, as it is provided in the third publication included in the present thesis (see section 5.3). The most commonly used units are in wet weight (ww), dry weight (dw) and lipid weight (lw) basis. The ww unit is mostly used for fresh tissue, thereby avoiding water lost. Dw is used for samples easy to dry, often used for liver tissue. Lw is calculated after lipid extraction of the samples and it is useful to compare different tissues, particularly when body condition is different or in the case of non-fresh samples. In this thesis the unit used for concentration of POPs was in lw basis. Aguilar et al. (2002) stated that the average value of 70% of lipid content could be used for unit conversion to ww. Regarding trace elements both dw and ww units were shown in this thesis, using the conversion factor obtained after the freeze-drying.
- **Mixture of pollutants**: the wide range of chemical mixtures analysed make comparison between datasets complicated.
- **Sample size:** when comparing small groups of animals, quite frequent in cetacean researches, the results and statistical analysis should be used with caution (Zar, 2010).

**Age and growth curves:**

As age is an important parameter in this thesis related to bioaccumulation of pollutants, attempt has been made for an accurate approach on this variable. Five categories were used i.e. newborn, calf, juvenile, subadult and adult, based on body length and gonadal appearance (Geraci & Lounsbury 1993), however, it was complicated due to the lack of data from teeth and bones, necessary for more precise age information (Butti et al., 2007).

In addition, many criteria exist related to the interpretation of data to define an age group, which may change depending on the geographical habitat and if they are offshore or coastal animals.

By gathering the information of the literature checked in this thesis (see table 1 for references), we attempted to establish an age criterion based on the total length and gonadal development of BNDs stranded on the Canary Islands:

- <u>Newborn</u>: 1-1.5m. Existence of vibrissal crypts at the snout, fetal folds and bent dorsal fin.
- <u>Calf</u>: 1-1.5m. Without characteristics of newborn. First appearance of teeth.
- <u>Juvenile</u>: 1.5-2m

- Subadult: 2-2.5m in females and 2-2.6m in males. Length could vary depending on the gonadal histological finding. For instance, the sexual mature female with the shortest length observed in the registrations of strandings in the last 20 years on the Canary Islands measured 228cm and the rest of adult females were above 253cm. Regarding males, 285cm was the longest length found in a sexually immature individual (subadult) and all adults were above 260cm (See table 6 in section 8.3 of erratum).
- Adult: >2.5m in females and > 2.6m in males.

Although we assumed that our samples are from coastal bottlenose dolphins, it is possible that specimens from the larger offshore form had been also included. To date, there is no specific criterion to identify offshore individuals from this marine area. Thus, the inclusion of these animals may be responsible for some of the variability in length at different age categories, seen in this study.

On the other hand, the bioaccumulation of chemicals also depends on the growth curve of the species (Bellante et al., 2012). In general it follows a slow growth in the first years and then gets a growth spurt to an impasse at sexual maturity (Marsili and Focardi, 1996). Unfortunately, no information on growth has been published on BND from the Canary Islands so a positive correlation between age and total length has been considered excluding the possible plateau effect of the growth curve, as other authors do before (Andre et al., 1991).

**Thresholds:**

The thresholds of pollutant levels associated with negative health effects established in the literature are often based on terrestrial mammals what could lead to an error (Fair and Becker, 2000). Scientists should ideally compare thresholds based on the same species, tissue and unit concentration, what it is quite difficult to achieve due to the lack of information (AMAP, 2002).

**Sample tissue:**

Other challenges that all studies on cetaceans have to face are the limitations for sampling because of the poor accessibility to these animals. The selection of tissues for the analysis of chemical residues is also important. In this thesis biopsy samples from live free ranging dolphins and blubber and liver from stranded death animals have been studied.

- **Liver samples from stranded animals:**
  This tissue seems to be the target organ for Hg accumulation and other xenobiotics, being responsible for the metabolism and detoxification of pollutants (Frodello et al., 2000).

- **Blubber samples from stranded animals:**

  Lipophilic xenobiotics accumulate in the blubber of MMs, representing approximately the 90% of total burden of most of the OCs sequestered in the body of the animal (Aguilar et al., 2002).

- Lipid content and body condition:

Regarding blubber as a target for chemical residue, it is important to understand the structure of this tissue. Few studies of the blubber (Gómez-Campos et al., 2015) revealed stratification of blubber into outer, middle and inner layers which may reflects functional differences. They found that the number of adipocytes and area were largest in the middle layer. The authors stated that the outer, middle and inner layers may be responsible for structural support, thermoregulation and energy mobilization respectively. Differences between several body positions were also observed, with an increasing gradient from dorsal to ventral samples in the number of adipocytes and lipid content. In this way, it is known that the blubber provide insulator and buoyancy functions but also the ventral blubber may serve as an energy reserve.

Lipophilic OCs dynamics in the body must be related to lipid mobilization. Thus, nutritive condition can affect OC burdens, being the lipid content and thickness of blubber possible indicators of the body condition (Aguilar et al., 1999). Several situations could lead the animals to lose weight, such as migration, reproductive activity or disease. Aguilar et al. (1999) stated that xenobiotics may be distributed to vital organs or remain in the blubber. The increase of OCs in body tissues following lipid metabolism has been observed in several species. For example, PCBs found in striped dolphins from the Mediterranean Sea, increased with the depletion of lipid stores, indicating a build-up in individuals in poor nutritive status (Aguilar et al., 1999). In addition, other limitation is the difference in the affinity of pollutants to different lipid classes (Krahn et al., 2003). In this way, PCBs prefer neutral lipids and therefore, PCB levels in brain are usually low due to the high amount of phospholipids (Reijnders, 1986). Aguilar (1985) claimed that PCBs and DDTs have higher affinity for triglycerides in cetaceans.

- Seasons:

Furthermore, lipid content are affected by seasonal or physiological fluctuations, but this seasonal changes appear to be more important in mysticetes than odontocetes (Aguilar et al., 1999), so it doesn’t seem to be an important factor to considered in this thesis. However, some authors found lower POP levels in dolphins in the cold season but it is still under investigation (Balmer et al., 2015).

In addition, the season of the strandings was not taken into consideration in this thesis as other authors do, because there is no remarkable temperature difference between seasons in this marine area compared to other locations.

- **Biopsy samples from live free-ranging animals:**

The biopsies samples were obtained through remote biopsy darting, thus, neither age nor sex were possible to determined; however, an attempt was made to obtain them from subadults and adults individuals and assuming that sampling occurred randomly according to the sex variable. This fact could be important to interpret differences in OC concentrations found between death and live animals, as in the comparison all age categories of the stranded animals have been. Additionally, there is no information of the gender of live dolphins sampled, so it is quite difficult to compared individuals from the same age and sex category group. However, to minimise the error, POP analyses in all types of samples were conducted at the same laboratory, which ensures the comparability between data.

Other challenge was the low weight of the blubber obtained from these biopsy samples, what made the extraction procedure more complicated.

**Large amount of information related to pollutants:**

One of the challenges faced in during this thesis has been the great number of publications dealing with the topic of pollutants what required a hard work to filter the information.

## 8.2. Recommendations for future toxicological researches

Toxicological data in cetaceans from the Canary Islands is largely limited; this thesis represents the beginning of an extensive study field with a wide variety of open fronts, some of them described below:

- Continuation of monitoring studies in the Canary Islands, increasing the target cetacean species (resident and migratory odontocete) for comparison between species and with other geographic areas. Enlarge the sample size of toxicological studies is essential as a small number of animals may be of limited value in comparing information on chemical residues. In general, on-going studies of POP residues in cetaceans worldwide are necessary for their persistency in the environment and to be able to obtain long-term datasets, temporal and spatial trends for conservation purposes.
- Study of pollutants in stranded whales due to the absence of data (Torres et al., 2015).
- Analysis of other tissues, such as milk to investigate the pollutant transfer from the mother. Also investigate other body locations and different blubber layers, improving the lipid extraction process to enhance the fat recovery.
- Improve knowledge of diet and ecological factors which could integrate the toxicological study of marine mammal populations.
- Emerging contaminants.
- Study of Biotoxins: natural toxins responsible for many of the latest mass mortality events occurred in cetacean populations.
- Microplastics studies: contamination by microplastic is likely to be a growing problem in the future (Browne, 2015). This material provide not only harmful compounds integrated in the plastic itself (e.g. monomers and additives), but also by-products of manufacturing (petroleum derivatives) and metals or POPs from the ocean which accumulate on the plastic (Rochman, 2015).

- Biomarkers: MM are exposed to a mixture of a large number of xenobiotics, what make necessary a strategy to assess the effect of the exposure to this chemical cocktail through non-invasive approaches.
- Risk assessment of effects at individual or population level.

Below, some of the subjects shown above are discussed:

**Emerging contaminants:**

Thousands of new chemicals are released to the environment every year, thus toxicological technology is continuously evolving. Example of emerging pollutants:

- Halogenated flame retardants (HFR):
    - Brominated flame retardants (BFRs), such as polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs). They are used in plastics, textile industry, electronic devices etc. They may be carcinogenic, neurotoxic and endocrine disrupters (Krivoshiev et al., 2015; Zhou et al., 2009).
    - Perfluorinated chemicals (PFCs). They have a broad range of functions in the industry, in detergents, solvents, packaging, as FRs in furniture and kitchen utensils, etc.
- Pharmaceutical and abused drugs.
- Hormones.
- Personal care products; e.g. benzophenone in sun filters suggested to be an endocrine disruptor (Bae et al., 2016).
- Nanomaterials (e.g. silver particles). Used in metallurgy, ceramic, textile industry etc. (EEA, 2015).

These novel xenobiotics are currently being detected in MMs, with an unknown potential impact (Law, 2014) what made them priority compounds for further research. In fact they are also named as contaminants of emerging concern (CECs) because the health risk for the environment is still unknown (Lunardi et al., 2016).

PBDEs and other BFRs are similar to PCBs in structure and toxicity, but most of these chemicals have not yet been banned, being considered as the “new PCB problem”. Some studies in MMs stated that HFRs are increasing in the last years indicating continuous input of these xenobiotics (Baron et al., 2015; Bossart, 2007; Ross et al., 2009). However, no trend was found for PBDE levels in belugas in the study period of 1997-2013 (Simond et al., 2017); Law et al. (2010) observed a decline of these brominated compounds in porpoises of UK from 1998 to 2008 and other authors stated that levels of HFRs found in UK porpoises do not seem to be of great concern at that time (Papachlimitzou et al., 2015). Further research should be undertaken in this respect.

Regarding PFAs several studies have been conducted in cetaceans (Huber et al., 2012; White et al., 2015) with the intention to get spatial differences and a temporal tendency.

**Biotoxins:**

Many unusual mortality events have been linked to natural toxins produced by HABs (Fair and Becker, 2000; Gulland and Hall, 2007). The most frequently reported biotoxins associated to MM die offs are the following:

- Saxitoxins: in mortalities of humpback whales and Mediterranean monk seals.
- Domoic acid: in mortalities of California sea lions, sea otters and gray whales.
- Brevetoxins: in mortalities of BND and Florida manatees.
- Ciguatoxins: may be involved in the poor survival of Hawaiian monk seals.

However, any of these biotoxins associated to MM mortalities have not yet been confirmed as the only causative agents of these die-offs. Further investigation is required.

**Biomarkers:**

A biomarker could be defined as "a xenobiotically induced alteration in cellular or biochemical components or processes, structures or functions that is measurable in a biological system or sample (Gil and Pla, 2001). Thus, a biomarker is a biological response to a xenobiotic or a group of xenobiotics. Additionally, the measurement of a chemical compound in a sample is an indicator of exposure, being also considered as a biological marker itself.

In the last 20 years the use of biological markers has been increased to assess the exposure, toxic effects and individual susceptibility to xenobiotics in order to prevent the long-term risks (Gil and Pla, 2001).

Considering that MM are exposed to complicated chemical mixtures, it is important to integrate biomarker studies in the contaminant monitoring programs (Fair and Becker, 2000).

Examples of biomarker researches in MM:

- Levels of retinoid (vitamin A) in storage tissues (mainly in blubber and liver) of MMs vary at exposure to OC compounds and the balance is restored with the decreasing of these xenobiotics. For that reason, retinoids may be considered as good biomarkers of exposure and impact of pollutants on cetacean populations (Desforges et al., 2013; Tornero et al., 2005).
- Cytochrome P450 (CYP) is a superfamily of important enzymes involved in the oxidative metabolism of endogenous and xenobiotic compounds. Particularly, the CYP1 family is responsible for the metabolism of polyhalogenated aromatic compounds (Nebert et al., 2000). The AhR previously activated by these chemicals, controls the expression of CYP1 genes (CYP1A1, 1A2, and 1B1). Thus, a chronic induction of CYP1 may result on a persistent metabolic activation of xenobiotics and endogenous compounds with the consequent production of reactive oxygen species (Nebert and Dalton, 2006). Therefore, Iwata et al. (2015) used the expression level of CYP1 as a biomarker of exposure and toxicity of DL pollutants in Baikal seals.

- Bachman et al. (2015) found a positively correlation between CYP1A1 expression in the liver with most POP compounds analysed, but not found in the blubber (only for HCH). Therefore, the authors support the use of liver CYP1A1 as a biomarker of POP exposure to stranded Pacific Island cetaceans.
- Study on BNDs from Florida found that endothelial CYP1A1 expression changes with the length of the skin biopsy and total PCBs were positively correlated to this expression. The authors support that pollutants are the main contributors to CYP1A1 expression, rather than age, sex or other biological factors (Wilson et al., 2007).
- Benzo(alpha)pyrene monoxigenase (BPMO) activity was measured in biopsies obtained from live cetaceans inhabiting the Ligurian Sea and OCs were found. The authors stated that CYP1A1 (BPMO) induction in these samples may represent an early evidence of exposure to endocrine disruptors (Fossi et al., 2006).
- Jia et al. (2015) evaluated the threats posed by POPs to their local dolphin population (the Indo-Pacific humpback dolphin inhabited the Pearl River Estuary in China, where high concentrations of POPs have been reported), using an in vitro system. They selected POPs found with high levels in the tissues of the dolphins to treat a cultured skin fibroblast cells (ScSF cells) of the dolphin, and analysed the expression of the ecological stresses biomarkers CYP1A1, AhR and heat-shock-protein (HSP70). The results showed that CYP1A1, AhR and HSP70 were up-regulated after exposed to different compounds. Moreover, they carried out comet assay experiments (method to measure DNA damage) and revealed that the DDT produced higher DNA damage to ScSF cells than other chemicals.
- Lehnert et al. (2014) established biomarkers of the xenobiotic metabolism and immune system. The mRNA transcription of AhR, aryl hydrocarbon receptor nuclear translocator (ARNT), the peroxisome proliferator-activated receptor (PPARalpha) and cytokine IL-2 and HSP70 was measured in blood of grey seal in permanent care comparing to rehabilitating harbour seal. They found higher levels of HSP70 and cytokine biomarkers in pups at admission suggesting that this may be due to stress, dehydration or pollutant exposure in lactation.
- An ex vivo assay using skin biopsies from BNDs first treated with PFOA and BPA and subsequently analysed to global gene expression. They obtained a transcriptomic signature of each sample providing information of exposure and health effects, as the genes affected were implicated in immune functions, stress, lipid regulation and development (Lunardi et al., 2016).
- It is worth pointing out that some studies support the use of epidermis as a bioindicator of Hg for its correlation with the Hg level in internal tissues (Caceres-Saez et al., 2015).

**Health risk assessment:**

To study potential associations between xenobiotics and health status, a more effective risk assessment concerning these pollutants is needed. The response to stress is multidimensional, what highlights the need of a multi-disciplinary research in toxicology, pathology, ecology among other fields. With the anthropogenic pollutant level observed in this thesis and after obtaining all the necessary pathological information, the data will be crossed to assess any possible association. For instance, this kind of monitoring combination was conducted in Baltic seals resulting in general improvement of the health status thanks to this multidisciplinary approach (Fair and Becker, 2000). In this way, a non-invasive methodology (Weijs and Zaccaroni, 2015) and the handling of cetacean cell lines for chemical risk assessment is also required (Burkard et al., 2015).

## 8.3. Erratum

Review of the age categories: as mentioned before (section 8.1), the age category previously assigned to each specimen has been changed based in total length data and several gonadal histological findings obtained subsequently to the publication of the articles. We re-assigned the corrected age categories to each individual, showed in the following table:

Table 6. Details of stranded BNDs, with the age considered before and the currently corrected age.

| SPECIMEN | SEX | TL (cm) | AGE | Corrected AGE |
|---|---|---|---|---|
| CET 043 | Female | NA | Adult | Adult |
| **CET 078** | **Male** | **294** | **ND/Subadult** | **Adult** |
| CET 093 | Male | 257 | Juvenile | Subadult |
| CET 094 | Male | 260 | Juvenile | Subadult |
| CET 099 | Female | 212 | Juvenile | Subadult |
| **CET 171** | **Female** | **238** | **Adult** | **Subadult** |
| **CET 203** | **Male** | **300** | **SubAdult** | **Adult** |
| CET 209 | Female | 215 | Juvenile | Subadult |
| CET 231 | Female | 298 | Adult | Adult |
| CET 286 | Male | 245 | Juvenile | Subadult |
| **CET 296** | **Female** | **296** | **SubAdult** | **Adult** |
| CET 305 | Female | 250 | SubAdult | Subadult |
| CET 311 | Male | 265 | SubAdult | Subadult |
| CET 314 | Female | 210 | SubAdult | Subadult |
| CET 403 | Male | 258 | SubAdult | Subadult |
| CET 407 | Female | 305 | Adult | Adult |
| CET 420 | Female | 279 | Adult | Adult |
| **CET 450** | **Female** | **260** | **SubAdult** | **Adult** |
| CET 458 | Female | 205 | SubAdult | Subadult |
| CET 505 | Male | 203 | Juvenile | Subadult |
| CET 509 | Male | 260 | Adult | Adult |
| CET 526 | Female | 228 | Adult | Adult |
| CET 543 | Male | 327 | Adult | Adult (old) |
| CET 562 | Female | 287 | Adult | Adult |
| CET 564 | Male | 306 | Adult | Adult |
| CET 584 | Female | 137 | Calf | Calf |
| CET 592 | Female | 152 | Newborn | Newborn |
| CET 595 | Male | 278 | SubAdult | Subadult |
| CET 635 | Male | 285 | SubAdult | Subadult |
| CET 640 | Male | 315 | Adult (old) | Adult (old) |
| CET 662 | Female | 260 | Adult | Adult |

Most of the changes corrected juveniles to subadults by applying the criterion established after publication of the articles in this thesis (see section 8.1), however, five of these corrections should be highlighted (shown in bold fond in the table).

See below some mistakes found in the review of the articles of this thesis after publication and other suggestions:

- 1st publication (see section 5.1):
    - There is a mistake in the introduction “Extensive research on the levels of chemical contamination <u>of this species</u> has been done all around the world” should be replaced for “extensive research… <u>of cetaceans</u>…” as the references indicated after this paragraph are researches on different cetacean species not only BND.
    - The percentage value of fat of liver tissue shown in the section 2.4. is wrong; the corrected data ranged from 0.85 to 19.42% (mean 5.82%).
    - The mean value of tPAHs shown in section 3 (results and discussion) is in fact the median data. The correct average is 38 ng/g.
    - There is a mistake when the predominant compounds are indicated, as it is p,p’-DDE etc. instead of p,p’-DDT etc.
    - In page 54, indicates that acenaphtlylene, anthracene among others were not detected in any samples, should say except for liver sample of cetacean 505 which shows 8.2 ng/g level of anthracene.

- 2nd publication (see section 5.2):
    - The title should specify “live free-ranging common BND” as both, live and stranded death animals are free-ranging dolphins.
    - In the graphical abstract the word “OCPs” next to the Y axis is duplicated by mistake and the font should be greater to be more readable.

- 3rd publication (see section 5.3):
    - In the abstract, it is mentioned that Hg level in the marine environment has tripled in recent years and it should be highlighted that it is consistent with the temporal trend of Hg level observed in the blubber in the study period of this thesis.
    - There is a mistake in Fig.4. It should indicate Se/Hg instead Hg/Se.
    - Table 3: the location shown as Australia (south coast) corresponds to Indian Ocean, not to Pacific Ocean, in fact the authors (Lavery et al., 2008) hold that it is the southern ocean.

## 8.4. Tables of results

Table 7. shows the distinct variables recorded for each specimen to be considered as possible influencing factors in the pollutant concentrations. As such, stranding condition, age class, sex, bodily and decomposition status were categorised.

| Specimen | Year | Sex[a] | TL (cm) | Age | Location[b] | Body condition | Conservation state[c] | Pathology entity[d] |
|---|---|---|---|---|---|---|---|---|
| CET 043 | 1997 | Female | ND | Adult | TF | Very poor | Fresh | NPL |
| CET 078 | 1999 | Male | 294 | Adult | GC | Poor | Very fresh | NPL |
| CET 093 | 1999 | Male | 257 | Subadult | TF | Poor | Fresh | IF |
| CET 094 | 1999 | Male | 260 | Subadult | TF | Good | Fresh | IF |
| CET 099 | 2000 | Female | 212 | Subadult | GC | Poor | Advanced Aut. | NPL |
| CET 171 | 2002 | Female | 238 | Subadult | TF | Good | Fresh | IF |
| CET 203 | 2003 | Male | 300 | Adult | FV | ND | Very advanced Aut. | ND |
| CET 209 | 2003 | Female | 215 | Subadult | GC | Poor | Moderate | NPL |
| CET 231 | 2004 | Female (P) | 298 | Adult | TF | ND | Very advanced Aut. | ND |
| CET 286 | 2005 | Male | 245 | Subadult | GC | Good | Advanced Aut. | NPNL |
| CET 296 | 2005 | Female | 296 | Adult | GC | Good | Very fresh | NPNL |
| CET 305 | 2005 | Female | 250 | Subadult | LZ | Moderate | Very fresh | NPNL |
| CET 311 | 2005 | Male | 265 | Subadult | FV | Poor | Fresh | NPL |
| CET 314 | 2005 | Female | 210 | Subadult | GC | Good | Advanced Aut. | NPNL |
| CET 403 | 2007 | Male | 258 | Subadult | TF | Moderate | Advanced Aut. | ND |
| CET 407 | 2008 | Female | 305 | Adult | TF | Poor | Fresh | NPL |
| CET 420 | 2008 | Female (P) | 279 | Adult | GC | Moderate | Advanced Aut. | NPNL |
| CET 450 | 2008 | Female | 260 | Adult | TF | Moderate | Very fresh | NPNL |
| CET 458 | 2008 | Female | 205 | Subadult | TF | Good | Very advanced Aut. | NPNL |
| CET 505 | 2009 | Male | 203 | Subadult | TF | ND | Advanced Aut. | IIT |
| CET 509 | 2009 | Male | 260 | Adult | TF | Moderate | Moderate | IIT |
| CET 526 | 2010 | Female | 228 | Adult | TF | Good | Fresh | NPNL |
| CET 543 | 2010 | Male | 327 | Adult (old) | LZ | ND | Advanced Aut. | INPL |
| CET 562 | 2011 | Female | 287 | Adult | TF | ND | Advanced Aut. | ND |
| CET 564 | 2011 | Male | 306 | Adult | LZ | Good | Fresh | NPNL |
| CET 584 | 2011 | Female | 137 | Calf | TF | ND | Advanced Aut. | IIT |
| CET 592 | 2011 | Female | 152 | Newborn | LG | Good | Moderate | NPNL |
| CET 595 | 2011 | Male | 278 | Subadult | LZ | Very poor | Fresh | NPL |
| CET 635 | 2012 | Male | 285 | Subadult | GC | Moderate | Fresh | NPNL |
| CET 640 | 2012 | Male | 315 | Adult (old) | LZ | Moderate | Very fresh | NPNL |
| CET 662 | 2013 | Female | 260 | Adult | TF | Very poor | Very fresh | NPL |

[a]Female (P), pregnant female. [b]LZ, Lanzarote; FV, Fuerteventura; GC, Gran Canaria; TF, Tenerife; LG, La Gomera. [C]Aut., autolysis. [d]NPL, natural pathology associated with a significant loss of body condition; NPNL, natural pathology non-associated with loss of body condition; IF, interaction with fishing activities; IIT, intra or interspecific physical trauma. ND = data not determined/no data.

Table 8. Levels of congeners of PCBs in blubber samples from stranded BND (ng $g^{-1}$ lw). TEQ-PCBs in pg $g^{-1}$ lw. PCB 77 was not detected in any sample. SD (standard deviation).

| BLUBBER | PCB 28 | PCB 52 | PCB 81 | PCB 101 | PCB 105 | PCB 114 | PCB 118 | PCB 123 | PCB 126 | PCB 138 | PCB 153 | PCB 156 | PCB 157 | PCB 167 | PCB 169 | PCB 180 | PCB 189 | ∑M-PCB | ∑DL-PCB | TEQ-PCBs | ∑PCB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET 78 | 22.0 | 203.4 | 55.5 | 461.8 | 172.5 | 22.2 | 580.2 | 30.6 | 1682.1 | 13056.1 | 14283.9 | 431.7 | 94.8 | 0.0 | 0.0 | 20984.9 | 182.2 | 49592.2 | 3251.9 | 168277.0 | 52263.9 |
| CET 93 | 48.2 | 28.9 | 1.8 | 24.1 | 39.1 | 9.2 | 77.2 | 6.7 | 161.7 | 860.7 | 1086.0 | 49.4 | 12.8 | 36.4 | 0.0 | 1965.7 | 18.1 | 4090.8 | 412.5 | 16179.0 | 4426.1 |
| CET 94 | 88.7 | 144.8 | 21.2 | 362.2 | 121.5 | 20.1 | 448.4 | 5.6 | 421.2 | 3791.3 | 3984.3 | 197.1 | 36.3 | 115.9 | 0.0 | 5156.4 | 48.1 | 13976.1 | 1435.3 | 42157.2 | 14963.1 |
| CET 99 | 99.3 | 810.1 | 139.9 | 2761.1 | 990.9 | 127.8 | 3855.8 | 64.0 | 4756.7 | 38528.7 | 43603.6 | 1954.9 | 410.7 | 0.0 | 0.0 | 58205.4 | 463.3 | 147863.9 | 12764.0 | 475944.4 | 156772.1 |
| CET 171 | 50.8 | 426.6 | 45.6 | 1380.9 | 660.2 | 0.0 | 1877.4 | 126.5 | 966.7 | 12097.0 | 12414.4 | 653.3 | 128.7 | 0.0 | 0.0 | 8080.3 | 62.3 | 36327.5 | 4520.8 | 96787.1 | 38970.9 |
| CET 203 | 83.0 | 84.7 | 24.4 | 169.2 | 121.9 | 20.0 | 234.6 | 9.7 | 342.9 | 3389.3 | 3295.3 | 105.8 | 34.2 | 71.7 | 0.0 | 3423.7 | 24.6 | 10679.8 | 989.7 | 34314.6 | 11434.9 |
| CET 209 | 21.7 | 167.9 | 24.9 | 590.4 | 190.0 | 24.0 | 740.4 | 48.3 | 705.8 | 4881.5 | 6148.3 | 266.4 | 47.5 | 188.1 | 0.0 | 7214.0 | 46.3 | 19764.1 | 2281.8 | 70636.1 | 21305.6 |
| CET 231 | 15.1 | 59.0 | 0.1 | 261.4 | 75.6 | 13.8 | 281.1 | 15.1 | 211.9 | 1913.3 | 2274.9 | 99.7 | 19.1 | 69.3 | 0.0 | 2305.8 | 14.4 | 7110.5 | 800.1 | 21210.7 | 7629.5 |
| CET 286 | 21.5 | 344.2 | 44.8 | 695.7 | 266.7 | 30.4 | 895.9 | 50.6 | 945.6 | 8912.1 | 10084.3 | 270.1 | 84.9 | 167.2 | 0.0 | 8756.3 | 51.9 | 29710.0 | 2808.2 | 94631.7 | 31622.3 |
| CET 296 | 68.3 | 87.6 | 19.4 | 284.4 | 136.2 | 24.4 | 456.6 | 60.8 | 1541.6 | 6863.6 | 7766.5 | 531.1 | 86.6 | 0.0 | 0.0 | 17692.6 | 317.7 | 33219.7 | 3174.5 | 154209.3 | 35937.6 |
| CET 305 | 40.9 | 222.3 | 45.4 | 660.2 | 235.3 | 43.2 | 1156.1 | 17.0 | 2301.2 | 20142.1 | 23281.7 | 554.6 | 159.7 | 0.0 | 0.0 | 30781.8 | 277.9 | 76285.1 | 4790.3 | 230208.9 | 79919.3 |
| CET 311 | 11.3 | 58.5 | 0.0 | 568.8 | 0.0 | 42.1 | 1640.1 | 0.0 | 11017.2 | 14503.2 | 22676.6 | 1909.8 | 531.7 | 0.0 | 0.0 | 37240.6 | 544.0 | 76699.3 | 15684.7 | 1101855.1 | 90743.9 |
| CET 314 | 67.2 | 1641.0 | 316.2 | 4431.4 | 1441.3 | 187.4 | 5776.7 | 251.3 | 6174.8 | 63051.3 | 67352.4 | 2385.5 | 603.4 | 0.0 | 0.0 | 64511.7 | 500.0 | 206831.7 | 17636.5 | 617906.9 | 218691.5 |
| CET 403 | 121.8 | 543.5 | 45.2 | 867.4 | 498.9 | 34.8 | 1250.4 | 91.8 | 633.5 | 7807.1 | 9397.3 | 260.4 | 75.3 | 0.0 | 0.0 | 5921.8 | 43.2 | 25909.2 | 2933.4 | 63433.1 | 27592.3 |
| CET 407 | 172.6 | 313.6 | 88.7 | 1203.9 | 488.0 | 41.8 | 1864.9 | 100.8 | 1161.0 | 8860.5 | 9778.9 | 515.9 | 121.3 | 321.9 | 0.0 | 11523.6 | 98.6 | 33717.8 | 4802.6 | 116228.5 | 36655.5 |
| CET 420 | 4.7 | 30.0 | 0.0 | 287.1 | 122.6 | 0.0 | 433.5 | 0.0 | 632.5 | 3049.9 | 3638.3 | 161.6 | 51.2 | 124.6 | 0.0 | 5123.8 | 39.4 | 12567.3 | 1565.5 | 63279.3 | 13699.3 |
| CET 450 | 106.2 | 370.8 | 65.1 | 1154.7 | 242.5 | 19.3 | 1041.5 | 73.9 | 707.1 | 5386.1 | 5904.5 | 295.7 | 68.0 | 201.2 | 0.0 | 7063.8 | 54.2 | 21027.6 | 2768.5 | 70792.5 | 22754.7 |
| CET 458 | 22.6 | 60.0 | 12.2 | 174.5 | 38.0 | 0.0 | 193.1 | 13.3 | 180.0 | 885.5 | 952.5 | 68.0 | 11.8 | 38.3 | 0.0 | 1679.4 | 10.2 | 3967.7 | 564.8 | 18011.1 | 4339.4 |
| CET 505 | 13.8 | 191.8 | 40.5 | 476.7 | 204.9 | 10.9 | 786.6 | 20.5 | 677.3 | 4889.9 | 5448.7 | 169.2 | 62.8 | 151.8 | 0.0 | 6564.5 | 44.9 | 18371.9 | 2169.6 | 67788.6 | 19754.8 |
| CET 509 | 102.4 | 400.6 | 19.6 | 619.9 | 256.2 | 0.0 | 891.2 | 55.0 | 268.4 | 3831.6 | 3877.4 | 212.5 | 54.2 | 0.0 | 0.0 | 2588.4 | 21.5 | 12311.5 | 1778.7 | 26895.1 | 13199.0 |
| CET 543 | 73.1 | 1108.3 | 187.1 | 1844.1 | 623.0 | 0.0 | 2033.4 | 120.5 | 3872.8 | 44168.5 | 46586.4 | 892.4 | 322.6 | 742.1 | 0.0 | 38862.1 | 359.8 | 134676.0 | 9153.7 | 387484.9 | 141796.4 |
| CET 562 | 14.8 | 35.0 | 5.0 | 83.2 | 31.5 | 0.0 | 126.4 | 0.0 | 119.4 | 777.5 | 893.7 | 22.1 | 7.2 | 28.1 | 0.0 | 952.9 | 3.4 | 2883.6 | 343.3 | 11952.0 | 3100.4 |
| CET 564 | 367.6 | 386.9 | 27.1 | 972.4 | 445.5 | 0.0 | 1815.5 | 31.7 | 2333.4 | 15694.3 | 18093.6 | 955.1 | 176.8 | 607.1 | 0.0 | 31906.8 | 425.0 | 69237.1 | 6817.2 | 233480.4 | 74238.8 |
| CET 584 | 101.5 | 416.4 | 28.8 | 1025.7 | 472.0 | 46.7 | 1567.4 | 152.3 | 498.4 | 6319.7 | 6261.6 | 408.9 | 98.3 | 0.0 | 0.0 | 4427.8 | 29.6 | 20120.0 | 3302.4 | 49935.7 | 21855.1 |
| CET 595 | 85.3 | 788.1 | 67.4 | 1257.5 | 512.0 | 41.8 | 1968.6 | 84.9 | 651.2 | 12214.5 | 11729.0 | 367.8 | 114.2 | 0.0 | 17.7 | 5605.6 | 33.8 | 33648.6 | 3859.4 | 65761.7 | 35539.3 |
| Mean | 73.0 | 357.0 | 53.0 | 904.7 | 335.5 | 30.4 | 1279.7 | 57.2 | 1718.6 | 12235.0 | 13632.5 | 549.6 | 136.6 | 114.5 | 0.7 | 15541.6 | 148.6 | 44023.6 | 4424.4 | 171974.4 | 47168.2 |
| SD | 74.8 | 383.7 | 70.1 | 958.8 | 333.7 | 42.3 | 1268.6 | 59.6 | 2465.0 | 15048.6 | 16319.3 | 631.0 | 159.8 | 189.8 | 3.5 | 17931.9 | 178.3 | 50751.0 | 4644.6 | 246552.9 | 53849.2 |
| Median | 67.2 | 222.3 | 28.8 | 619.9 | 235.3 | 20.1 | 895.9 | 48.3 | 705.8 | 6863.6 | 7766.5 | 295.7 | 84.9 | 36.4 | 0.0 | 7063.8 | 48.1 | 25909.2 | 2933.4 | 70636.1 | 27592.3 |

Table 9. Levels of congeners of PCBs in liver samples from stranded BND (ng $g^{-1}$ lw). TEQ-PCBs in pg $g^{-1}$ lw. PCB 77 was not detected in any sample. SD (standard deviation).

| LIVER | PCB 28 | PCB 52 | PCB 81 | PCB 101 | PCB 105 | PCB 114 | PCB 118 | PCB 123 | PCB 126 | PCB 138 | PCB 153 | PCB 156 | PCB 157 | PCB 167 | PCB 169 | PCB 180 | PCB 189 | ∑M-PCB | ∑DL-PCB | TEQ-PCBs | ∑PCB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET 43 | 0.9 | 2.3 | 0.4 | 7.9 | 2.1 | 0.0 | 8.0 | 0.2 | 12.2 | 78.4 | 85.2 | 5.1 | 0.7 | 3.0 | 0.0 | 144.0 | 1.4 | 326.7 | 33.1 | 1222.4 | 351.8 |
| CET 78 | 0.9 | 3.2 | 0.9 | 6.7 | 2.5 | 0.0 | 7.7 | 0.4 | 33.9 | 212.0 | 249.4 | 7.6 | 1.7 | 5.1 | 0.0 | 397.2 | 3.3 | 877.1 | 63.1 | 3390.2 | 932.5 |
| CET 93 | 0.9 | 4.1 | 0.7 | 9.3 | 0.0 | 0.0 | 5.8 | 0.5 | 20.0 | 137.4 | 207.8 | 4.7 | 1.5 | 3.4 | 0.0 | 273.4 | 2.6 | 638.8 | 39.2 | 2001.4 | 672.2 |
| CET 94 | 1.0 | 3.6 | 0.5 | 8.1 | 2.3 | 0.3 | 8.3 | 0.0 | 9.8 | 79.1 | 89.2 | 4.0 | 0.9 | 2.9 | 0.0 | 109.6 | 1.0 | 298.8 | 29.9 | 977.0 | 320.4 |
| CET 99 | 1.2 | 4.1 | 0.5 | 14.9 | 5.7 | 0.0 | 19.0 | 1.2 | 16.0 | 124.9 | 138.8 | 9.5 | 1.9 | 6.0 | 0.0 | 177.8 | 1.7 | 480.7 | 61.6 | 1601.9 | 523.2 |
| CET 171 | 1.3 | 3.4 | 0.7 | 12.0 | 4.1 | 0.5 | 15.4 | 0.4 | 35.9 | 192.0 | 234.2 | 11.5 | 2.0 | 7.9 | 0.0 | 421.1 | 3.8 | 879.3 | 82.3 | 3588.1 | 946.1 |
| CET 203 | 1.3 | 9.8 | 2.2 | 23.7 | 9.3 | 1.6 | 36.7 | 1.5 | 72.3 | 629.2 | 642.8 | 17.1 | 6.3 | 13.7 | 0.0 | 732.7 | 8.1 | 2076.1 | 168.8 | 7230.7 | 2208.3 |
| CET 209 | 1.5 | 1.9 | 0.4 | 5.8 | 2.6 | 0.2 | 8.9 | 0.2 | 14.9 | 89.6 | 104.4 | 5.7 | 0.9 | 0.0 | 0.0 | 177.8 | 1.5 | 389.9 | 35.3 | 1495.0 | 416.4 |
| CET 286 | 1.2 | 2.1 | 0.3 | 4.6 | 2.2 | 0.4 | 6.5 | 0.0 | 7.2 | 75.3 | 80.3 | 2.6 | 0.7 | 1.9 | 0.0 | 83.0 | 0.6 | 253.1 | 22.4 | 718.9 | 269.0 |
| CET 296 | 1.5 | 0.5 | 0.0 | 0.7 | 0.5 | 0.1 | 1.4 | 0.1 | 2.4 | 11.9 | 12.9 | 1.0 | 0.2 | 0.3 | 0.0 | 28.8 | 0.5 | 57.8 | 6.5 | 239.1 | 62.9 |
| CET 305 | 0.5 | 4.1 | 0.5 | 14.8 | 6.7 | 0.9 | 23.7 | 0.3 | 15.7 | 160.1 | 171.4 | 9.5 | 2.7 | 6.2 | 0.0 | 166.2 | 1.3 | 540.8 | 67.3 | 1568.0 | 584.4 |
| CET 311 | 0.4 | 0.6 | 0.0 | 3.5 | 0.0 | 0.0 | 11.4 | 0.0 | 19.4 | 12.1 | 33.1 | 8.3 | 1.2 | 0.0 | 0.0 | 143.8 | 3.8 | 204.9 | 44.2 | 1936.2 | 237.6 |
| CET 314 | 0.7 | 3.0 | 0.1 | 8.0 | 2.9 | 0.3 | 10.7 | 0.3 | 13.6 | 121.7 | 133.0 | 5.0 | 1.4 | 0.0 | 0.0 | 142.3 | 1.0 | 419.4 | 35.3 | 1364.0 | 444.0 |
| CET 403 | 1.3 | 3.3 | 0.0 | 5.5 | 2.9 | 0.2 | 9.5 | 0.3 | 4.5 | 58.8 | 60.5 | 2.2 | 0.7 | 1.8 | 0.0 | 55.5 | 0.4 | 194.3 | 22.4 | 447.1 | 207.3 |
| CET 407 | 0.9 | 6.0 | 0.0 | 57.4 | 24.5 | 0.0 | 86.7 | 0.0 | 126.5 | 610.0 | 727.7 | 32.3 | 10.2 | 24.9 | 0.0 | 1024.8 | 7.9 | 2513.5 | 313.1 | 12655.8 | 2739.9 |
| CET 420 | 1.1 | 3.0 | 0.6 | 8.7 | 1.9 | 0.0 | 9.7 | 0.7 | 9.0 | 44.3 | 47.6 | 3.4 | 0.6 | 1.9 | 0.0 | 84.0 | 0.5 | 198.4 | 28.2 | 900.6 | 217.0 |
| CET 450 | 3.0 | 7.0 | 1.0 | 16.6 | 6.3 | 0.0 | 25.3 | 0.0 | 23.9 | 155.5 | 178.7 | 4.4 | 1.4 | 5.6 | 0.0 | 190.6 | 0.7 | 576.7 | 68.7 | 2390.4 | 620.1 |
| CET 458 | 1.3 | 3.9 | 0.8 | 10.4 | 4.3 | 0.0 | 15.8 | 1.7 | 7.6 | 53.5 | 53.3 | 3.5 | 0.3 | 2.8 | 0.0 | 48.9 | 0.1 | 187.1 | 36.8 | 758.3 | 208.1 |
| CET 505 | 2.3 | 15.1 | 2.4 | 24.6 | 11.0 | 0.0 | 35.7 | 0.9 | 42.7 | 294.9 | 314.8 | 8.3 | 3.6 | 8.7 | 0.0 | 422.1 | 2.5 | 1109.5 | 115.9 | 4270.9 | 1189.6 |
| CET 509 | 0.8 | 1.1 | 0.0 | 1.9 | 1.2 | 0.2 | 4.0 | 0.1 | 1.0 | 14.3 | 14.6 | 0.8 | 0.2 | 0.6 | 0.0 | 11.1 | 0.1 | 47.8 | 8.2 | 104.2 | 52.1 |
| CET 543 | 0.9 | 2.9 | 0.0 | 4.8 | 1.9 | 0.2 | 6.1 | 0.2 | 11.7 | 125.7 | 129.1 | 2.8 | 0.8 | 0.0 | 0.0 | 126.8 | 0.9 | 396.3 | 24.7 | 1172.5 | 414.9 |
| CET 562 | 1.5 | 2.2 | 0.4 | 8.9 | 3.5 | 0.0 | 12.7 | 0.0 | 22.5 | 98.6 | 109.7 | 5.3 | 1.4 | 4.1 | 0.0 | 226.2 | 1.3 | 459.9 | 51.2 | 2255.4 | 498.4 |
| CET 564 | 0.8 | 0.4 | 0.0 | 0.7 | 0.6 | 0.1 | 1.7 | 0.1 | 1.9 | 12.9 | 14.7 | 0.9 | 0.2 | 0.4 | 0.0 | 27.2 | 0.2 | 58.3 | 6.2 | 191.5 | 62.7 |
| CET 584 | 0.4 | 1.0 | 0.0 | 3.3 | 2.1 | 0.0 | 6.9 | 0.2 | 5.2 | 55.3 | 60.3 | 2.5 | 0.7 | 0.0 | 0.2 | 61.5 | 0.5 | 188.6 | 18.2 | 525.1 | 199.9 |
| CET 592 | 0.9 | 2.5 | 0.0 | 8.6 | 4.5 | 0.4 | 14.3 | 1.3 | 6.7 | 66.5 | 70.3 | 4.9 | 1.2 | 0.0 | 0.0 | 72.6 | 0.6 | 235.7 | 34.0 | 673.4 | 255.4 |
| CET 595 | 0.4 | 2.9 | 0.1 | 12.8 | 5.3 | 0.8 | 24.8 | 0.5 | 53.3 | 383.3 | 467.6 | 14.0 | 3.2 | 0.0 | 0.0 | 728.0 | 7.9 | 1619.8 | 109.8 | 5330.4 | 1704.8 |
| Mean | 1.1 | 3.6 | 0.5 | 10.9 | 4.3 | 0.2 | 16.0 | 0.4 | 22.7 | 149.9 | 170.4 | 6.8 | 1.8 | 3.9 | 0.0 | 233.7 | 2.1 | 585.7 | 58.7 | 2269.6 | 628.4 |
| SD | 0.6 | 3.1 | 0.6 | 11.3 | 4.9 | 0.4 | 17.2 | 0.5 | 27.1 | 163.3 | 183.7 | 6.6 | 2.2 | 5.5 | 0.0 | 251.6 | 2.4 | 619.0 | 63.9 | 2712.8 | 664.8 |
| Median | 1.0 | 3.0 | 0.4 | 8.3 | 2.7 | 0.1 | 10.2 | 0.2 | 14.3 | 94.1 | 107.0 | 4.9 | 1.2 | 2.4 | 0.0 | 143.9 | 1.1 | 393.1 | 36.1 | 1429.5 | 415.6 |

Table 10. Levels of OCPs in blubber samples from stranded BND (ng $g^{-1}$ lw). No detectable values of δ-HCH, heptachlor, aldrin, endrin or β-endosulfan were found in any sample. SD (standard deviation).

| BLUBBER | HCB | α-HCH | β-HCH | γ-HCH | ΣHCH | o,p-DDE | p,p-DDE | o,p-DDD | p,p-DDD o,p-DDT | p,p-DDT | ΣDDT | p,p-DDE/ΣDDT | ΣDDT/ΣPCB | Dieldrin | α-Endosulfan | Endos. sulfate | ΣCyclodienes | cis-Chlordane | trans-Chlordane | ΣChlordane | Dicofol | Methoxychlor | Mirex | ΣOCPs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET 78 | 52.0 | 0.0 | 9.8 | 5.1 | 14.9 | 76.7 | 36237.9 | 116.7 | 988.2 | 3688.6 | 41108.0 | 0.9 | 0.8 | 457.5 | 0.0 | 17.9 | 475.5 | 110.6 | 38.4 | 149.0 | 8.1 | 0.0 | 840.2 | 42647.7 |
| CET 93 | 0.6 | 0.0 | 2.7 | 0.2 | 2.9 | 7.2 | 3488.9 | 29.3 | 105.3 | 178.9 | 3809.5 | 0.9 | 0.9 | 1174.7 | 0.0 | 0.2 | 1174.9 | 5.7 | 59.8 | 65.5 | 5.7 | 0.0 | 220.6 | 5279.7 |
| CET 94 | 11.4 | 0.0 | 7.4 | 1.1 | 8.4 | 42.4 | 10136.3 | 42.2 | 473.0 | 1131.3 | 11825.2 | 0.9 | 0.8 | 2433.0 | 0.0 | 0.4 | 2433.5 | 19.9 | 149.1 | 169.0 | 11.4 | 0.0 | 501.1 | 14959.9 |
| CET 99 | 601.4 | 0.0 | 19.9 | 12.8 | 32.7 | 405.8 | 87323.7 | 527.9 | 4631.4 | 23959. | 116848. | 0.7 | 0.7 | 2722.9 | 0.0 | 81.0 | 2803.9 | 983.9 | 319.8 | 1303.8 | 35.3 | 0.0 | 3835.4 | 125460.9 |
| CET 171 | 241.8 | 0.0 | 18.8 | 11.2 | 30.0 | 119.1 | 28445.8 | 197.2 | 2778.7 | 12809. | 44350.3 | 0.6 | 1.1 | 1761.5 | 0.0 | 42.9 | 1804.4 | 778.9 | 297.0 | 1075.9 | 33.5 | 0.0 | 1007.7 | 48543.7 |
| CET 203 | 15.3 | 223.2 | 0.0 | 1.5 | 224. | 36.3 | 10795.8 | 156.5 | 217.7 | 157.2 | 11363.4 | 1.0 | 1.0 | 1241.1 | 0.0 | 1.1 | 1242.2 | 26.5 | 25.9 | 52.4 | 11.3 | 0.0 | 314.7 | 13224.0 |
| CET 209 | 261.8 | 0.0 | 13.0 | 5.0 | 17.9 | 84.3 | 12389.7 | 269.0 | 1173.5 | 1063.0 | 14979.6 | 0.8 | 0.7 | 554.9 | 0.0 | 6.9 | 561.9 | 207.0 | 63.8 | 270.9 | 13.6 | 0.0 | 330.7 | 16436.4 |
| CET 231 | 20.3 | 0.0 | 3.3 | 1.3 | 4.6 | 25.4 | 7081.5 | 118.5 | 310.7 | 55.7 | 7591.7 | 0.9 | 1.0 | 201.2 | 0.0 | 0.1 | 201.3 | 25.4 | 10.1 | 35.5 | 6.5 | 0.0 | 156.8 | 8016.8 |
| CET 286 | 138.3 | 0.0 | 9.1 | 6.3 | 15.3 | 124.4 | 33032.0 | 336.3 | 291.2 | 757.8 | 34541.7 | 1.0 | 1.1 | 490.9 | 0.0 | 0.1 | 491.1 | 135.2 | 40.1 | 175.3 | 7.6 | 0.0 | 516.9 | 35886.2 |
| CET 296 | 108.6 | 0.0 | 0.0 | 1.6 | 1.6 | 30.9 | 13482.7 | 40.7 | 602.1 | 3472.9 | 17629.4 | 0.8 | 0.5 | 697.2 | 0.0 | 3.1 | 700.3 | 189.9 | 102.6 | 292.5 | 7.2 | 0.0 | 3031.1 | 21770.7 |
| CET 305 | 30.3 | 0.0 | 0.0 | 2.7 | 2.7 | 110.3 | 48519.8 | 86.6 | 720.6 | 1285.5 | 50722.9 | 1.0 | 0.6 | 760.3 | 0.0 | 16.2 | 776.5 | 16.0 | 36.0 | 52.0 | 5.3 | 0.0 | 1932.8 | 53522.4 |
| CET 311 | 138.1 | 2.1 | 74.8 | 24.7 | 101. | 3.2 | 396653.6 | 695.0 | 5963.4 | 7015.3 | 410330. | 1.0 | 4.5 | 15952. | 0.0 | 113.0 | 16065.1 | 559.4 | 907.3 | 1466.7 | 70.5 | 0.0 | 6607.3 | 434779.7 |
| CET 314 | 320.4 | 0.0 | 41.6 | 37.6 | 79.2 | 944.4 | 348979.4 | 998.4 | 8151.6 | 23855. | 382929. | 0.9 | 1.8 | 2545.9 | 0.0 | 121.1 | 2667.0 | 1081.2 | 235.9 | 1317.1 | 38.3 | 0.0 | 3649.5 | 391001.1 |
| CET 403 | 523.3 | 61.0 | 18.9 | 8.4 | 88.3 | 165.0 | 37176.2 | 182.5 | 1639.5 | 2640.2 | 41803.4 | 0.9 | 1.5 | 1223.4 | 0.0 | 11.9 | 1235.3 | 353.5 | 157.5 | 511.0 | 8.0 | 0.0 | 369.9 | 44539.2 |
| CET 407 | 175.0 | 0.0 | 11.6 | 0.0 | 11.6 | 244.1 | 21084.0 | 319.6 | 1506.9 | 0.0 | 23154.6 | 0.9 | 0.6 | 2585.7 | 0.0 | 0.0 | 2585.7 | 228.5 | 72.3 | 300.8 | 96.0 | 0.0 | 172.9 | 26496.6 |
| CET 420 | 1.7 | 0.0 | 39.7 | 54.1 | 93.9 | 39.6 | 4652.3 | 120.3 | 424.2 | 0.0 | 5236.5 | 0.9 | 0.4 | 140.0 | 0.0 | 0.0 | 140.0 | 22.7 | 0.0 | 22.7 | 5.7 | 11.0 | 76.6 | 5587.9 |
| CET 450 | 362.5 | 0.0 | 16.7 | 0.0 | 16.7 | 96.5 | 8165.1 | 193.2 | 921.6 | 17.1 | 9393.6 | 0.9 | 0.4 | 1039.9 | 0.0 | 0.0 | 1039.9 | 192.4 | 50.5 | 242.9 | 37.5 | 35.4 | 408.7 | 11537.1 |
| CET 458 | 117.0 | 0.0 | 63.5 | 34.9 | 98.3 | 12.8 | 1222.1 | 42.8 | 119.4 | 17.7 | 1414.8 | 0.9 | 0.3 | 354.7 | 0.0 | 0.0 | 354.7 | 43.3 | 12.1 | 55.4 | 0.0 | 9.6 | 108.5 | 2158.4 |
| CET 505 | 58.7 | 0.0 | 5.9 | 7.3 | 13.2 | 61.1 | 12218.6 | 165.9 | 691.0 | 41.0 | 13177.6 | 0.9 | 0.7 | 471.1 | 906.7 | 0.0 | 1377.8 | 112.2 | 18.0 | 130.2 | 5.8 | 7.4 | 456.2 | 15226.9 |
| CET 509 | 417.8 | 0.0 | 11.7 | 12.8 | 24.4 | 0.0 | 10062.1 | 47.3 | 929.4 | 1450.7 | 12489.5 | 0.8 | 0.9 | 704.8 | 0.0 | 10.3 | 715.1 | 272.4 | 69.3 | 341.7 | 3.1 | 0.0 | 166.3 | 14158.0 |
| CET 543 | 710.0 | 0.0 | 28.0 | 14.1 | 42.1 | 319.8 | 146275.8 | 373.1 | 2305.1 | 3379.8 | 152653. | 1.0 | 1.1 | 1565.8 | 0.0 | 92.3 | 1658.1 | 638.3 | 159.7 | 797.9 | 10.6 | 0.0 | 4748.1 | 160620.3 |
| CET 562 | 28.8 | 0.0 | 34.4 | 26.0 | 60.4 | 11.2 | 1650.4 | 50.6 | 119.9 | 0.0 | 1832.2 | 0.9 | 0.6 | 139.8 | 147.2 | 0.0 | 287.0 | 16.6 | 0.0 | 16.6 | 1.3 | 5.7 | 67.2 | 2299.1 |
| CET 564 | 238.4 | 242.6 | 36.1 | 10.3 | 289. | 70.9 | 21298.8 | 116.9 | 1504.8 | 4731.5 | 27722.9 | 0.8 | 0.4 | 4665.7 | 0.0 | 0.4 | 4666.1 | 266.6 | 360.8 | 627.4 | 33.8 | 0.0 | 2836.1 | 36413.7 |
| CET 584 | 761.7 | 0.0 | 3.8 | 2.8 | 6.7 | 43.9 | 20939.5 | 85.1 | 1322.6 | 7374.3 | 29765.4 | 0.7 | 1.4 | 754.7 | 0.0 | 18.1 | 772.8 | 881.6 | 254.3 | 1136.0 | 25.2 | 0.0 | 535.3 | 33003.0 |
| CET 595 | 788.9 | 0.0 | 33.5 | 18.6 | 52.1 | 273.7 | 52653.9 | 272.4 | 1653.5 | 2471.7 | 57325.2 | 0.9 | 1.6 | 1410.2 | 0.0 | 37.3 | 1447.5 | 395.1 | 141.1 | 536.3 | 12.9 | 0.0 | 293.5 | 60456.2 |
| Mean | 245.0 | 21.2 | 20.2 | 12.0 | 53.3 | 134.0 | 54958.6 | 223.4 | 1581.8 | 4062.2 | 60960.0 | 0.9 | 1.0 | 1842.0 | 42.2 | 23.0 | 1907.1 | 302.5 | 143.3 | 445.8 | 19.8 | 2.8 | 1327.4 | 64961.0 |
| SD | 253.1 | 64.9 | 19.7 | 13.8 | 70.1 | 199.3 | 100964.1 | 228.7 | 1955.2 | 6700.9 | 107050. | 0.1 | 0.8 | 3124.3 | 182.5 | 37.5 | 3131.1 | 328.9 | 191.5 | 464.7 | 22.9 | 7.5 | 1763.5 | 111220.8 |
| Median | 138.3 | 0.0 | 13.0 | 7.3 | 24.4 | 70.9 | 20939.5 | 156.5 | 929.4 | 1285.5 | 23154.6 | 0.9 | 0.8 | 1039.9 | 0.0 | 3.1 | 1174.9 | 192.4 | 69.3 | 270.9 | 10.6 | 0.0 | 456.2 | 26496.6 |

Table 11. Levels of OCPs in liver samples from stranded BND (ng $g^{-1}$ lw). No detectable values of δ-HCH, heptachlor, aldrin, endrin or beta-endosulphan were found in any sample. SD (standard deviation).

| LIVER | HCB | α-HCH | β-HCH | γ-HCH | ∑HCH | o,p-DDE | p,p-DDE | o,p-DDD | p,p-DDD o,p-DDT | p,p-DDT | ∑DDT | p,p-DDE/ ∑DDT | ∑DDT/ ∑PCB | Dieldrin | α-Endosulph. | Endos. sulfate | ∑Cyclodienes | cis-Chlordane | trans-Chlordane | ∑Chlordane | Dicofol | Methoxychlor | Mirex | ∑OCPs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET 43 | 8.6 | 1.1 | 1.4 | 1.1 | 3.6 | 0.7 | 199.4 | 7.1 | 36.4 | 1.0 | 244.7 | 0.8 | 0.7 | 18.6 | 0.0 | 0.1 | 18.7 | 5.8 | 1.9 | 7.7 | 0.1 | 0.0 | 19.7 | 303.2 |
| CET 78 | 1.4 | 0.0 | 0.4 | 0.6 | 1.0 | 1.2 | 458.9 | 8.5 | 29.6 | 0.5 | 498.8 | 0.9 | 0.5 | 9.4 | 0.0 | 0.0 | 9.4 | 1.6 | 1.3 | 2.9 | 0.1 | 0.0 | 17.3 | 531.0 |
| CET 93 | 4.1 | 0.0 | 0.3 | 0.4 | 0.7 | 3.1 | 538.2 | 15.7 | 1.7 | 0.5 | 559.4 | 1.0 | 0.8 | 10.0 | 0.0 | 0.0 | 10.0 | 0.4 | 1.2 | 1.6 | 0.1 | 0.0 | 24.6 | 600.5 |
| CET 94 | 2.4 | 0.0 | 1.0 | 0.9 | 1.9 | 0.9 | 199.5 | 6.0 | 19.0 | 0.8 | 226.1 | 0.9 | 0.7 | 15.0 | 0.0 | 0.1 | 15.1 | 0.2 | 0.5 | 0.7 | 0.1 | 0.0 | 12.4 | 258.7 |
| CET 99 | 10.1 | 0.0 | 0.7 | 0.9 | 1.6 | 1.0 | 264.9 | 8.8 | 56.1 | 1.4 | 332.2 | 0.8 | 0.6 | 23.8 | 0.0 | 0.0 | 23.9 | 5.7 | 1.7 | 7.4 | 0.1 | 0.0 | 38.3 | 413.7 |
| CET 171 | 7.1 | 0.0 | 0.7 | 1.0 | 1.6 | 1.8 | 318.8 | 10.6 | 45.2 | 1.9 | 378.3 | 0.8 | 0.4 | 20.0 | 0.0 | 0.0 | 20.0 | 3.4 | 1.2 | 4.5 | 0.1 | 0.0 | 42.0 | 453.7 |
| CET 203 | 6.2 | 19.4 | 0.3 | 0.2 | 19.9 | 7.1 | 1706.6 | 40.4 | 51.9 | 1.7 | 1807.7 | 0.9 | 0.8 | 24.4 | 0.0 | 1.1 | 25.5 | 4.6 | 1.3 | 5.9 | 0.3 | 0.0 | 84.8 | 1950.2 |
| CET 209 | 3.7 | 1.7 | 0.2 | 0.2 | 2.0 | 0.8 | 158.4 | 5.9 | 26.1 | 1.0 | 192.2 | 0.8 | 0.5 | 19.3 | 0.0 | 0.0 | 19.3 | 3.2 | 1.0 | 4.3 | 0.2 | 0.0 | 11.1 | 232.8 |
| CET 286 | 1.6 | 5.4 | 0.2 | 0.2 | 5.8 | 0.9 | 213.9 | 3.3 | 11.1 | 1.2 | 230.4 | 0.9 | 0.9 | 13.2 | 0.0 | 0.0 | 13.2 | 0.4 | 0.6 | 1.1 | 0.2 | 0.0 | 5.3 | 257.5 |
| CET 296 | 0.3 | 0.9 | 0.1 | 0.2 | 1.2 | 0.1 | 23.4 | 0.5 | 1.4 | 1.9 | 27.3 | 0.9 | 0.4 | 11.1 | 0.0 | 0.0 | 11.1 | 0.3 | 0.7 | 1.0 | 0.2 | 0.0 | 5.5 | 46.6 |
| CET 305 | 0.9 | 0.8 | 0.5 | 0.5 | 1.7 | 3.2 | 447.7 | 7.5 | 28.4 | 66.6 | 553.5 | 0.8 | 0.9 | 16.3 | 0.0 | 0.3 | 16.6 | 3.1 | 0.3 | 3.4 | 0.2 | 0.0 | 25.3 | 601.6 |
| CET 311 | 12.8 | 0.0 | 0.0 | 0.0 | 0.0 | 6.8 | 2050.3 | 11.6 | 30.7 | 7.7 | 2107.0 | 1.0 | 8.9 | 20.9 | 0.0 | 0.0 | 20.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 67.5 | 2208.3 |
| CET 314 | 1.3 | 0.0 | 0.2 | 0.1 | 0.3 | 1.7 | 496.3 | 9.0 | 22.5 | 1.3 | 530.8 | 0.9 | 1.2 | 10.7 | 0.0 | 0.0 | 10.7 | 2.3 | 0.1 | 2.4 | 0.1 | 0.0 | 8.4 | 553.9 |
| CET 403 | 4.7 | 7.3 | 0.0 | 0.0 | 7.3 | 1.2 | 202.0 | 3.8 | 15.0 | 2.2 | 224.2 | 0.9 | 1.1 | 14.9 | 0.0 | 0.0 | 14.9 | 1.9 | 0.7 | 2.6 | 0.2 | 0.0 | 3.6 | 257.5 |
| CET 407 | 0.3 | 0.0 | 7.9 | 10.8 | 18.8 | 7.9 | 930.5 | 24.1 | 84.8 | 0.0 | 1047.3 | 0.9 | 0.4 | 28.0 | 0.0 | 0.0 | 28.0 | 4.5 | 0.0 | 4.5 | 1.1 | 2.2 | 15.3 | 1117.6 |
| CET 420 | 5.9 | 0.0 | 3.2 | 1.7 | 4.9 | 0.6 | 61.1 | 2.1 | 6.0 | 0.0 | 69.9 | 0.9 | 0.3 | 17.7 | 0.0 | 0.0 | 17.7 | 2.2 | 0.6 | 2.8 | 0.0 | 0.5 | 5.4 | 107.0 |
| CET 450 | 5.8 | 0.0 | 6.9 | 5.2 | 12.1 | 2.2 | 330.1 | 10.1 | 24.0 | 0.0 | 366.4 | 0.9 | 0.6 | 28.0 | 29.4 | 0.0 | 57.4 | 3.3 | 0.0 | 3.3 | 0.3 | 1.1 | 13.4 | 459.8 |
| CET 458 | 9.1 | 0.0 | 4.9 | 5.1 | 10.0 | 0.0 | 87.4 | 2.9 | 19.0 | 0.0 | 109.3 | 0.8 | 0.5 | 37.1 | 0.0 | 0.0 | 37.1 | 4.0 | 1.2 | 5.2 | 1.5 | 0.0 | 3.0 | 175.2 |
| CET 505 | 22.9 | 0.0 | 4.9 | 3.4 | 8.3 | 3.3 | 485.0 | 14.3 | 48.8 | 0.0 | 551.4 | 0.9 | 0.5 | 51.1 | 0.0 | 0.0 | 51.1 | 11.4 | 2.1 | 13.5 | 0.5 | 0.6 | 25.9 | 674.1 |
| CET 509 | 0.3 | 0.0 | 0.5 | 0.8 | 1.3 | 0.2 | 28.4 | 0.5 | 2.8 | 2.1 | 34.1 | 0.8 | 0.7 | 10.9 | 0.0 | 0.0 | 11.0 | 0.8 | 0.2 | 1.1 | 0.1 | 0.0 | 0.8 | 48.7 |
| CET 543 | 3.3 | 0.0 | 0.2 | 0.1 | 0.3 | 0.7 | 360.3 | 5.4 | 4.7 | 1.8 | 372.9 | 1.0 | 0.9 | 11.4 | 0.0 | 0.2 | 11.6 | 1.1 | 0.5 | 1.6 | 0.1 | 0.0 | 14.4 | 404.2 |
| CET 562 | 2.6 | 0.0 | 1.6 | 0.8 | 2.4 | 0.0 | 78.3 | 1.6 | 11.1 | 0.0 | 91.0 | 0.9 | 0.2 | 133.2 | 0.0 | 0.0 | 133.2 | 1.1 | 0.0 | 1.1 | 0.4 | 0.0 | 9.6 | 240.3 |
| CET 564 | 0.3 | 4.2 | 0.1 | 0.1 | 4.4 | 0.1 | 15.7 | 0.2 | 1.3 | 0.6 | 17.9 | 0.9 | 0.3 | 7.0 | 0.0 | 0.0 | 7.0 | 0.0 | 0.2 | 0.2 | 0.1 | 0.0 | 2.3 | 32.2 |
| CET 584 | 0.0 | 0.0 | 0.6 | 0.3 | 0.9 | 0.6 | 132.3 | 2.6 | 3.3 | 1.4 | 140.1 | 0.9 | 0.7 | 11.9 | 0.0 | 0.0 | 11.9 | 0.2 | 0.4 | 0.6 | 0.1 | 0.0 | 5.8 | 159.5 |
| CET 592 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 179.4 | 3.3 | 37.2 | 4.8 | 225.1 | 0.8 | 0.9 | 16.6 | 0.0 | 0.1 | 16.8 | 7.2 | 1.5 | 8.7 | 0.3 | 0.0 | 15.5 | 266.9 |
| CET 595 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 1.9 | 627.8 | 2.2 | 4.1 | 7.5 | 643.5 | 1.0 | 0.4 | 14.1 | 0.0 | 0.5 | 14.6 | 1.6 | 0.2 | 1.8 | 0.1 | 0.0 | 69.1 | 729.2 |
| Mean | 4.5 | 1.6 | 1.4 | 1.3 | 4.3 | 1.9 | 407.5 | 8.0 | 23.9 | 4.2 | 445.4 | 0.9 | 1.0 | 22.9 | 1.1 | 0.1 | 24.1 | 2.7 | 0.7 | 3.5 | 0.3 | 0.2 | 21.0 | 503.2 |
| SD | 5.2 | 4.1 | 2.2 | 2.4 | 5.5 | 2.2 | 486.3 | 8.6 | 20.9 | 12.9 | 505.5 | 0.1 | 1.6 | 24.5 | 5.8 | 0.2 | 25.4 | 2.7 | 0.6 | 3.1 | 0.3 | 0.5 | 22.2 | 526.9 |
| Median | 2.9 | 0.0 | 0.4 | 0.4 | 1.8 | 0.9 | 239.4 | 5.9 | 20.8 | 1.3 | 288.5 | 0.9 | 0.6 | 16.5 | 0.0 | 0.0 | 16.7 | 2.0 | 0.6 | 2.7 | 0.1 | 0.0 | 13.9 | 353.7 |

Table 12. Levels of PAHs in blubber samples from stranded BND (ng $g^{-1}$ lw). Acenaphthylene, anthracene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene were not detected in any sample. TEQ-PAHs in pg $g^{-1}$ lw. SD (standard deviation).

| BLUBBER | Naphta. | Acenaphtene | Fluorene | Phenanthrene | Fluoranthene | Pyrene | Chrysene | Benzo (a) anthracene | Benzo (b) fluoranthene | Benzo (k) fluoranthene | Benzo (a) pyrene | Benzo (g,h,i) perylene | TEQ-PAHs | ∑PAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET 78 | 0.0 | 22.4 | 14.0 | 93.5 | 35.0 | 104.0 | 41.6 | 0.0 | 16.0 | 3.4 | 22.8 | 0.0 | 23.7 | 352.7 |
| CET 93 | 43.6 | 164.0 | 8.7 | 209.2 | 101.0 | 277.3 | 98.9 | 0.0 | 36.7 | 7.3 | 51.8 | 0.0 | 53.8 | 998.5 |
| CET 94 | 0.0 | 74.3 | 13.8 | 317.5 | 146.6 | 430.6 | 134.9 | 0.0 | 59.0 | 8.9 | 81.8 | 0.0 | 84.8 | 1267.4 |
| CET 99 | 0.0 | 163.2 | 50.5 | 406.8 | 110.0 | 335.5 | 140.9 | 37.5 | 56.3 | 8.2 | 66.5 | 0.0 | 73.2 | 1375.2 |
| CET 171 | 0.0 | 0.0 | 4.5 | 122.1 | 41.5 | 103.7 | 50.6 | 0.0 | 18.1 | 3.9 | 17.3 | 0.0 | 18.3 | 361.7 |
| CET 203 | 288.5 | 418.2 | 58.1 | 553.3 | 128.8 | 410.5 | 153.6 | 0.0 | 48.7 | 8.3 | 61.5 | 0.0 | 64.8 | 2129.6 |
| CET 209 | 31.3 | 27.7 | 40.9 | 54.3 | 6.4 | 12.7 | 4.1 | 0.0 | 0.0 | 0.0 | 1.2 | 0.0 | 1.4 | 178.5 |
| CET 231 | 265.6 | 87.5 | 15.6 | 74.6 | 9.6 | 26.4 | 10.3 | 0.0 | 3.1 | 0.6 | 3.0 | 0.0 | 3.3 | 496.3 |
| CET 286 | 563.7 | 90.7 | 85.0 | 129.6 | 15.3 | 34.5 | 13.8 | 0.0 | 4.2 | 0.7 | 3.8 | 0.0 | 4.3 | 941.2 |
| CET 296 | 106.6 | 135.4 | 42.3 | 286.8 | 85.2 | 151.5 | 68.9 | 20.3 | 24.2 | 4.5 | 22.9 | 0.0 | 26.5 | 948.7 |
| CET 305 | 16.4 | 46.5 | 148.0 | 243.1 | 63.2 | 161.1 | 79.7 | 17.7 | 25.6 | 5.1 | 32.7 | 0.0 | 36.2 | 839.3 |
| CET 311 | 1013.8 | 0.0 | 24.3 | 1711.0 | 936.7 | 1515.4 | 560.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 9.8 | 5761.3 |
| CET 314 | 0.0 | 45.0 | 51.6 | 158.0 | 53.5 | 142.2 | 55.4 | 0.0 | 19.6 | 2.4 | 20.5 | 0.0 | 21.7 | 548.3 |
| CET 403 | 0.0 | 134.2 | 58.2 | 167.4 | 40.7 | 132.5 | 27.1 | 11.5 | 7.4 | 1.1 | 6.1 | 0.0 | 8.0 | 586.2 |
| CET 407 | 180.4 | 53.3 | 73.5 | 604.3 | 239.9 | 382.6 | 64.5 | 290.1 | 95.1 | 21.3 | 112.9 | 35.5 | 145.4 | 2153.4 |
| CET 420 | 53.2 | 21.6 | 33.8 | 44.1 | 0.0 | 49.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 202.1 |
| CET 450 | 69.1 | 82.8 | 97.1 | 252.0 | 111.6 | 80.9 | 0.0 | 43.4 | 28.7 | 12.0 | 0.0 | 11.3 | 5.4 | 788.8 |
| CET 458 | 258.2 | 106.5 | 132.4 | 191.7 | 77.7 | 83.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.5 | 850.1 |
| CET 505 | 0.0 | 0.0 | 2.8 | 16.9 | 13.8 | 18.4 | 0.0 | 6.4 | 5.9 | 1.3 | 7.1 | 2.7 | 7.9 | 75.3 |
| CET 509 | 22.8 | 240.9 | 69.2 | 108.8 | 23.4 | 73.5 | 14.8 | 0.0 | 4.2 | 0.6 | 5.3 | 0.0 | 5.8 | 563.6 |
| CET 543 | 0.0 | 79.2 | 6.7 | 184.1 | 43.4 | 105.8 | 33.6 | 0.0 | 0.0 | 0.0 | 9.9 | 0.0 | 10.6 | 462.8 |
| CET 562 | 52.5 | 41.1 | 54.4 | 258.9 | 15.5 | 38.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.4 | 461.2 |
| CET 564 | 409.8 | 439.1 | 180.9 | 1931.3 | 463.2 | 1356.3 | 207.4 | 0.0 | 111.1 | 17.2 | 136.1 | 0.0 | 143.4 | 5252.5 |
| CET 584 | 0.0 | 41.9 | 52.2 | 167.7 | 41.3 | 112.5 | 28.7 | 8.2 | 9.2 | 1.8 | 11.0 | 0.0 | 12.5 | 474.4 |
| CET 595 | 26.7 | 665.6 | 36.1 | 172.3 | 38.5 | 130.2 | 32.7 | 9.0 | 8.7 | 1.4 | 8.1 | 0.0 | 9.8 | 1129.2 |
| Mean | 136.1 | 127.2 | 54.2 | 338.4 | 113.7 | 250.8 | 72.9 | 17.8 | 23.3 | 4.4 | 27.3 | 2.0 | 30.9 | 1167.9 |
| SD | 235.4 | 159.7 | 45.9 | 469.6 | 196.9 | 377.9 | 115.9 | 57.9 | 30.0 | 5.7 | 37.4 | 7.4 | 41.6 | 1409.1 |
| Median | 31.3 | 79.2 | 50.5 | 184.1 | 43.4 | 112.5 | 33.6 | 0.0 | 9.2 | 1.8 | 9.9 | 0.0 | 10.6 | 788.8 |

Table 13. Levels of PAHs in liver samples from stranded BND (ng $g^{-1}$ lw). Acenaphthylene, indeno(1,2,3-cd)pyrene, dibenzo (a,h)anthracene and benzo(g,h,i) perylene were not detected in any sample. TEQ-PAHs in pg $g^{-1}$ lw. SD (standard deviation).

| LIVER | Naphta. | Acenaphtene | Fluorene | Phenanthrene | Anthracene | Fluoranthene | Pyrene | Chrysene | Benzo (a) anthracene | Benzo (b) fluoranthene | Benzo (k) fluoranthene | Benzo (a) pyrene | TEQ-PAHs | ∑PAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET 43 | 0.5 | 0.9 | 1.8 | 5.7 | 0.0 | 1.6 | 4.6 | 1.4 | 0.5 | 0.4 | 0.1 | 0.4 | 0.5 | 17.8 |
| CET 78 | 0.0 | 1.8 | 3.8 | 6.8 | 0.0 | 1.1 | 3.1 | 1.0 | 0.2 | 0.3 | 0.0 | 0.3 | 0.4 | 18.4 |
| CET 93 | 1.3 | 0.0 | 0.2 | 7.1 | 0.0 | 0.7 | 1.9 | 0.6 | 0.0 | 0.2 | 0.0 | 0.3 | 0.3 | 12.1 |
| CET 94 | 0.6 | 0.8 | 0.6 | 6.4 | 0.0 | 1.3 | 3.3 | 0.9 | 0.2 | 0.2 | 0.0 | 0.3 | 0.3 | 14.7 |
| CET 99 | 0.6 | 1.8 | 5.5 | 7.5 | 0.0 | 2.0 | 4.3 | 1.5 | 0.6 | 0.4 | 0.1 | 0.5 | 0.6 | 24.8 |
| CET 171 | 0.8 | 1.6 | 0.7 | 8.5 | 0.0 | 2.3 | 5.9 | 1.5 | 0.4 | 0.5 | 0.1 | 0.4 | 0.4 | 22.6 |
| CET 203 | 0.0 | 0.0 | 0.5 | 9.9 | 0.0 | 1.9 | 5.6 | 14.0 | 0.0 | 0.7 | 0.6 | 0.8 | 1.0 | 34.0 |
| CET 209 | 0.0 | 0.0 | 2.0 | 12.2 | 0.0 | 2.8 | 6.0 | 3.8 | 0.9 | 0.9 | 0.2 | 0.9 | 1.1 | 29.7 |
| CET 286 | 0.0 | 0.0 | 1.8 | 7.4 | 0.0 | 1.5 | 4.0 | 6.8 | 0.6 | 0.4 | 0.1 | 0.5 | 0.6 | 23.0 |
| CET 296 | 1.1 | 0.0 | 7.1 | 12.4 | 0.0 | 2.9 | 6.4 | 2.9 | 1.0 | 0.9 | 0.1 | 0.8 | 1.0 | 35.6 |
| CET 305 | 0.4 | 0.0 | 0.0 | 3.7 | 0.0 | 2.2 | 6.3 | 2.3 | 0.4 | 0.7 | 0.1 | 0.8 | 0.9 | 16.9 |
| CET 311 | 0.0 | 0.0 | 17.3 | 6.6 | 0.0 | 3.3 | 8.4 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 36.8 |
| CET 314 | 0.7 | 0.0 | 0.5 | 5.7 | 0.0 | 2.2 | 6.9 | 2.3 | 0.0 | 0.7 | 0.1 | 0.8 | 0.8 | 19.9 |
| CET 403 | 0.0 | 51.1 | 0.7 | 6.5 | 0.0 | 2.2 | 5.9 | 3.1 | 0.0 | 0.6 | 0.1 | 0.9 | 1.0 | 71.0 |
| CET 407 | 10.6 | 4.3 | 6.8 | 8.8 | 0.0 | 0.0 | 9.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 40.4 |
| CET 420 | 12.9 | 5.3 | 6.6 | 9.6 | 0.0 | 3.9 | 4.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 42.5 |
| CET 450 | 10.5 | 8.2 | 10.9 | 51.8 | 0.0 | 3.1 | 7.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 92.2 |
| CET 458 | 13.7 | 11.3 | 0.0 | 0.0 | 0.0 | 13.7 | 19.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 58.1 |
| CET 505 | 8.3 | 0.0 | 34.3 | 97.8 | 8.2 | 6.4 | 7.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 162.8 |
| CET 509 | 0.7 | 0.0 | 0.2 | 4.2 | 0.0 | 2.0 | 5.1 | 2.2 | 0.4 | 0.8 | 0.2 | 1.0 | 1.0 | 16.8 |
| CET 543 | 1.1 | 0.0 | 0.6 | 6.2 | 0.0 | 1.6 | 5.3 | 2.3 | 0.5 | 0.7 | 0.1 | 0.8 | 0.9 | 19.1 |
| CET 562 | 14.0 | 9.0 | 11.3 | 65.7 | 0.0 | 5.8 | 9.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 115.7 |
| CET 564 | 0.6 | 0.0 | 2.3 | 6.4 | 0.0 | 1.3 | 3.3 | 2.0 | 0.5 | 0.4 | 0.1 | 0.4 | 0.5 | 17.5 |
| CET 584 | 0.4 | 0.0 | 0.0 | 1.2 | 0.0 | 1.6 | 5.1 | 2.3 | 0.0 | 0.8 | 0.1 | 0.8 | 0.8 | 12.3 |
| CET 592 | 0.8 | 0.0 | 0.0 | 3.4 | 0.0 | 1.8 | 4.0 | 2.5 | 0.5 | 0.8 | 0.2 | 0.9 | 1.0 | 14.9 |
| CET 595 | 0.0 | 0.0 | 0.0 | 2.7 | 0.0 | 1.4 | 3.8 | 1.3 | 0.0 | 0.4 | 0.1 | 0.4 | 0.4 | 10.2 |
| Mean | 3.1 | 3.7 | 4.4 | 14.0 | 0.3 | 2.7 | 6.1 | 2.1 | 0.3 | 0.4 | 0.1 | 0.5 | 0.5 | 37.7 |
| SD | 4.9 | 10.2 | 7.5 | 22.5 | 1.6 | 2.6 | 3.4 | 2.8 | 0.3 | 0.3 | 0.1 | 0.4 | 0.4 | 36.2 |
| Median | 0.7 | 0.0 | 1.2 | 6.7 | 0.0 | 2.0 | 5.4 | 1.5 | 0.1 | 0.4 | 0.1 | 0.4 | 0.5 | 22.8 |

Table 14. Levels of PCBs in blubber biopsies from live free-ranging BND (ng $g^{-1}$ lw). TEQ-PCBs in pg $g^{-1}$ lw. PCB 77 and PCB 169 were not detected in any sample. SD (standard deviation).

| | PCB 28 | PCB 52 | PCB 81 | PCB 101 | PCB 105 | PCB 114 | PCB 118 | PCB 123 | PCB 126 | PCB 138 | PCB 153 | PCB 156 | PCB 157 | PCB 167 | PCB 180 | PCB 189 | ∑M-PCB | ∑DL-PCB | TEQ-PCBs | ∑PCB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 855.4 | 455.7 | 65.5 | 829.8 | 347.8 | 72.6 | 1269.0 | 9.5 | 9.5 | 7496.6 | 8156.3 | 380.9 | 108.4 | 249.7 | 8655.3 | 73.1 | 27718.0 | 2586.2 | 1046.6 | 29035.2 |
| B2 | 1228.0 | 347.9 | 0.0 | 174.6 | 197.3 | 224.6 | 345.3 | 724.2 | 724.2 | 1278.5 | 1284.9 | 101.3 | 106.0 | 45.8 | 1380.1 | 4.3 | 6039.2 | 2473.2 | 72474.4 | 8167.1 |
| B3 | 3197.5 | 1401.1 | 66.6 | 1450.4 | 526.8 | 0.0 | 1628.3 | 507.7 | 507.7 | 4537.2 | 4142.1 | 337.1 | 94.1 | 0.0 | 4564.6 | 32.4 | 20921.4 | 3700.7 | 50887.3 | 22993.8 |
| B4 | 614.4 | 324.8 | 2.9 | 498.6 | 252.8 | 51.9 | 794.6 | 470.2 | 470.2 | 5569.7 | 5904.9 | 262.5 | 65.2 | 133.6 | 5055.7 | 32.5 | 18762.7 | 2536.6 | 47087.4 | 20504.6 |
| B5 | 1642.9 | 1110.5 | 2.2 | 1969.5 | 966.3 | 117.4 | 2993.9 | 1308.5 | 1308.5 | 18055.8 | 17626.3 | 980.6 | 194.2 | 574.8 | 15495.5 | 83.2 | 58894.4 | 8529.4 | 131063.3 | 64430.0 |
| B6 | 2605.8 | 1427.3 | 3.3 | 1540.9 | 794.4 | 96.9 | 2286.2 | 851.3 | 851.3 | 10633.4 | 10818.2 | 658.8 | 141.0 | 370.0 | 9386.9 | 2.1 | 38698.6 | 6055.3 | 85282.3 | 42467.6 |
| B7 | 1463.2 | 986.7 | 5.2 | 2092.4 | 1198.4 | 144.1 | 3262.9 | 1385.1 | 1385.1 | 19331.9 | 18153.9 | 1124.0 | 298.5 | 499.9 | 16301.3 | 109.5 | 61592.2 | 9412.7 | 138754.7 | 67742.1 |
| B8 | 1058.4 | 425.1 | 1.9 | 437.7 | 345.7 | 65.7 | 697.9 | 89.7 | 89.7 | 1673.6 | 1352.2 | 158.9 | 11.3 | 72.7 | 1250.3 | 4.6 | 6895.3 | 1538.1 | 9011.2 | 7735.5 |
| B9 | 545.7 | 144.3 | 1.0 | 188.0 | 212.1 | 43.6 | 448.2 | 118.0 | 118.0 | 2248.4 | 2038.5 | 213.4 | 11.3 | 39.1 | 2651.6 | 14.9 | 8264.7 | 1219.7 | 11834.0 | 9036.2 |
| B10 | 1509.4 | 746.7 | 155.3 | 949.9 | 693.7 | 64.5 | 1503.3 | 633.1 | 633.1 | 8740.4 | 7994.1 | 633.4 | 123.5 | 267.0 | 7233.6 | 49.2 | 28677.4 | 4756.2 | 63476.7 | 31930.3 |
| B11 | 2178.9 | 1012.4 | 4.5 | 763.8 | 588.9 | 102.9 | 1487.3 | 774.0 | 774.0 | 10944.9 | 10858.5 | 497.1 | 69.0 | 194.1 | 10293.7 | 52.3 | 37539.6 | 4544.1 | 77509.3 | 40596.4 |
| B12 | 690.1 | 356.4 | 1.8 | 567.2 | 403.7 | 76.9 | 985.8 | 164.9 | 164.9 | 2708.1 | 2346.1 | 224.0 | 16.8 | 71.2 | 2137.0 | 3.8 | 9790.7 | 2113.7 | 16549.3 | 10918.6 |
| B13 | 872.8 | 519.1 | 94.4 | 614.2 | 388.7 | 90.5 | 979.6 | 606.0 | 606.0 | 7525.8 | 7833.1 | 401.3 | 79.4 | 207.6 | 8785.3 | 32.3 | 27130.0 | 3485.9 | 60711.2 | 29636.3 |
| B14 | 1780.4 | 1095.7 | 199.1 | 2634.4 | 2196.4 | 185.9 | 5015.8 | 904.6 | 904.6 | 15895.6 | 12233.2 | 1631.4 | 356.1 | 622.0 | 12198.7 | 94.6 | 50853.8 | 12110.6 | 90854.7 | 57948.6 |
| B15 | 2134.0 | 1462.2 | 320.0 | 3012.9 | 2238.2 | 224.9 | 5507.2 | 2107.0 | 2107.0 | 29857.0 | 27353.2 | 1898.4 | 499.9 | 0.0 | 24070.6 | 172.9 | 93397.1 | 15075.5 | 211173.3 | 102965.3 |
| B16 | 1483.4 | 654.4 | 143.1 | 825.0 | 615.9 | 124.0 | 1379.8 | 338.4 | 338.4 | 4668.5 | 4155.3 | 612.7 | 55.0 | 186.9 | 4659.0 | 23.3 | 17825.5 | 3817.6 | 33984.2 | 20263.2 |
| B17 | 1069.7 | 543.0 | 137.7 | 895.3 | 653.9 | 110.8 | 1662.5 | 349.9 | 349.9 | 5597.3 | 4994.8 | 443.1 | 66.0 | 194.0 | 4007.4 | 28.9 | 18769.9 | 3996.7 | 35140.3 | 21104.2 |
| B18 | 1575.4 | 712.2 | 141.7 | 801.2 | 583.2 | 48.9 | 1054.9 | 332.0 | 332.0 | 5149.3 | 4541.1 | 330.1 | 68.4 | 122.8 | 3627.4 | 24.0 | 17461.5 | 3038.0 | 33320.8 | 19444.6 |
| B19 | 1348.1 | 830.5 | 6.9 | 1526.3 | 928.9 | 108.0 | 2449.6 | 658.6 | 658.6 | 9023.5 | 8794.6 | 793.5 | 179.6 | 376.7 | 7759.6 | 47.3 | 31732.2 | 6207.7 | 66031.0 | 35490.3 |
| B20 | 492.3 | 292.0 | 16.3 | 483.7 | 261.5 | 66.2 | 659.1 | 304.7 | 304.7 | 3470.9 | 3579.7 | 248.5 | 51.1 | 127.6 | 3900.1 | 31.3 | 12877.7 | 2071.0 | 30529.9 | 14289.6 |
| B21 | 855.9 | 517.0 | 6.1 | 937.8 | 658.9 | 0.0 | 1573.1 | 466.0 | 466.0 | 5850.3 | 5537.4 | 408.8 | 89.4 | 184.4 | 4931.7 | 14.3 | 20203.3 | 3867.2 | 46707.3 | 22497.4 |
| B22 | 872.9 | 414.5 | 1.7 | 583.3 | 352.6 | 43.9 | 887.6 | 276.7 | 276.7 | 3361.9 | 3023.4 | 324.9 | 52.4 | 80.5 | 2813.3 | 14.2 | 11956.9 | 2311.3 | 27732.9 | 13380.6 |
| B23 | 1854.0 | 759.2 | 1.8 | 751.0 | 706.1 | 138.0 | 1185.5 | 236.1 | 236.1 | 4877.9 | 4106.2 | 406.0 | 53.6 | 118.3 | 3534.1 | 13.7 | 17067.9 | 3095.2 | 23695.5 | 18977.7 |
| B24 | 50.0 | 6.7 | 0.0 | 0.2 | 0.5 | 0.1 | 1.9 | 0.3 | 0.3 | 57.8 | 62.8 | 2.8 | 0.4 | 0.0 | 60.7 | 0.0 | 240.1 | 6.3 | 32.0 | 244.5 |
| B25 | 1239.5 | 510.6 | 47.9 | 402.1 | 340.0 | 0.0 | 749.4 | 229.7 | 229.7 | 2248.0 | 1879.3 | 235.2 | 12.1 | 66.9 | 2728.9 | 19.5 | 9757.7 | 1930.3 | 23030.1 | 10938.6 |
| B26 | 1828.9 | 722.1 | 99.1 | 701.9 | 441.7 | 61.5 | 937.4 | 81.8 | 81.8 | 1925.5 | 1298.0 | 284.0 | 61.0 | 78.0 | 1306.5 | 3.8 | 8720.4 | 2130.0 | 8266.8 | 9913.1 |
| B27 | 1888.2 | 777.2 | 2.9 | 844.6 | 490.1 | 82.5 | 1317.7 | 145.9 | 145.9 | 3046.0 | 2239.8 | 382.7 | 50.2 | 77.6 | 2317.6 | 11.5 | 12431.1 | 2707.1 | 14672.0 | 13820.6 |
| B28 | 1135.9 | 498.3 | 1.9 | 507.5 | 364.0 | 48.6 | 810.4 | 92.2 | 92.2 | 2024.9 | 1628.4 | 237.0 | 6.3 | 64.9 | 1488.6 | 8.9 | 8093.9 | 1726.4 | 9265.8 | 9009.9 |
| B29 | 2074.0 | 901.4 | 3.6 | 946.0 | 729.4 | 173.8 | 1534.6 | 137.8 | 137.8 | 3239.5 | 2308.1 | 459.3 | 35.3 | 149.9 | 2418.7 | 6.2 | 13422.2 | 3367.7 | 13882.2 | 15255.4 |
| B30 | 1656.2 | 800.3 | 163.3 | 1093.6 | 836.7 | 102.4 | 1693.4 | 144.9 | 144.9 | 3834.4 | 2628.3 | 549.9 | 97.5 | 119.1 | 2101.5 | 0.1 | 13807.7 | 3852.2 | 14641.6 | 15966.5 |

(Continuation of table 14)

| | PCB 28 | PCB 52 | PCB 81 | PCB 101 | PCB 105 | PCB 114 | PCB 118 | PCB 123 | PCB 126 | PCB 138 | PCB 153 | PCB 156 | PCB 157 | PCB 167 | PCB 180 | PCB 189 | ∑M-PCB | ∑DL-PCB | TEQ-PCBs | ∑PCB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B31 | 741.2 | 308.9 | 0.4 | 286.6 | 156.7 | 19.8 | 350.5 | 16.5 | 16.5 | 658.8 | 503.8 | 70.5 | 2.2 | 13.4 | 278.7 | 0.4 | 3128.6 | 646.7 | 1664.3 | 3424.8 |
| B32 | 2760.9 | 1134.3 | 2.5 | 1148.4 | 680.9 | 174.0 | 1588.9 | 193.8 | 193.8 | 3728.4 | 3276.8 | 383.3 | 12.9 | 142.9 | 2778.1 | 7.3 | 16415.8 | 3380.3 | 19476.0 | 18207.2 |
| B33 | 2446.1 | 1047.0 | 112.7 | 786.6 | 539.0 | 77.0 | 1086.8 | 493.3 | 493.3 | 5748.6 | 5716.0 | 331.0 | 20.1 | 0.0 | 4858.0 | 17.9 | 21689.2 | 3171.0 | 49437.2 | 23773.4 |
| B34 | 1963.7 | 873.4 | 1.7 | 1237.5 | 863.3 | 150.2 | 2052.1 | 393.4 | 393.4 | 5888.3 | 5278.1 | 486.6 | 48.4 | 234.8 | 4424.9 | 25.0 | 21718.1 | 4648.9 | 39472.8 | 24314.9 |
| B35 | 4098.9 | 1561.1 | 183.7 | 1114.3 | 524.9 | 0.0 | 1574.9 | 290.7 | 290.7 | 4108.1 | 3770.5 | 384.0 | 45.0 | 0.0 | 2721.6 | 13.6 | 18949.5 | 3307.4 | 29207.7 | 20682.0 |
| B36 | 1676.7 | 825.7 | 1.4 | 1166.3 | 430.7 | 136.0 | 1784.0 | 332.0 | 332.0 | 5350.7 | 4317.2 | 505.1 | 36.0 | 183.9 | 4377.9 | 15.5 | 19498.5 | 3756.5 | 33301.1 | 21471.0 |
| B37 | 527.0 | 2337.8 | 309.5 | 7352.9 | 3858.3 | 463.4 | 12654.6 | 7018.2 | 7018.2 | 72607.4 | 74762.1 | 4471.0 | 1068.8 | 2276.8 | 73775.0 | 568.0 | 244016.8 | 39706.8 | 702881.0 | 271068.9 |
| B38 | 401.7 | 1710.3 | 326.7 | 3625.4 | 1224.7 | 166.8 | 5076.7 | 9921.9 | 9921.9 | 81431.2 | 93149.9 | 1817.7 | 556.4 | 1511.8 | 126373. | 796.2 | 311769.0 | 31320.8 | 992921.0 | 338013.0 |
| B39 | 362.3 | 1353.4 | 196.7 | 3074.7 | 1222.6 | 100.7 | 4045.6 | 2424.9 | 2424.9 | 28015.3 | 31061.3 | 1030.7 | 311.0 | 816.4 | 23217.7 | 158.3 | 91130.2 | 12731.9 | 242852.4 | 99816.5 |
| B40 | 417.0 | 5252.9 | 618.5 | 11278.5 | 6077.9 | 780.0 | 18689.2 | 12930.4 | 12930.4 | 142825. | 144287. | 5773.9 | 1914.2 | 3186.4 | 135116. | 932.0 | 457867.5 | 63832.8 | 1294729. | 503011.0 |
| B41 | 1048.7 | 3378.3 | 395.9 | 7814.6 | 4206.0 | 478.9 | 14021.8 | 8062.3 | 8062.3 | 88028.2 | 87348.2 | 3676.4 | 1096.9 | 2284.4 | 83413.2 | 579.0 | 285052.9 | 42863.8 | 807381.5 | 313894.9 |
| B42 | 1125.4 | 1466.2 | 294.0 | 2952.8 | 2031.9 | 0.0 | 5221.4 | 6259.0 | 6259.0 | 59538.0 | 64612.1 | 1919.0 | 562.2 | 1288.4 | 76253.3 | 517.0 | 211169.2 | 24352.0 | 626526.8 | 230299.8 |
| B43 | 2642.7 | 2782.2 | 367.6 | 5108.4 | 1849.4 | 251.0 | 5787.2 | 3706.6 | 3706.6 | 38876.0 | 40978.9 | 1859.1 | 536.0 | 922.6 | 36695.8 | 236.8 | 132871.0 | 19222.7 | 371222.2 | 146306.5 |
| B44 | 902.5 | 2246.0 | 243.8 | 4916.9 | 2521.1 | 350.5 | 8639.1 | 5731.9 | 5731.9 | 56910.5 | 57718.8 | 2583.2 | 701.8 | 1458.1 | 58800.0 | 427.7 | 190133.8 | 28389.2 | 573937.2 | 209883.9 |
| B45 | 689.8 | 2145.5 | 196.4 | 3779.4 | 2292.8 | 220.7 | 5958.5 | 4665.1 | 4665.1 | 47204.9 | 50479.1 | 1971.4 | 661.9 | 1222.1 | 49139.1 | 336.2 | 159396.2 | 22190.3 | 467093.1 | 175628.1 |
| B46 | 1704.4 | 842.4 | 2.4 | 1356.6 | 1289.3 | 163.1 | 2489.2 | 493.8 | 493.8 | 6270.1 | 5961.5 | 598.0 | 115.1 | 283.0 | 5844.4 | 39.0 | 24468.6 | 5966.8 | 49546.5 | 27946.2 |
| B47 | 275.6 | 1580.9 | 177.8 | 2950.0 | 1217.9 | 162.3 | 4474.7 | 3175.2 | 3175.2 | 31764.2 | 35765.6 | 999.2 | 327.2 | 876.8 | 32680.8 | 204.8 | 109491.8 | 14791.1 | 317921.1 | 119808.2 |
| B48 | 525.8 | 1351.0 | 179.2 | 3395.5 | 1319.5 | 151.6 | 4152.0 | 2189.8 | 2189.8 | 21777.0 | 24559.6 | 1092.3 | 303.8 | 772.0 | 21719.7 | 165.3 | 77480.6 | 12515.3 | 219333.7 | 85843.8 |
| B49 | 1094.7 | 1496.4 | 164.9 | 3741.4 | 1841.0 | 170.4 | 4636.0 | 1731.7 | 1731.7 | 18665.2 | 19744.8 | 1240.3 | 325.3 | 684.9 | 17913.5 | 136.2 | 67292.0 | 12662.5 | 173547.1 | 75318.4 |
| B50 | 575.9 | 1294.0 | 125.3 | 3465.2 | 1497.9 | 208.1 | 5003.9 | 2426.7 | 2426.7 | 23829.2 | 25940.3 | 1390.9 | 376.3 | 748.1 | 24782.9 | 179.1 | 84891.4 | 14382.9 | 243062.0 | 94270.5 |
| B51 | 482.6 | 445.3 | 2.0 | 1035.8 | 379.6 | 38.6 | 1169.3 | 688.4 | 688.4 | 6023.1 | 6745.9 | 381.5 | 78.0 | 779.6 | 6810.1 | 45.6 | 22712.2 | 4251.0 | 68946.9 | 25793.9 |
| B52 | 2750.8 | 1223.3 | 2.9 | 1465.1 | 868.4 | 182.3 | 2239.1 | 1305.5 | 1305.5 | 11620.5 | 13409.0 | 596.4 | 110.2 | 382.1 | 16215.4 | 96.4 | 48923.2 | 7088.7 | 130719.5 | 53772.8 |
| B53 | 565.7 | 1785.7 | 297.1 | 3013.5 | 1412.1 | 191.7 | 4828.2 | 6726.5 | 6726.5 | 69010.5 | 74435.8 | 1284.4 | 525.6 | 915.8 | 71800.8 | 431.4 | 225440.2 | 23339.3 | 673225.8 | 243951.3 |
| B54 | 764.4 | 1533.6 | 217.5 | 4918.8 | 1680.0 | 291.1 | 6769.5 | 4795.6 | 4795.6 | 44389.0 | 52770.2 | 2120.4 | 487.3 | 1530.1 | 59308.3 | 419.7 | 170453.9 | 23106.9 | 480165.4 | 186791.2 |
| B55 | 324.3 | 511.3 | 0.4 | 1122.9 | 561.6 | 123.5 | 1986.0 | 1506.3 | 1506.3 | 14325.1 | 15223.3 | 568.1 | 163.4 | 337.3 | 14727.3 | 106.9 | 48220.3 | 6859.9 | 150793.8 | 53094.2 |
| B56 | 1820.9 | 846.6 | 12.0 | 1036.6 | 619.1 | 106.4 | 1620.4 | 726.5 | 726.5 | 8060.6 | 8374.4 | 434.0 | 33.8 | 190.5 | 9968.8 | 43.1 | 31728.4 | 4512.2 | 72767.2 | 34620.2 |
| B57 | 908.0 | 4049.8 | 499.5 | 10922.3 | 4244.5 | 476.1 | 14854.6 | 10947.9 | 10947.9 | 103445. | 114865. | 5029.2 | 1171.7 | 3215.4 | 120969. | 744.1 | 370014.6 | 52130.9 | 1096164. | 407290.9 |
| B58 | 401.5 | 159.4 | 9.2 | 200.8 | 92.2 | 10.4 | 227.6 | 25.8 | 25.8 | 540.6 | 548.9 | 43.9 | 7.9 | 13.1 | 452.3 | 1.7 | 2531.1 | 457.6 | 2592.1 | 2761.0 |
| B59 | 495.1 | 5700.1 | 734.9 | 17152.1 | 4937.2 | 792.0 | 18440.4 | 21633.3 | 21633.3 | 203252. | 212055. | 9199.7 | 2559.0 | 4402.4 | 223500. | 1585.0 | 680595.1 | 85917.2 | 2165452. | 748071.9 |
| B60 | 710.0 | 8568.1 | 1166.8 | 21991.0 | 11786.2 | 1449.4 | 39455.6 | 27603.7 | 27603.7 | 284379. | 290718. | 10761.9 | 3163.2 | 5868.3 | 279622. | 2002.2 | 925446.0 | 130860.9 | 2763781. | 1016851. |
| B61 | 273.2 | 1811.4 | 172.3 | 4329.8 | 1965.3 | 255.9 | 6918.6 | 3835.3 | 3835.3 | 37974.0 | 40679.2 | 1767.3 | 484.2 | 1058.0 | 39521.1 | 274.5 | 131507.3 | 20566.7 | 384081.5 | 145155.4 |
| B62 | 95.6 | 507.1 | 1.1 | 1046.7 | 427.3 | 0.0 | 1533.2 | 1925.1 | 1925.1 | 18383.8 | 19863.4 | 394.5 | 124.6 | 309.3 | 18957.6 | 103.9 | 60387.3 | 6744.0 | 192654.5 | 65598.2 |
| B63 | 99.1 | 350.0 | 0.8 | 859.7 | 505.2 | 0.0 | 1735.6 | 1382.3 | 1382.3 | 13580.1 | 13938.9 | 504.4 | 168.0 | 317.4 | 14850.0 | 101.3 | 45413.4 | 6097.6 | 138375.8 | 49775.4 |
| B64 | 335.3 | 353.6 | 39.8 | 970.0 | 263.2 | 32.7 | 1023.2 | 558.8 | 558.8 | 5465.4 | 5966.2 | 297.3 | 64.7 | 197.9 | 5247.8 | 39.8 | 19361.6 | 3076.1 | 55962.0 | 21414.5 |
| Mean | 1215.8 | 1306.1 | 136.8 | 2650.6 | 1323.5 | 169.5 | 4049.9 | 2665.1 | 2665.1 | 27633.6 | 28714.1 | 1268.6 | 331.5 | 683.1 | 28816.1 | 193.1 | 94386.1 | 13486.1 | 266872.6 | 103822.3 |
| SD | 842.7 | 1422.6 | 205.3 | 3868.0 | 1798.9 | 227.5 | 6101.4 | 4912.6 | 4912.6 | 48751.3 | 50678.9 | 1960.8 | 574.8 | 1081.3 | 51297.1 | 360.3 | 161173.7 | 21777.7 | 491811.3 | 176960.4 |
| Median | 1053.5 | 860.0 | 56.7 | 1118.6 | 687.3 | 109.4 | 1677.9 | 673.5 | 673.5 | 7793.2 | 8075.2 | 504.8 | 107.2 | 242.2 | 8207.4 | 44.3 | 28197.7 | 4596.5 | 67489.0 | 30783.3 |

Table 15. Levels of OCPs in blubber biopsies from live free-ranging BND (ng $g^{-1}$ lw). No detectable values of α-HCH, δ-HCH, heptachlor, aldrin, dieldrin, and endosulfan derivates were found in any sample. SD (standard deviation).

| | HCB | β-HCH | Lindane | ∑HCH | o,p-DDE | p,p-DDE | o,p-DDD | p,p-DDD | o,p-DDT | p,p-DDT | ∑DDT | p,p-DDE/∑DDT | ∑DDT/∑PCB | Endrin | ∑Ciclodienes | cis-Chlordane | trans-Chlordane | ∑Chlordane | Dicofol | Methoxyc. | Mirex | ∑OCPs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 43.2 | 90.5 | 24.6 | 115.1 | 104.0 | 20060.6 | 209.2 | 269.8 | 271.0 | 1284.0 | 22198.6 | 0.9 | 0.8 | 8887.5 | 8887.5 | 717.8 | 483.8 | 1201.6 | 111.9 | 0.0 | 712.5 | 33270.4 |
| B2 | 193.8 | 128.5 | 63.8 | 192.3 | 24.8 | 2657.5 | 0.0 | 116.1 | 81.8 | 664.8 | 3545.0 | 0.7 | 0.4 | 4425.3 | 4425.3 | 373.5 | 126.2 | 499.7 | 95.5 | 0.0 | 80.5 | 9032.1 |
| B3 | 389.2 | 513.1 | 341.5 | 854.6 | 0.0 | 25362.8 | 445.4 | 625.1 | 492.6 | 3198.6 | 30124.5 | 0.8 | 1.3 | 17815.1 | 17815.1 | 1241.5 | 761.9 | 2003.4 | 471.8 | 0.0 | 570.1 | 52228.6 |
| B4 | 26.6 | 65.4 | 12.5 | 77.9 | 78.1 | 18444.7 | 201.2 | 378.4 | 224.3 | 1069.4 | 20396.0 | 0.9 | 1.0 | 4531.0 | 4531.0 | 361.6 | 194.4 | 556.0 | 75.8 | 0.0 | 462.2 | 26125.6 |
| B5 | 218.1 | 199.1 | 207.6 | 406.7 | 289.5 | 49827.7 | 418.6 | 1697.3 | 577.0 | 6020.6 | 58830.7 | 0.8 | 0.9 | 29619.8 | 29619.8 | 2044.5 | 1386.3 | 3430.8 | 196.8 | 0.0 | 1293.5 | 93996.5 |
| B6 | 240.2 | 288.2 | 156.4 | 444.6 | 204.7 | 46979.0 | 526.0 | 946.2 | 441.8 | 3923.4 | 53021.1 | 0.9 | 1.2 | 44135.2 | 44135.2 | 2904.5 | 1723.1 | 4627.6 | 299.1 | 0.0 | 1145.5 | 103913.3 |
| B7 | 0.0 | 103.1 | 48.3 | 151.4 | 167.8 | 49253.0 | 409.3 | 825.0 | 848.2 | 4336.3 | 55839.6 | 0.9 | 0.8 | 36738.6 | 36738.6 | 3228.7 | 1484.5 | 4713.2 | 148.9 | 0.0 | 1246.8 | 98838.4 |
| B8 | 8.4 | 145.9 | 78.9 | 224.8 | 12.4 | 2544.7 | 0.0 | 160.5 | 105.7 | 1015.1 | 3838.5 | 0.7 | 0.5 | 17278.4 | 17278.4 | 1325.3 | 690.9 | 2016.2 | 62.6 | 0.0 | 125.7 | 23554.6 |
| B9 | 0.0 | 44.4 | 0.0 | 44.4 | 4.7 | 3248.0 | 0.0 | 78.0 | 75.3 | 687.9 | 4093.9 | 0.8 | 0.5 | 11484.6 | 11484.6 | 747.2 | 214.1 | 961.3 | 27.7 | 0.0 | 308.1 | 16920.0 |
| B10 | 7.8 | 141.4 | 114.6 | 256.0 | 80.8 | 17698.4 | 161.1 | 368.4 | 389.7 | 4061.5 | 22759.9 | 0.8 | 0.7 | 29486.0 | 29486.0 | 2038.6 | 1462.8 | 3501.4 | 137.3 | 0.0 | 694.8 | 56843.1 |
| B11 | 18.2 | 214.4 | 125.3 | 339.7 | 84.6 | 36996.1 | 557.0 | 682.0 | 337.1 | 4173.8 | 42830.7 | 0.9 | 1.1 | 26092.8 | 26092.8 | 2420.8 | 1024.8 | 3445.6 | 176.2 | 0.0 | 1101.6 | 74004.8 |
| B12 | 0.0 | 59.6 | 16.0 | 75.6 | 22.1 | 4053.3 | 0.0 | 122.8 | 173.1 | 1377.0 | 5748.4 | 0.7 | 0.5 | 15564.9 | 15564.9 | 1104.4 | 259.6 | 1364.0 | 62.7 | 0.0 | 114.9 | 22930.3 |
| B13 | 4.5 | 79.1 | 36.8 | 115.9 | 46.1 | 12328.0 | 0.0 | 505.7 | 193.0 | 2251.5 | 15324.3 | 0.8 | 0.5 | 25706.7 | 25706.7 | 1977.9 | 510.6 | 2488.5 | 54.9 | 0.0 | 424.4 | 44119.3 |
| B14 | 10.5 | 36.3 | 0.0 | 36.3 | 134.0 | 23002.4 | 0.0 | 971.1 | 810.9 | 12345.0 | 37263.4 | 0.6 | 0.6 | 55643.4 | 55643.4 | 4618.9 | 1524.0 | 6142.9 | 230.4 | 0.0 | 1016.5 | 100343.5 |
| B15 | 32.5 | 255.7 | 179.8 | 435.6 | 91.6 | 66183.7 | 373.3 | 2041.1 | 1067.0 | 23617.0 | 93373.7 | 0.7 | 0.9 | 60699.0 | 60699.0 | 4771.0 | 2765.0 | 7536.0 | 219.2 | 0.0 | 2613.2 | 164909.2 |
| B16 | 5.8 | 148.6 | 104.2 | 252.8 | 44.3 | 3703.9 | 148.2 | 336.7 | 250.7 | 3186.6 | 7670.2 | 0.5 | 0.4 | 35881.3 | 35881.3 | 2031.4 | 552.3 | 2583.7 | 79.2 | 0.0 | 585.9 | 47058.9 |
| B17 | 2.9 | 145.8 | 58.5 | 204.2 | 71.7 | 9634.9 | 288.2 | 237.3 | 296.6 | 2830.4 | 13359.2 | 0.7 | 0.6 | 26001.8 | 26001.8 | 2005.8 | 1063.8 | 3069.6 | 111.8 | 0.0 | 269.9 | 43019.4 |
| B18 | 35.1 | 194.2 | 145.7 | 339.9 | 52.4 | 10969.3 | 0.0 | 153.5 | 303.8 | 2300.9 | 13779.8 | 0.8 | 0.7 | 31843.3 | 31843.3 | 2299.0 | 920.7 | 3219.7 | 142.5 | 0.0 | 325.0 | 49685.3 |
| B19 | 453.5 | 150.3 | 102.9 | 253.2 | 133.1 | 22142.8 | 263.5 | 1145.8 | 596.5 | 8269.1 | 32550.9 | 0.7 | 0.9 | 23598.1 | 23598.1 | 1825.8 | 1219.5 | 3045.3 | 154.3 | 0.0 | 937.4 | 60992.6 |
| B20 | 66.7 | 43.0 | 11.1 | 54.1 | 35.5 | 7760.1 | 84.6 | 323.3 | 173.8 | 2193.2 | 10570.6 | 0.7 | 0.7 | 10576.5 | 10576.5 | 772.3 | 451.2 | 1223.6 | 59.4 | 0.0 | 445.6 | 22996.5 |
| B21 | 20.3 | 29.1 | 8.0 | 37.1 | 95.6 | 14333.2 | 0.0 | 414.7 | 420.9 | 4512.1 | 19776.6 | 0.7 | 0.9 | 13485.9 | 13485.9 | 700.6 | 613.7 | 1314.3 | 137.9 | 0.0 | 439.5 | 35211.5 |
| B22 | 12.9 | 142.1 | 86.8 | 228.8 | 40.0 | 5651.0 | 113.5 | 164.8 | 173.8 | 1519.8 | 7662.9 | 0.7 | 0.6 | 15617.1 | 15617.1 | 1344.4 | 332.9 | 1677.3 | 93.0 | 0.0 | 241.1 | 25533.2 |
| B23 | 5.7 | 192.6 | 140.9 | 333.5 | 41.5 | 7106.5 | 206.4 | 167.3 | 315.4 | 2533.2 | 10370.4 | 0.7 | 0.5 | 31590.9 | 31590.9 | 2659.3 | 1097.6 | 3756.9 | 178.7 | 0.0 | 144.5 | 46380.4 |
| B24 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 170.7 | 1.9 | 0.4 | 0.3 | 15.1 | 188.6 | 0.9 | 0.8 | 47.4 | 47.4 | 1.2 | 2.9 | 4.1 | 1.1 | 1027.4 | 1.2 | 1269.7 |
| B25 | 52.5 | 146.1 | 92.8 | 238.9 | 32.8 | 2729.5 | 0.0 | 103.5 | 160.6 | 1436.4 | 4462.7 | 0.6 | 0.4 | 13428.2 | 13428.2 | 1155.3 | 275.8 | 1431.0 | 86.9 | 0.0 | 596.5 | 20296.8 |
| B26 | 35.7 | 335.4 | 213.8 | 549.2 | 32.8 | 871.8 | 87.5 | 196.1 | 157.0 | 1401.3 | 2746.4 | 0.3 | 0.3 | 23986.6 | 23986.6 | 2313.5 | 886.6 | 3200.1 | 189.0 | 0.0 | 20.4 | 30727.4 |
| B27 | 16.4 | 249.8 | 96.1 | 345.9 | 48.3 | 3311.2 | 0.0 | 192.1 | 232.2 | 1932.5 | 5716.3 | 0.6 | 0.4 | 35632.7 | 35632.7 | 2850.5 | 817.0 | 3667.5 | 220.3 | 0.0 | 90.0 | 45689.0 |
| B28 | 25.3 | 240.8 | 86.9 | 327.7 | 32.0 | 1874.1 | 140.1 | 102.7 | 173.3 | 1617.3 | 3939.5 | 0.5 | 0.4 | 28721.6 | 28721.6 | 2147.5 | 829.5 | 2977.0 | 128.0 | 0.0 | 71.7 | 36190.7 |
| B29 | 0.0 | 271.1 | 34.1 | 305.2 | 76.8 | 1496.6 | 0.0 | 382.5 | 237.4 | 2766.9 | 4960.3 | 0.3 | 0.3 | 43140.5 | 43140.5 | 4466.7 | 945.3 | 5412.0 | 488.9 | 0.0 | 26.0 | 54332.7 |
| B30 | 0.0 | 245.2 | 168.5 | 413.7 | 81.2 | 1290.4 | 0.0 | 366.3 | 365.8 | 3302.8 | 5406.6 | 0.2 | 0.3 | 40199.7 | 40199.7 | 2902.4 | 1297.7 | 4200.1 | 176.9 | 0.0 | 9.1 | 50406.1 |

(Continuation of table 15)

| | HCB | β-HCH | Lindane | ∑HCH | o,p-DDE | p,p-DDE | o,p-DDD | p,p-DDD | o,p-DDT | p,p-DDT | ∑DDT | p,p-DDE/∑DDT | ∑DDT/∑PCB | Endrin | ∑Ciclodienes | cis-Chlordane | trans-Chlordane | ∑Chlordane | Dicofol | Methoxyc. | Mirex | ∑OCPs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B31 | 1.6 | 111.1 | 23.9 | 135.0 | 7.8 | 342.0 | 0.0 | 59.1 | 0.0 | 609.9 | 1018.8 | 0.3 | 0.3 | 17322.2 | 17322.2 | 1299.5 | 420.2 | 1719.7 | 55.6 | 0.0 | 1.2 | 20254.1 |
| B32 | 37.4 | 465.9 | 254.6 | 720.5 | 73.6 | 5308.0 | 0.0 | 251.5 | 297.9 | 2556.3 | 8487.3 | 0.6 | 0.5 | 54457.8 | 54457.8 | 3867.3 | 1878.5 | 5745.8 | 245.2 | 0.0 | 195.4 | 69889.3 |
| B33 | 38.9 | 389.9 | 115.7 | 505.6 | 101.2 | 22342.4 | 0.0 | 307.3 | 339.7 | 1963.5 | 25054.0 | 0.9 | 1.1 | 32245.2 | 32245.2 | 2772.0 | 1035.9 | 3807.9 | 177.7 | 0.0 | 445.5 | 62274.7 |
| B34 | 26.5 | 289.2 | 83.6 | 372.8 | 47.7 | 9373.9 | 0.0 | 281.1 | 309.6 | 3358.0 | 13370.3 | 0.7 | 0.5 | 48053.6 | 48053.6 | 3553.9 | 1145.1 | 4699.1 | 244.7 | 0.0 | 350.5 | 67117.5 |
| B35 | 322.6 | 384.6 | 221.2 | 605.9 | 112.9 | 7617.6 | 0.0 | 199.4 | 201.4 | 2953.1 | 11084.4 | 0.7 | 0.5 | 39780.5 | 39780.5 | 3173.7 | 1950.9 | 5124.6 | 261.7 | 0.0 | 185.5 | 57365.1 |
| B36 | 26.9 | 125.6 | 69.2 | 194.7 | 63.9 | 7423.2 | 146.4 | 278.4 | 316.4 | 3405.2 | 11633.5 | 0.6 | 0.5 | 29632.8 | 29632.8 | 2269.6 | 871.1 | 3140.8 | 201.3 | 0.0 | 277.0 | 45107.1 |
| B37 | 452.4 | 69.3 | 38.8 | 108.0 | 591.9 | 222702.7 | 1006.4 | 4514.8 | 2056.6 | 29283.7 | 260155.9 | 0.9 | 1.0 | 10666.2 | 10666.2 | 1048.8 | 3261.7 | 4310.5 | 168.6 | 0.0 | 9584.0 | 285445.7 |
| B38 | 674.3 | 47.0 | 32.5 | 79.5 | 678.0 | 252703.0 | 772.1 | 2411.9 | 1593.1 | 8697.2 | 266855.3 | 0.9 | 0.8 | 9388.0 | 9388.0 | 909.9 | 1250.8 | 2160.7 | 70.6 | 0.0 | 8497.3 | 287725.6 |
| B39 | 698.5 | 25.5 | 14.3 | 39.7 | 906.7 | 83259.4 | 646.1 | 2010.0 | 1171.6 | 5043.7 | 93037.5 | 0.9 | 0.9 | 7101.8 | 7101.8 | 963.8 | 2122.4 | 3086.2 | 62.6 | 0.0 | 1518.6 | 105545.0 |
| B40 | 1171.8 | 23.8 | 8.2 | 32.0 | 620.3 | 499767.6 | 1442.6 | 9421.3 | 3681.8 | 68020.1 | 582953.6 | 0.9 | 1.2 | 8657.0 | 8657.0 | 916.5 | 3573.2 | 4489.7 | 294.5 | 0.0 | 14296.9 | 611895.5 |
| B41 | 605.4 | 52.1 | 17.6 | 69.8 | 256.3 | 294415.0 | 1026.2 | 6296.0 | 2259.7 | 33156.2 | 337409.3 | 0.9 | 1.1 | 16103.3 | 16103.3 | 1222.8 | 1940.1 | 3162.9 | 234.5 | 0.0 | 9431.8 | 367017.0 |
| B42 | 162.8 | 69.9 | 25.2 | 95.1 | 488.9 | 228232.5 | 619.0 | 2976.5 | 1768.6 | 15720.9 | 249806.4 | 0.9 | 1.1 | 15754.2 | 15754.2 | 1468.4 | 1918.2 | 3386.6 | 145.6 | 0.0 | 8003.8 | 277354.6 |
| B43 | 705.1 | 171.9 | 31.7 | 203.6 | 1011.4 | 135329.1 | 852.4 | 3021.2 | 2116.7 | 9856.4 | 152187.2 | 0.9 | 1.0 | 35257.8 | 35257.8 | 3514.0 | 2077.3 | 5591.4 | 244.7 | 0.0 | 3487.9 | 197677.6 |
| B44 | 402.5 | 106.9 | 54.7 | 161.5 | 661.5 | 184016.5 | 865.8 | 4248.2 | 1741.2 | 17545.4 | 209078.6 | 0.9 | 1.0 | 13351.0 | 13351.0 | 1212.7 | 1776.9 | 2989.6 | 248.3 | 0.0 | 7063.3 | 233294.7 |
| B45 | 1077.7 | 75.8 | 0.0 | 75.8 | 647.7 | 153598.7 | 744.1 | 4019.2 | 1882.3 | 23463.6 | 184355.7 | 0.8 | 1.0 | 9492.1 | 9492.1 | 739.3 | 1596.8 | 2336.1 | 114.8 | 0.0 | 5959.9 | 203412.0 |
| B46 | 269.0 | 94.9 | 98.4 | 193.3 | 239.9 | 12672.7 | 162.1 | 585.3 | 594.7 | 5490.5 | 19745.3 | 0.6 | 0.7 | 25503.3 | 25503.3 | 1843.5 | 711.4 | 2554.9 | 208.0 | 0.0 | 779.6 | 49253.4 |
| B47 | 925.2 | 82.4 | 4.2 | 86.6 | 748.5 | 119782.8 | 706.3 | 2508.4 | 1366.2 | 8439.5 | 133551.8 | 0.9 | 1.1 | 5394.2 | 5394.2 | 449.6 | 1221.0 | 1670.6 | 48.0 | 0.0 | 3904.2 | 145580.6 |
| B48 | 712.1 | 34.0 | 2.7 | 36.7 | 684.4 | 66903.5 | 584.1 | 2659.3 | 1073.6 | 9066.6 | 80971.4 | 0.8 | 0.9 | 8317.5 | 8317.5 | 1044.7 | 2301.3 | 3346.0 | 89.3 | 0.0 | 2767.6 | 96240.5 |
| B49 | 1118.0 | 107.5 | 69.0 | 176.5 | 656.9 | 56365.1 | 568.8 | 2807.8 | 1003.1 | 11104.1 | 72505.8 | 0.8 | 1.0 | 16170.4 | 16170.4 | 1594.8 | 2028.3 | 3623.2 | 137.7 | 0.0 | 2450.5 | 96182.0 |
| B50 | 916.0 | 40.2 | 15.3 | 55.5 | 520.1 | 84709.3 | 472.2 | 2312.9 | 903.2 | 9627.6 | 98545.3 | 0.9 | 1.0 | 6597.7 | 6597.7 | 695.0 | 1080.0 | 1774.9 | 71.6 | 0.0 | 3286.5 | 111247.5 |
| B51 | 279.1 | 63.3 | 36.9 | 100.2 | 105.5 | 19724.3 | 108.4 | 518.9 | 260.2 | 2030.8 | 22748.1 | 0.9 | 0.9 | 6814.0 | 6814.0 | 492.8 | 510.4 | 1003.2 | 69.0 | 0.0 | 849.6 | 31863.2 |
| B52 | 251.0 | 335.9 | 197.0 | 533.0 | 114.3 | 26246.4 | 0.0 | 548.7 | 497.0 | 4982.5 | 32388.8 | 0.8 | 0.6 | 26542.6 | 26542.6 | 2185.1 | 1170.3 | 3355.4 | 295.0 | 0.0 | 1224.5 | 64590.2 |
| B53 | 429.3 | 54.0 | 52.7 | 106.7 | 862.4 | 263542.9 | 731.0 | 1882.5 | 1822.9 | 6841.0 | 275682.7 | 1.0 | 1.1 | 8885.6 | 8885.6 | 779.4 | 1139.7 | 1919.1 | 132.4 | 0.0 | 7621.6 | 294777.4 |
| B54 | 679.2 | 91.7 | 51.8 | 143.5 | 848.7 | 150939.7 | 612.3 | 2854.0 | 1758.1 | 17695.8 | 174708.6 | 0.9 | 0.9 | 8948.3 | 8948.3 | 739.2 | 1210.3 | 1949.5 | 192.7 | 0.0 | 5955.8 | 192577.6 |
| B55 | 179.7 | 0.0 | 8.0 | 8.0 | 100.0 | 44020.2 | 161.2 | 600.8 | 373.9 | 2378.8 | 47634.9 | 0.9 | 0.9 | 4724.3 | 4724.3 | 283.4 | 503.5 | 786.9 | 45.4 | 0.0 | 1392.4 | 54771.5 |
| B56 | 17.8 | 218.8 | 0.0 | 218.8 | 144.1 | 17240.6 | 0.0 | 423.9 | 470.5 | 5138.6 | 23417.7 | 0.7 | 0.7 | 27063.9 | 27063.9 | 2003.8 | 863.9 | 2867.7 | 327.7 | 0.0 | 663.0 | 54576.6 |
| B57 | 3063.9 | 95.3 | 46.5 | 141.8 | 0.0 | 308409.2 | 1082.7 | 7807.4 | 3280.9 | 54562.5 | 375142.7 | 0.8 | 0.9 | 14212.3 | 14212.3 | 1389.0 | 3896.1 | 5285.1 | 194.8 | 0.0 | 7813.8 | 405854.4 |
| B58 | 16.6 | 52.9 | 9.4 | 62.4 | 14.8 | 533.4 | 0.0 | 14.0 | 75.3 | 744.6 | 1382.0 | 0.4 | 0.5 | 4213.9 | 4213.9 | 308.7 | 0.0 | 308.7 | 73.1 | 0.0 | 20.3 | 6077.0 |
| B59 | 949.7 | 97.0 | 10.1 | 107.1 | 851.3 | 714168.9 | 1486.9 | 9077.9 | 3990.8 | 54255.6 | 783831.3 | 0.9 | 1.0 | 8978.3 | 8978.3 | 2028.2 | 7058.8 | 9087.0 | 180.7 | 0.0 | 25972.1 | 829106.2 |
| B60 | 2087.6 | 62.8 | 48.1 | 110.9 | 2287.6 | 1142163.9 | 2667.1 | 14048.0 | 7281.3 | 83762.3 | 1252210.1 | 0.9 | 1.2 | 14340.5 | 14340.5 | 1558.7 | 8129.9 | 9688.5 | 261.3 | 0.0 | 29541.5 | 1308240.3 |
| B61 | 801.0 | 18.4 | 8.9 | 27.3 | 526.9 | 135514.3 | 681.7 | 2656.1 | 1199.5 | 9664.6 | 150243.0 | 0.9 | 1.0 | 3463.9 | 3463.9 | 332.5 | 899.3 | 1231.8 | 45.9 | 0.0 | 4842.0 | 160654.8 |
| B62 | 41.0 | 7.6 | 0.0 | 7.6 | 299.2 | 71839.0 | 269.0 | 752.6 | 558.4 | 1920.8 | 75639.1 | 0.9 | 1.2 | 2015.2 | 2015.2 | 141.7 | 572.0 | 713.7 | 15.8 | 0.0 | 1988.4 | 80420.7 |
| B63 | 17.1 | 13.0 | 7.4 | 20.4 | 102.4 | 40418.2 | 145.9 | 661.0 | 296.6 | 3215.4 | 44839.5 | 0.9 | 0.9 | 1344.6 | 1344.6 | 90.2 | 202.8 | 293.0 | 9.6 | 0.0 | 1656.3 | 48180.4 |
| B64 | 180.8 | 31.2 | 16.4 | 47.7 | 92.2 | 17313.5 | 95.0 | 370.2 | 177.7 | 1240.7 | 19289.4 | 0.9 | 0.9 | 3828.0 | 3828.0 | 293.8 | 305.3 | 599.1 | 21.7 | 0.0 | 488.6 | 24455.3 |
| Mean | 332.3 | 137.6 | 69.2 | 206.9 | 275.5 | 91728.5 | 363.9 | 1679.1 | 885.4 | 9806.9 | 104739.3 | 0.8 | 0.8 | 20413.8 | 20413.8 | 1664.7 | 1352.2 | 3016.9 | 153.3 | 16.1 | 2947.4 | 131825.7 |
| SD | 533.9 | 116.5 | 73.5 | 183.9 | 384.3 | 183585.7 | 474.5 | 2620.7 | 1196.2 | 16248.4 | 202926.4 | 0.2 | 0.3 | 14736.6 | 14736.6 | 1160.1 | 1399.2 | 1988.5 | 101.1 | 128.4 | 5434.6 | 207020.2 |
| Median | 47.8 | 100.1 | 47.3 | 147.4 | 100.6 | 21101.7 | 161.6 | 533.8 | 381.8 | 3381.6 | 24235.9 | 0.8 | 0.9 | 15928.8 | 15928.8 | 1366.7 | 1049.9 | 3017.4 | 140.2 | 0.0 | 746.1 | 57104.1 |

Table 16. Levels of PAHs in blubber biopsies from live free-ranging BND (ng $g^{-1}$ lw). Acenaphthylene was not detected in any sample. TEQ-PAHs in pg $g^{-1}$ lw. SD (standard deviation).

| | Naphtalene | Acenaphtene | Fluorene | Phenanthrene | Anthracene | Fluoranthene | Pyrene | Chrysene | Benzo (a) anthracene | Benzo (b) fluoranthene | Benzo (k) fluoranthene | Benzo (a) pyrene | Indeno (1,2,3,c-d) pyrene | Dibenz (a,h) anthracene | Benzo (g,h,i) perylene | TEQ-PAHs | ∑PAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | 411.5 | 242.1 | 232.3 | 6818.9 | 2173.6 | 1122.6 | 2538.6 | 944.6 | 295.8 | 334.9 | 52.5 | 359.7 | 0.0 | 0.0 | 0.0 | 435.1 | 15527.3 |
| B2 | 1341.0 | 1928.8 | 4070.3 | 16098.1 | 5336.0 | 950.5 | 1551.0 | 526.0 | 0.0 | 229.3 | 46.3 | 343.1 | 103.5 | 0.0 | 224.7 | 439.8 | 32748.6 |
| B3 | 10118.2 | 0.0 | 3932.0 | 16119.1 | 0.0 | 2215.1 | 3564.7 | 2181.6 | 727.9 | 609.4 | 0.0 | 907.6 | 153.4 | 84.3 | 350.6 | 1137.3 | 40963.9 |
| B4 | 686.2 | 0.0 | 330.1 | 3429.8 | 0.0 | 737.5 | 2353.2 | 746.3 | 187.9 | 347.0 | 59.2 | 429.5 | 37.8 | 0.0 | 0.0 | 470.4 | 9344.5 |
| B5 | 1634.2 | 346.2 | 350.9 | 8248.2 | 0.0 | 2062.4 | 5455.4 | 1458.2 | 394.6 | 621.4 | 124.4 | 871.9 | 0.0 | 0.0 | 0.0 | 949.5 | 21567.8 |
| B6 | 5065.7 | 501.7 | 666.9 | 12674.7 | 0.0 | 2888.6 | 7312.3 | 1967.4 | 409.1 | 811.8 | 140.9 | 981.0 | 0.0 | 0.0 | 0.0 | 1074.6 | 33419.9 |
| B7 | 2606.1 | 304.8 | 170.3 | 1942.8 | 0.0 | 2102.6 | 4673.6 | 2219.2 | 0.0 | 1019.2 | 188.4 | 1146.7 | 0.0 | 0.0 | 0.0 | 1189.9 | 16373.9 |
| B8 | 1355.0 | 362.1 | 427.4 | 4050.0 | 0.0 | 876.0 | 1889.5 | 729.0 | 166.6 | 311.2 | 59.1 | 369.6 | 0.0 | 0.0 | 0.0 | 404.5 | 10595.7 |
| B9 | 1445.7 | 0.0 | 130.9 | 1457.5 | 0.0 | 645.6 | 1742.7 | 588.2 | 0.0 | 240.6 | 45.9 | 285.2 | 0.0 | 0.0 | 0.0 | 297.9 | 6582.4 |
| B10 | 1355.3 | 0.0 | 262.9 | 5326.4 | 0.0 | 1252.9 | 3612.0 | 1775.9 | 0.0 | 717.1 | 100.7 | 931.4 | 57.2 | 0.0 | 0.0 | 973.5 | 15391.9 |
| B11 | 2593.4 | 0.0 | 311.5 | 7752.1 | 0.0 | 1891.2 | 5146.6 | 1286.9 | 0.0 | 509.7 | 79.2 | 574.1 | 0.0 | 0.0 | 0.0 | 607.9 | 20144.6 |
| B12 | 2226.9 | 0.0 | 0.0 | 1798.5 | 0.0 | 905.4 | 2345.8 | 1180.2 | 0.0 | 493.0 | 68.9 | 623.9 | 0.0 | 0.0 | 0.0 | 646.3 | 9642.5 |
| B13 | 1221.9 | 286.2 | 297.0 | 3865.1 | 0.0 | 994.2 | 2778.0 | 994.4 | 213.4 | 417.7 | 69.2 | 533.0 | 0.0 | 0.0 | 0.0 | 577.1 | 11670.1 |
| B14 | 2279.2 | 0.0 | 0.0 | 5179.8 | 0.0 | 2245.2 | 6301.3 | 4400.2 | 0.0 | 1941.4 | 320.3 | 2488.0 | 182.4 | 153.2 | 0.0 | 2739.8 | 25491.0 |
| B15 | 2202.9 | 346.6 | 197.2 | 7403.2 | 0.0 | 2820.9 | 7189.6 | 3505.8 | 788.2 | 1470.4 | 273.2 | 1815.8 | 129.4 | 0.0 | 0.0 | 1977.6 | 28143.2 |
| B16 | 1397.6 | 328.1 | 184.7 | 5701.4 | 0.0 | 850.7 | 4582.9 | 2049.0 | 0.0 | 821.1 | 144.6 | 1017.9 | 0.0 | 0.0 | 0.0 | 1059.3 | 17078.0 |
| B17 | 1363.8 | 0.0 | 120.3 | 3848.0 | 0.0 | 1367.0 | 3295.3 | 1682.8 | 0.0 | 654.4 | 114.3 | 844.2 | 0.0 | 0.0 | 0.0 | 877.3 | 13289.9 |
| B18 | 1120.5 | 410.4 | 343.7 | 6917.7 | 0.0 | 1534.9 | 4154.0 | 1704.7 | 266.4 | 612.2 | 112.8 | 769.3 | 58.2 | 0.0 | 0.0 | 839.0 | 18004.8 |
| B19 | 3260.5 | 606.0 | 808.6 | 6932.5 | 0.0 | 1427.4 | 3498.6 | 1668.8 | 314.4 | 586.0 | 102.7 | 751.8 | 0.0 | 0.0 | 0.0 | 819.5 | 19957.3 |
| B20 | 469.3 | 190.4 | 190.1 | 2265.8 | 0.0 | 632.8 | 1601.6 | 631.5 | 0.0 | 219.9 | 42.1 | 264.7 | 0.0 | 0.0 | 0.0 | 278.4 | 6508.1 |
| B21 | 822.0 | 528.8 | 399.6 | 3672.1 | 0.0 | 1235.7 | 4027.6 | 1284.4 | 0.0 | 640.5 | 114.1 | 742.9 | 0.0 | 0.0 | 0.0 | 772.6 | 13467.6 |
| B22 | 1343.4 | 269.9 | 224.8 | 3388.4 | 0.0 | 1170.1 | 2875.3 | 823.7 | 220.0 | 316.8 | 49.0 | 378.7 | 27.5 | 0.0 | 0.0 | 423.0 | 11087.7 |
| B23 | 3741.0 | 275.8 | 351.1 | 7156.3 | 0.0 | 2024.4 | 5813.3 | 1987.0 | 389.5 | 807.3 | 141.7 | 968.1 | 0.0 | 0.0 | 0.0 | 1051.8 | 23655.5 |
| B24 | 552.6 | 0.0 | 205.0 | 416.2 | 587.3 | 33.3 | 61.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.6 | 1856.2 |
| B25 | 1913.7 | 430.2 | 895.1 | 6493.5 | 0.0 | 1082.1 | 3828.1 | 948.0 | 0.0 | 392.7 | 55.1 | 422.4 | 0.0 | 0.0 | 0.0 | 448.7 | 16460.9 |
| B26 | 6411.0 | 0.0 | 360.4 | 7368.7 | 0.0 | 1596.0 | 3720.3 | 1284.0 | 0.0 | 502.3 | 99.0 | 599.8 | 0.0 | 0.0 | 0.0 | 631.7 | 21941.6 |
| B27 | 7253.2 | 623.4 | 401.3 | 6499.1 | 0.0 | 1664.5 | 5417.4 | 1729.1 | 375.2 | 711.3 | 120.4 | 804.7 | 0.0 | 0.0 | 0.0 | 881.8 | 25599.6 |
| B28 | 1800.2 | 281.7 | 346.8 | 4322.4 | 0.0 | 1259.8 | 3141.8 | 1198.0 | 243.9 | 475.8 | 72.6 | 585.9 | 0.0 | 0.0 | 0.0 | 636.9 | 13728.9 |
| B29 | 1409.5 | 321.6 | 250.4 | 8903.8 | 0.0 | 2634.9 | 5618.9 | 3333.4 | 936.0 | 1414.3 | 213.3 | 1398.5 | 0.0 | 0.0 | 0.0 | 1559.1 | 26434.6 |
| B30 | 739.4 | 268.1 | 110.0 | 3916.0 | 0.0 | 1922.7 | 5582.8 | 2514.1 | 509.8 | 1032.5 | 164.9 | 1197.2 | 0.0 | 0.0 | 0.0 | 1296.8 | 17957.5 |

(Continuation of table 16)

| | Naphtalene | Acenaphtene | Fluorene | Phenanthrene | Anthracene | Fluoranthene | Pyrene | Chrysene | Benzo (a) anthracene | Benzo (b) fluoranthene | Benzo (k) fluoranthene | Benzo (a) pyrene | Indeno (1,2,3,c-d) pyrene | Dibenz (a,h) anthracene | Benzo (g,h,i) perylene | TEQ-PAHs | ∑PAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B31 | 625.6 | 104.3 | 59.3 | 1670.1 | 0.0 | 766.6 | 2085.2 | 569.8 | 0.0 | 333.5 | 71.8 | 293.2 | 0.0 | 0.0 | 0.0 | 307.5 | 6579.4 |
| B32 | 7471.6 | 2748.9 | 931.8 | 10002.5 | 0.0 | 2579.0 | 5221.9 | 2038.0 | 515.6 | 1000.6 | 155.6 | 1228.9 | 60.6 | 0.0 | 0.0 | 1337.2 | 33955.1 |
| B33 | 1890.8 | 441.8 | 639.0 | 8237.5 | 0.0 | 1915.8 | 5587.3 | 1533.6 | 360.7 | 681.3 | 112.2 | 658.1 | 0.0 | 0.0 | 0.0 | 733.8 | 22057.9 |
| B34 | 2008.5 | 1575.9 | 499.9 | 7918.1 | 0.0 | 2113.8 | 4724.8 | 2148.7 | 516.7 | 1022.0 | 195.4 | 1233.7 | 0.0 | 0.0 | 0.0 | 1334.3 | 23957.5 |
| B35 | 733.1 | 1911.7 | 2829.2 | 16399.0 | 0.0 | 4484.7 | 11718.9 | 2629.3 | 0.0 | 829.2 | 147.5 | 894.2 | 0.0 | 0.0 | 0.0 | 965.7 | 42576.8 |
| B36 | 0.0 | 298.6 | 190.8 | 4733.6 | 0.0 | 1836.5 | 4542.6 | 2070.8 | 427.2 | 933.8 | 170.6 | 1109.3 | 0.0 | 0.0 | 0.0 | 1195.0 | 16313.8 |
| B37 | 186.8 | 420.7 | 132.6 | 1952.5 | 0.0 | 533.2 | 1333.6 | 357.2 | 89.4 | 191.3 | 36.0 | 197.7 | 0.0 | 0.0 | 0.0 | 216.5 | 5431.0 |
| B38 | 181.8 | 873.4 | 132.6 | 1310.4 | 0.0 | 277.1 | 716.9 | 259.4 | 65.2 | 118.1 | 24.0 | 140.7 | 0.0 | 0.0 | 0.0 | 153.6 | 4099.5 |
| B39 | 199.8 | 593.1 | 231.1 | 1652.4 | 0.0 | 228.3 | 501.8 | 331.5 | 0.0 | 181.3 | 31.5 | 238.0 | 18.7 | 0.0 | 0.0 | 248.0 | 4207.5 |
| B40 | 234.5 | 361.0 | 197.7 | 1874.2 | 0.0 | 375.2 | 1011.5 | 394.7 | 0.0 | 216.0 | 32.0 | 235.0 | 0.0 | 0.0 | 0.0 | 244.9 | 4931.8 |
| B41 | 0.0 | 329.1 | 303.6 | 4235.3 | 388.6 | 1017.7 | 2457.6 | 825.7 | 0.0 | 540.7 | 87.4 | 695.0 | 45.9 | 0.0 | 0.0 | 726.0 | 10926.5 |
| B42 | 323.3 | 489.1 | 85.0 | 4161.8 | 0.0 | 1181.2 | 2862.8 | 1222.2 | 291.1 | 739.7 | 146.8 | 1352.0 | 85.0 | 0.0 | 0.0 | 1419.0 | 12939.9 |
| B43 | 967.4 | 3574.3 | 578.3 | 13201.6 | 938.4 | 2339.3 | 6760.5 | 2156.3 | 400.5 | 1200.9 | 177.5 | 1366.1 | 0.0 | 0.0 | 0.0 | 1473.8 | 33661.3 |
| B44 | 0.0 | 193.7 | 89.9 | 4728.4 | 324.9 | 1078.9 | 2400.7 | 981.9 | 0.0 | 589.3 | 104.7 | 562.7 | 69.2 | 0.0 | 0.0 | 597.9 | 11124.2 |
| B45 | 18635.1 | 1747.9 | 651.6 | 3869.8 | 0.0 | 794.2 | 2218.3 | 780.2 | 0.0 | 452.6 | 85.9 | 481.3 | 44.8 | 0.0 | 0.0 | 506.5 | 29761.5 |
| B46 | 1026.9 | 3055.9 | 409.7 | 8164.3 | 0.0 | 1996.3 | 6033.5 | 2172.4 | 0.0 | 1037.2 | 161.7 | 1153.4 | 0.0 | 0.0 | 0.0 | 1203.7 | 25211.2 |
| B47 | 562.1 | 888.8 | 97.4 | 1345.1 | 0.0 | 306.1 | 879.7 | 415.5 | 0.0 | 253.5 | 53.8 | 359.1 | 33.6 | 0.0 | 0.0 | 372.3 | 5194.7 |
| B48 | 263.6 | 255.2 | 145.5 | 2376.2 | 0.0 | 563.3 | 1078.0 | 542.1 | 0.0 | 262.8 | 48.6 | 262.5 | 0.0 | 0.0 | 0.0 | 275.2 | 5797.7 |
| B49 | 410.5 | 1067.5 | 224.2 | 5559.1 | 0.0 | 1450.4 | 4139.1 | 1317.7 | 0.0 | 861.9 | 154.7 | 832.4 | 117.6 | 0.0 | 0.0 | 878.8 | 16135.2 |
| B50 | 6571.7 | 1313.3 | 427.4 | 2558.6 | 0.0 | 517.2 | 1708.3 | 444.9 | 0.0 | 226.3 | 36.4 | 248.3 | 22.4 | 0.0 | 0.0 | 262.8 | 14074.9 |
| B51 | 1680.7 | 288.9 | 827.1 | 2844.1 | 3308.0 | 380.7 | 727.0 | 278.0 | 78.8 | 133.8 | 20.7 | 143.8 | 0.0 | 0.0 | 0.0 | 193.8 | 10711.8 |
| B52 | 1759.3 | 4675.7 | 442.8 | 14661.5 | 0.0 | 2622.8 | 6504.1 | 2780.8 | 557.7 | 1159.8 | 208.5 | 1127.2 | 0.0 | 0.0 | 0.0 | 1248.7 | 36500.2 |
| B53 | 150.2 | 244.8 | 217.8 | 3288.1 | 0.0 | 743.4 | 1352.7 | 919.8 | 178.8 | 374.7 | 67.1 | 362.2 | 25.5 | 0.0 | 0.0 | 401.9 | 7925.0 |
| B54 | 308.9 | 676.2 | 104.0 | 4630.4 | 0.0 | 932.8 | 2271.4 | 957.2 | 206.0 | 430.0 | 82.6 | 483.1 | 30.3 | 0.0 | 0.0 | 529.3 | 11113.0 |
| B55 | 371.2 | 652.5 | 95.6 | 1440.2 | 0.0 | 369.1 | 709.7 | 350.8 | 77.8 | 201.0 | 34.6 | 193.7 | 0.0 | 0.0 | 0.0 | 209.9 | 4496.3 |
| B56 | 885.2 | 724.1 | 1783.3 | 8259.8 | 0.0 | 2327.1 | 6338.3 | 2409.8 | 552.8 | 1152.3 | 174.9 | 1236.2 | 99.0 | 0.0 | 0.0 | 1357.5 | 25942.8 |
| B57 | 301.8 | 945.7 | 487.2 | 4092.3 | 0.0 | 672.7 | 1714.8 | 464.3 | 0.0 | 341.5 | 70.2 | 289.6 | 29.8 | 0.0 | 0.0 | 308.3 | 9409.8 |
| B58 | 523.8 | 1539.4 | 599.9 | 2462.6 | 0.0 | 414.8 | 1168.1 | 358.0 | 0.0 | 190.6 | 29.5 | 194.4 | 19.4 | 0.0 | 0.0 | 206.8 | 7500.7 |
| B59 | 275.7 | 468.6 | 303.2 | 2671.9 | 0.0 | 624.5 | 1798.8 | 549.2 | 0.0 | 234.3 | 42.3 | 401.8 | 26.5 | 0.0 | 0.0 | 418.1 | 7396.8 |
| B60 | 510.0 | 1351.9 | 628.1 | 3676.7 | 0.0 | 838.8 | 2433.8 | 889.9 | 0.0 | 458.7 | 68.3 | 640.6 | 39.8 | 0.0 | 0.0 | 666.4 | 11536.6 |
| B61 | 268.4 | 623.5 | 402.6 | 1307.8 | 0.0 | 282.9 | 978.2 | 194.7 | 0.0 | 106.6 | 22.5 | 182.7 | 14.3 | 0.0 | 0.0 | 190.3 | 4384.2 |
| B62 | 114.0 | 100.6 | 0.0 | 401.6 | 0.0 | 117.6 | 393.5 | 134.8 | 0.0 | 59.8 | 5.5 | 66.6 | 0.0 | 0.0 | 0.0 | 69.5 | 1393.9 |
| B63 | 113.1 | 75.9 | 347.2 | 466.9 | 526.0 | 89.5 | 222.4 | 70.0 | 0.0 | 34.0 | 7.4 | 49.1 | 0.0 | 0.0 | 0.0 | 56.6 | 2001.6 |
| B64 | 189.7 | 124.2 | 425.4 | 1687.2 | 2002.4 | 265.9 | 634.2 | 190.1 | 47.6 | 88.4 | 17.4 | 94.8 | 0.0 | 0.0 | 0.0 | 125.6 | 5767.3 |
| Mean | 1873.2 | 681.1 | 501.4 | 5278.2 | 243.5 | 1268.5 | 3295.4 | 1266.9 | 168.8 | 560.9 | 94.7 | 662.7 | 23.9 | 3.7 | 9.0 | 717.8 | 15932.1 |
| SD | 2942.9 | 896.7 | 759.9 | 3942.3 | 855.5 | 869.7 | 2260.1 | 924.7 | 231.0 | 393.7 | 67.2 | 471.6 | 41.2 | 21.7 | 51.7 | 513.1 | 10232.8 |
| Median | 997.2 | 353.8 | 307.5 | 4127.0 | 0.0 | 1080.5 | 2820.4 | 988.2 | 0.0 | 484.4 | 75.9 | 580.0 | 0.0 | 0.0 | 0.0 | 619.8 | 13598.3 |

Table 17. Levels of heavy metals and toxic trace elements in blubber samples of stranded BND ($\mu g\ g^{-1}$ dw). SD (standard deviation).

| BLUBBER | Hg | Cu | Mn | Fe | Pb | Zn | Cd | Ni | Cr | As | Al | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET78 | 30.4 | 1.4 | 0.1 | 6.4 | 0.5 | 45.9 | 0.5 | 1.2 | 0.6 | 0.7 | 3.4 | 3.3 |
| CET93 | 79.9 | 31.8 | 0.0 | 36.5 | 0.1 | 73.1 | 0.0 | 15.8 | 1.3 | 1.2 | 16.7 | 11.9 |
| CET94 | 48.2 | 52.8 | 0.1 | 110.1 | 0.0 | 47.5 | 0.0 | 23.9 | 1.2 | 0.6 | 13.7 | 4.9 |
| CET99 | 36.1 | 15.4 | 0.1 | 73.1 | 0.2 | 27.3 | 0.1 | 3.8 | 0.4 | 0.3 | 15.4 | 5.1 |
| CET171 | 31.7 | 2.7 | 0.0 | 88.0 | 0.0 | 26.0 | 0.0 | 1.6 | 1.1 | 0.6 | 15.1 | 7.4 |
| CET203 | 41.9 | 2.8 | 0.1 | 279.2 | 0.0 | 67.3 | 0.0 | 0.4 | 0.8 | 0.6 | 9.4 | 5.8 |
| CET209 | 52.2 | 0.6 | 0.1 | 10.2 | 0.1 | 6.4 | 0.0 | 8.0 | 2.0 | 0.7 | 5.0 | 7.2 |
| CET231 | 69.8 | 23.5 | 0.0 | 140.0 | 0.0 | 41.0 | 0.0 | 14.7 | 0.4 | 1.6 | 15.8 | 11.1 |
| CET286 | 32.8 | 6.5 | 0.0 | 87.8 | 0.0 | 36.0 | 0.0 | 5.9 | 2.5 | 1.3 | 4.0 | 4.3 |
| CET296 | 51.1 | 1.4 | 0.3 | 16.7 | 0.0 | 12.4 | 0.0 | 0.4 | 0.1 | 1.0 | 3.7 | 6.0 |
| CET305 | 35.9 | 1.1 | 0.2 | 8.8 | 0.0 | 5.2 | 0.0 | 0.6 | 0.1 | 0.7 | 6.2 | 4.8 |
| CET311 | 65.4 | 6.7 | 0.1 | 88.9 | 0.0 | 28.5 | 0.0 | 7.2 | 0.8 | 0.7 | 6.2 | 8.6 |
| CET314 | 113.7 | 4.9 | 0.2 | 58.4 | 0.0 | 13.5 | 0.1 | 1.6 | 1.2 | 1.4 | 1.7 | 14.5 |
| CET403 | 80.3 | 1.3 | 3.0 | 125.4 | 0.1 | 41.6 | 0.1 | 2.9 | 2.2 | 0.7 | 63.7 | 8.9 |
| CET407 | 79.5 | 12.6 | 1.3 | 102.6 | 31.4 | 33.7 | 0.1 | 9.7 | 1.8 | 1.0 | 243.6 | 11.7 |
| CET420 | 90.2 | 1.2 | 0.3 | 10.5 | 0.1 | 13.5 | 0.1 | 0.9 | 0.0 | 1.9 | 0.1 | 6.7 |
| CET450 | 105.2 | 0.6 | 0.2 | 18.0 | 0.6 | 5.7 | 0.1 | 0.7 | 0.1 | 1.4 | 6.1 | 13.9 |
| CET458 | 113.6 | 3.8 | 0.2 | 47.8 | 1.2 | 5.4 | 0.1 | 1.2 | 0.3 | 1.1 | 4.5 | 7.2 |
| CET505 | 121.5 | 0.9 | 0.9 | 90.6 | 56.9 | 48.9 | 0.1 | 0.2 | 0.9 | 0.8 | 62.6 | 7.3 |
| CET509 | 73.2 | 0.8 | 0.1 | 10.9 | 0.1 | 8.2 | 0.0 | 0.4 | 0.0 | 1.0 | 20.5 | 2.1 |
| CET526 | 106.6 | 0.6 | 0.3 | 7.6 | 0.1 | 10.0 | 0.1 | 0.3 | 0.1 | 0.7 | 3.3 | 3.4 |
| CET543 | 80.8 | 0.7 | 0.2 | 57.2 | 0.1 | 9.2 | 0.1 | 0.4 | 1.3 | 1.5 | 27.0 | 4.5 |
| CET562 | 137.2 | 7.2 | 0.0 | 128.5 | 0.3 | 60.6 | 0.0 | 0.3 | 1.3 | 1.0 | 11.3 | 21.3 |
| CET564 | 141.7 | 6.5 | 0.0 | 41.1 | 0.0 | 16.0 | 0.0 | 5.1 | 0.9 | 0.7 | 16.2 | 16.1 |
| CET584 | 130.8 | 0.6 | 0.0 | 18.3 | 0.0 | 25.5 | 0.1 | 0.6 | 0.3 | 0.2 | 14.6 | 19.7 |
| CET592 | 93.1 | 5.8 | 0.2 | 28.2 | 0.1 | 4.5 | 0.1 | 2.0 | 0.1 | 0.1 | 3.3 | 6.6 |
| CET595 | 123.4 | 0.7 | 0.3 | 8.4 | 0.1 | 45.5 | 0.1 | 0.6 | 0.4 | 0.6 | 5.8 | 9.1 |
| CET635 | 125.6 | 24.5 | 12.2 | 101.0 | 0.1 | 9.1 | 0.1 | 17.5 | 1.5 | 1.7 | 96.8 | 13.7 |
| CET640 | 101.4 | 4.6 | 0.0 | 35.6 | 0.1 | 7.7 | 0.1 | 0.6 | 0.2 | 1.4 | 28.2 | 10.0 |
| CET662 | 104.1 | 2.0 | 0.2 | 18.6 | 0.1 | 28.1 | 0.1 | 1.5 | 0.2 | 1.5 | 5.2 | 14.8 |
| Mean | 83.2 | 7.5 | 0.7 | 61.8 | 3.1 | 26.8 | 0.1 | 4.3 | 0.8 | 1.0 | 24.3 | 9.1 |
| SD | 34.9 | 11.7 | 2.3 | 59.0 | 11.7 | 20.1 | 0.1 | 6.1 | 0.7 | 0.5 | 46.6 | 4.9 |
| Median | 80.5 | 2.7 | 0.2 | 44.4 | 0.1 | 25.7 | 0.1 | 1.4 | 0.7 | 0.9 | 10.3 | 7.3 |

Table 18. Levels of heavy metals and toxic trace elements in liver samples of stranded BND ($\mu g\ g^{-1}$ dw). SD (standard deviation).

| LIVER | Hg | Cu | Mn | Fe | Pb | Zn | Cd | Ni | Cr | As | Al | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CET43 | 87.1 | 38.8 | 15.9 | 1615.5 | 0.3 | 171.7 | 1.5 | 27.1 | 7.8 | 2.5 | 6.1 | 14.4 |
| CET78 | 429.6 | 19.2 | 9.9 | 465.3 | 0.1 | 165.8 | 1.5 | 2.8 | 1.6 | 0.7 | 6.3 | 157.1 |
| CET93 | 7.4 | 23.0 | 13.2 | 2389.8 | 0.1 | 120.2 | 2.3 | 0.3 | 1.2 | 2.7 | 5.8 | 35.9 |
| CET94 | 100.9 | 22.4 | 10.9 | 454.5 | 0.0 | 139.9 | 4.4 | 0.1 | 0.7 | 2.8 | 1.1 | 28.3 |
| CET99 | 84.2 | 32.8 | 5.4 | 3409.7 | 0.1 | 135.4 | 12.3 | 1.2 | 0.8 | 0.1 | 0.8 | 18.3 |
| CET171 | 156.0 | 30.7 | 12.6 | 601.3 | 0.0 | 119.0 | 4.1 | 0.7 | 2.2 | 1.8 | 4.8 | 42.9 |
| CET203 | 419.7 | 73.1 | 16.2 | 707.1 | 0.1 | 217.9 | 8.2 | 0.2 | 0.6 | 1.2 | 6.9 | 304.8 |
| CET209 | 181.1 | 41.0 | 6.2 | 874.3 | 0.0 | 312.9 | 3.1 | 0.2 | 0.8 | 0.5 | 2.5 | 51.0 |
| CET286 | 200.6 | 22.9 | 5.1 | 843.2 | 0.1 | 179.0 | 3.9 | 0.2 | 0.6 | 0.6 | 3.7 | 68.6 |
| CET296 | 298.5 | 16.2 | 10.6 | 569.7 | 0.0 | 100.5 | 1.5 | 12.0 | 4.2 | 0.6 | 5.5 | 107.0 |
| CET305 | 135.7 | 19.4 | 10.9 | 1762.4 | 0.1 | 199.7 | 3.9 | 1.6 | 1.4 | 0.4 | 2.3 | 31.0 |
| CET311 | 427.3 | 49.2 | 14.8 | 672.8 | 0.1 | 565.6 | 2.6 | 0.0 | 0.9 | 0.9 | 2.2 | 392.0 |
| CET314 | 253.0 | 24.6 | 13.9 | 272.4 | 0.0 | 133.0 | 4.8 | 0.1 | 5.2 | 1.0 | 3.6 | 82.7 |
| CET403 | 59.5 | 15.7 | 6.8 | 563.6 | 0.0 | 265.1 | 0.7 | 0.1 | 0.6 | 0.7 | 103.0 | 4.9 |
| CET407 | 699.8 | 105.8 | 4.8 | 9515.7 | 0.9 | 162.9 | 3.3 | 4.0 | 1.2 | 1.4 | 0.7 | 2040.2 |
| CET420 | 470.2 | 27.1 | 5.6 | 1344.1 | 0.8 | 157.6 | 7.1 | 0.5 | 0.6 | 1.8 | 2.1 | 521.5 |
| CET450 | 223.8 | 34.2 | 6.5 | 619.1 | 0.1 | 118.5 | 2.4 | 0.2 | 0.8 | 3.2 | 1.3 | 63.4 |
| CET458 | 96.2 | 15.2 | 10.6 | 588.8 | 58.2 | 172.4 | 0.8 | 0.2 | 0.9 | 0.9 | 10.8 | 7.6 |
| CET505 | 77.7 | 11.0 | 10.3 | 321.6 | 0.5 | 186.1 | 0.6 | 0.1 | 0.8 | 0.8 | 290.7 | 6.9 |
| CET509 | 77.1 | 14.7 | 7.8 | 345.0 | 0.0 | 173.7 | 0.4 | 1.9 | 1.3 | 1.2 | 3.4 | 4.7 |
| CET526 | 61.4 | 9.5 | 4.0 | 369.5 | 0.1 | 203.4 | 0.3 | 0.2 | 0.7 | 0.4 | 0.7 | 2.8 |
| CET543 | 398.5 | 33.8 | 2.9 | 890.8 | 0.1 | 134.7 | 3.0 | 0.1 | 0.5 | 0.8 | 26.6 | 345.8 |
| CET562 | 381.9 | 51.6 | 12.1 | 739.0 | 0.1 | 295.0 | 1.8 | 0.2 | 0.8 | 2.8 | 8.3 | 370.3 |
| CET564 | 518.4 | 39.5 | 7.6 | 1117.1 | 0.0 | 78.4 | 2.7 | 159.0 | 42.9 | 1.3 | 14.3 | 562.5 |
| CET584 | 96.2 | 42.0 | 18.9 | 528.2 | 0.0 | 446.8 | 0.0 | 0.4 | 0.8 | 1.2 | 428.6 | 11.0 |
| CET592 | 75.5 | 15.7 | 4.3 | 628.7 | 0.0 | 292.0 | 0.0 | 0.2 | 0.4 | 0.2 | 2.2 | 7.2 |
| CET595 | 366.5 | 24.6 | 13.8 | 290.7 | 0.0 | 113.8 | 2.3 | 0.1 | 0.8 | 0.9 | 1.1 | 268.5 |
| CET635 | 366.3 | 21.0 | 6.8 | 1721.8 | 0.0 | 90.8 | 1.6 | 0.6 | 0.8 | 0.2 | 1.1 | 176.0 |
| CET640 | 419.9 | 28.6 | 8.7 | 448.3 | 0.0 | 133.1 | 4.2 | 0.1 | 0.7 | 1.5 | 2.8 | 215.8 |
| CET662 | 422.4 | 20.6 | 11.9 | 652.4 | 0.1 | 426.7 | 1.0 | 0.2 | 0.9 | 0.8 | 5.3 | 217.7 |
| Mean | 253.1 | 30.8 | 9.6 | 1177.4 | 2.1 | 200.4 | 2.9 | 7.2 | 2.8 | 1.2 | 31.8 | 205.4 |
| SD | 177.8 | 19.8 | 4.2 | 1719.8 | 10.6 | 113.3 | 2.6 | 29.2 | 7.8 | 0.8 | 92.8 | 382.6 |
| Median | 212.2 | 24.6 | 10.1 | 640.6 | 0.1 | 168.8 | 2.3 | 0.2 | 0.8 | 0.9 | 3.6 | 66.0 |

Table 19. Trace element concentrations (ppm) in the blubber and liver of BND from the present study and other marine areas worldwide. Data expressed as individual, mean or (median) value, when available.

| Locality | Tissue (n) | Fe | Hg | Se | Zn | Al | Cu | Mn | Ni | Cd | Cr | Pb | As | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **NE Atlantic Ocean** | | | | | | | | | | | | | | |
| Canary islands, 1997-2013 | Blubber (30) | 61.82 (44.44)[a] | 83.36 (80.83)[a] | 8.96 (7.29)[a] | 26.77 (25.75)[a] | 24.31 (10.34)[a] | 7.53 (2.75)[a] | 0.70 (0.16)[a] | 4.33 (1.35)[a] | 0.07 (0.06)[a] | 0.81 (0.69)[a] | 3.08 (0.07)[a] | 0.95 (0.86)[a] | The present study |
| | | 28.38 (23.88)[b] | 45.33 (43.17)[b] | 4.63 (3.48)[b] | 11.80 (9.16)[b] | 10.60 (4.45)[b] | 3.10 (1.10)[b] | 0.34 (0.11)[b] | 1.86 (0.67)[b] | 0.03 (0.03)[b] | 0.39 (0.23)[b] | 1.26 (0.04)[b] | 0.51 (0.45)[b] | |
| | Liver (30) | 1177.42 (640.55)[a] | 261.56 (223.77)[a] | 211.20 (68.63)[a] | 200.38 (168.78)[a] | 31.82 (3.63)[a] | 30.81 (24.58)[a] | 9.64 (10.10)[a] | 7.15 (0.20)[a] | 2.87 (2.34)[a] | 2.77 (0.78)[a] | 2.07 (0.06)[a] | 1,20 (0.90)[a] | |
| | | 330.51 (184.27)[b] | 72.44 (72.79)[b] | 58.33 (20.61)[b] | 56.49 (47.65)[b] | 9.00 (1.05)[b] | 8.63 (6.89)[b] | 2.72 (2.87)[b] | 2.08 (0.06)[b] | 0.81 (0.67)[b] | 0.80 (0.22)[b] | 0.60 (0.02)[b] | 0,34 (0,26)[b] | |
| Canary islands, 1997-2005 | Blubber (8) | | | | 1.9 (0.9)[b] | <0.03[b] | 0.8 (0.5)[b] | | | <0.06[b] | 0.74 (0.65)[b] | 1.22 (1.20)[b] | | Esperón F, Thesis 2005 |
| | Liver (7) | 1709 (1501)[a] | 809.83 (320.67)[a] | 331.4 (104.9)[a] | 18.0 (10.1)[a] | 25.4 (20.0)[a] | 10.3 (9.5)[a] | | <0.09[a] | 0.52 (0.66)[a] | 1.33 (1.30)[a] | 4.88 (4.98)[a] | | |
| NW Iberian Peninsula, 2004-2008 | Liver (8) | 258[b] | 19.1[b] | 10.8[b] | 33.8[b] | | 4.5[b] | <2.5[b] | <0.2[b] | 1.2[b] | 0.27[b] | <0.07[b] | 0,99[b] | Méndez-Fernández P, et al., 2014 |
| France, 1999-2004 | Liver (10) | | 38[b] | | | | | | | | | | | Lahaye V, et al., 2006 |
| **Mediterranean Sea** | | | | | | | | | | | | | | |
| Croatia (Adriatic Sea), 2000-2002 | Liver (14) | | 450 (177)[b] | | | | | | | 0.63 (0.27)[b] | | 0.14 (0.11)[b] | 1.36 (0.45)[b] | Bilandzic N, et al., 2012 |
| Israel, 2004-2006 | Liver (7) | 386[b] | 35.6[b] | | 49.5[b] | | 11.4[b] | 2.3[b] | 18.2[b] | <0.04[b] | | | | Shoham-Frider E, et al., 2009 |

| Locality | Tissue (n) | Fe | Hg | Se | Zn | Al | Cu | Mn | Ni | Cd | Cr | Pb | As | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Israel, 1993-2001 | Blubber (14) | 40 (30)[b] | 1.5 (0.58)[b] | | 10 (8.9)[b] | | 0.36 (0.28)[b] | 0.42 (0.29)[b] | | 0.07 (0.07)[b] | | | | Roditi-Elasar et al., 2003 |
| | Liver (15) | 352 (268)[b] | 97 (32)[b] | | 44 (33)[b] | | 8.9 (7.3)[b] | 3.5 (2.8)[b] | | 0.49 (0.41)[b] | | | | |
| France, 1999-2004 | Liver (5) | | 204[b] | | | | | | | | | | | Lahaye V, et al., 2006 |
| Ligurian Sea, 1999-2002 | Liver (2) | 1337-3478[a] | 13.55-3737[a] | 8.73-1708[a] | 243-288[a] | | 21.8-95.1[a] | 10.71-14.93[a] | | 3.02[a] | | 0.155-0.457[a] | | Capelli R, et al., 2008 |
| Corsica, 1997 | Liver (1) | | 62[a] | | 196[a] | | 41[a] | | | 0.07[a] | | 2.8[a] | | Frodello J.P, et al., 2002 |
| Corsica, 1995 | Liver (1) | | 4250[a] | | | | | | | | | | | Frodello J.P, et al., 2000 |
| Tyrrhenian Sea, 1987-1989 | Liver | | 12.2-13150[a] | | | | | | | | | | | Leonzio C, et al., 1992 |
| **North Sea** | | | | | | | | | | | | | | |
| United Kingdom, 1991-2006 (18 species of MMs) | Liver (492) | | 25 (7.0)[b] | 13 (4.2)[b] | 59 (46)[b] | | 15 (9.8)[b] | | 0.11 (<nd)[b] | 0.8 (0.16)[b] | 0.25 (0.1)[b] | 0.06 (0.02)[b] | 0,66 (0,47)[b] | Law R.J, et al., 2012 |
| **NW Atlantic Ocean** | | | | | | | | | | | | | | |
| South Carolina, <1997 | Liver (34) | | 17.8[b] | 9.54[b] | 56.8[b] | | 10.78[b] | | | 0.051[b] | <0.10[b] | <0.10[b] | 0,28[b] | Beck et al., 1997 |
| South Carolina, 2000-2007 | Liver (8-12) | 732[a] | 34.3[a] | 14.5[a] | 253[a] | 19.6[a] | 43.7[a] | 15.0[a] | 0.041[a] | 0.266[a] | | 0.031[a] | 1,66[a] | Stavros H.C, et al., 2011. |
| Florida, 2004-2008 | Liver (15) | 1125[a] | 300[a] | 109[a] | 211[a] | 12.0[a] | 27.5[a] | 13.7[a] | nd | 0.142[a] | | 0.196[a] | 0,821a | |
| Texas, 1991-1992 | Liver (8-30) | | 212[a] | 124[a] | 290[a] | | 71[a] | | | 0.32[a] | | 0.30[a] | 1,3a | Meador J.P, et al., 1999 |

| Locality | Tissue (n) | Fe | Hg | Se | Zn | Al | Cu | Mn | Ni | Cd | Cr | Pb | As | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Florida, 1991-1992 | Liver (10-14) | | 304[a] | 65[a] | 118[a] | | 25[a] | | | 1.6[a] | | 0.09[a] | 2,0a | |
| New Jersey to Florida, 1987-1988 | Liver (59) | | 22[b] | 9[b] | 76[b] | | 8.3[b] | | | nd | | 0.23[b] | | Geraci J.R, 1989 |
| **Pacific Ocean** | | | | | | | | | | | | | | |
| Australia, 1995-1996 | Liver (2) | | 0.72-32[b] | 1.5-12[b] | 41-144[b] | | 8.5-18[b] | | 0.12-0.56[b] | 0.07-3.7[b] | 0.97-1.2[b] | 0.04-0.17[b] | 0,2-0,76[b] | Law R.J, et al., 2003 |
| Hong Kong, 1994-1995 | Blubber (3) | | <0.8-0.9[a] | 2.69-7.67[a] | 7.67-10.82[a] | | 0.85-15.0[a] | | 0.83-0.9[a] | <0.8[a] | <0.8[a] | <0.8-15.3[a] | <0,8-12,6[a] | Parsons E.C.M, et al., 2001 |
| | Liver (3) | | <0.8-299[a] | 19.1-40.7[a] | 49.6-84.1[a] | | 8.27-12.1[a] | | <0.8-0.92[a] | 0.87-2.35[a] | <0.8-7.82[a] | <0.9-5.48[a] | 1,73-23,0[a] | |
| **Indian Ocean (Southern Ocean)** | | | | | | | | | | | | | | |
| Australia, 1988-2004 | Liver (9-11) | | 213.94[b] | 70.19[b] | 40.20[b] | | 21.18[b] | | | 4.10[b] | | 0.074[b] | | Lavery T.J, et al., 2008 |
| Australia, 1988-2004 (T.aduncus) | Liver (50-63) | | 475.78[b] | 178.85[b] | 93.88[b] | | 19.67[b] | | | 6.45[b] | | 0.455[b] | | |
| **Dolphinarium** | | | | | | | | | | | | | | |
| Israel, 1995 | Liver (1) | | 1.7[b] | | 31[b] | | 4.2[b] | | | <0.1[b] | | 3.6[b] | | Shlosberg A, et al., 1997 |

[a] Data expressed in dry weigh basis;
[b] Data expressed in wet weight basis.

Table 20. Mercury and selenium molar concentrations and ratios in the liver of the studied BND.

| *Specimen* | *Hg nmol g$^{-1}$* | *Se nmol g$^{-1}$* | *Hg/Se* | *Se/Hg* |
|---|---|---|---|---|
| CET526 | 306.33 | 35.29 | 8.68 | 0.12 |
| CET509 | 384.53 | 59.46 | 6.47 | 0.15 |
| CET458 | 479.79 | 96.77 | 4.96 | 0.20 |
| CET403 | 296.76 | 61.90 | 4.79 | 0.21 |
| CET505 | 387.21 | 87.35 | 4.43 | 0.23 |
| CET592 | 376.14 | 91.67 | 4.10 | 0.24 |
| CET584 | 479.34 | 138.86 | 3.45 | 0.29 |
| CET043 | 434.46 | 182.78 | 2.38 | 0.42 |
| CET099 | 420.00 | 231.51 | 1.81 | 0.55 |
| CET305 | 676.30 | 392.03 | 1.73 | 0.58 |
| CET171 | 777.95 | 543.36 | 1.43 | 0.70 |
| CET094 | 503.18 | 358.34 | 1.40 | 0.71 |
| CET209 | 903.08 | 646.32 | 1.40 | 0.72 |
| CET450 | 1115.56 | 802.51 | 1.39 | 0.72 |
| CET314 | 1261.32 | 1047.58 | 1.20 | 0.83 |
| CET286 | 1000.28 | 869.21 | 1.15 | 0.87 |
| CET296 | 1488.34 | 1355.46 | 1.10 | 0.91 |
| CET078 | 2141.85 | 1990.19 | 1.08 | 0.93 |
| CET635 | 1825.97 | 2229.02 | 0.82 | 1.22 |
| CET640 | 2093.16 | 2732.97 | 0.77 | 1.31 |
| CET662 | 2105.73 | 2757.64 | 0.76 | 1.31 |
| CET203 | 2092.43 | 3859.99 | 0.54 | 1.84 |
| CET595 | 1827.33 | 3400.42 | 0.54 | 1.86 |
| CET543 | 1986.53 | 4379.82 | 0.45 | 2.20 |
| CET311 | 2130.11 | 4964.07 | 0.43 | 2.33 |
| CET562 | 1903.78 | 4689.29 | 0.41 | 2.46 |
| CET564 | 2584.13 | 7123.59 | 0.36 | 2.76 |
| CET420 | 2344.20 | 6604.15 | 0.35 | 2.82 |
| CET407 | 3488.72 | 25838.53 | 0.14 | 7.41 |

Data are expressed on dry weight basis (dw) and listed by Se/Hg, in ascending order. In bold: outlier for selenium.

# 8.5. List of abbreviations (alphabetical order)

AHR: aryl hydrocarbon receptor
Al: aluminium
ARNT: aryl hydrocarbon receptor nuclear translocator
As: arsenic
BFR: brominated flame retardant
BND: bottlenose dolphin
BPA: bisphenol A
BPMO: benzo(alpha)pyrene monoxigenase
Cd: cadmium
COPs: contaminanes orgánicos persistentes
Cr: chromium
Cu: copper
CYP: cytochrome P450
DDD: dichlorodiphenyldichloroethane
DDE: dichlorodiphenyldichloroethylene
DDT: dichlorodiphenyltrichloroethane
DL: dioxin like
Dw: dry weight
EH: El Hierro
Fe: iron
Fig.: figure
FV: Fuerteventura
GC: Gran Canaria
HAB: harmful algal blooms
HCB: hexachlorobenzene
HCH: hexachlorocyclohexane
Hg: mercury
HM: heavy metal
HSP70: heat-shock-protein
IMO: International Maritime Organization
IUCN: International Union for Conservation of Nature
IWC: International Whaling Commission
LG: La Gomera
LP: La Palma
Lw: lipid weight
LZ: Lanzarote
MeHg: methyl mercury
MM: marine mammal
MMC: Marine Mammal Commission
Mn: manganese
MPAs: Spanish Mediterranean Marine Protected Areas
MSFD: Marine Strategy Framework Directive
Ni: nickel
NOAA: National Oceanic and Atmospheric Administration
OC: organochlorine
OCP: organochlorine pesticide
OMR: outermost regions
PAH: polycyclic aromatic hydrocarbon/ hidrocarburos aromáticos policíclicos
Pb: lead
PBB: polybrominated biphenyl
PBDE: polybrominated diphenyl ether
PBT: persistent bioacumulative and toxic
PCB: polychlorinated biphenyl/bifenilos policlorados
PCDD: polychlorinated dibenzo-p-dioxin
PCDF: polychlorinated dibenzofuran
PFC: perfluorinated chemicals
PFOA: perfluorooctanoic acid
POCs: pesticidas organoclorados
PPARalpha: the peroxisome proliferator-activated receptor
PSSA: Particular Sensitive Sea Area
SACs: Special Areas of Conservation
SCANS: small cetacean abundance in European Atlantic waters and North Sea
SECAC: Society for the Study of Cetaceans in the Canary Archipelago.
Se: selenium
TCDD: 2,3,7,8-Tetrachlorodibenzodioxin
TEF: Toxic Equivalency Factors
TEQ: Toxic Equivalency to Dioxins
TF: Tenerife
UK: United Kingdom
UME: Unusual Mortality Events.
USEPA: United States Environmental Protection Agency
Ww: wet weight
Zn: zinc

# 8.6. List of figures and tables

## FIGURES

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Table 17. Levels of heavy metals and toxic trace elements in blubber samples of stranded BND (μg $g^{-1}$ dw). SD (standard deviation).

Table 18. Levels of heavy metals and toxic trace elements in liver samples of stranded BND (μg $g^{-1}$ dw). SD (standard deviation).

Table 19. Trace element concentrations (ppm) in the blubber and liver of BND from the present study and other marine areas worldwide. Data expressed as individual, mean or (median) value, when available.

Table 20. Mercury and selenium molar concentrations and ratios in the liver of the studied BND.

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TESIS DOCTORAL

# ASSESSMENT OF ANTHROPOGENIC CONTAMINANTS IN BOTTLENOSE DOLPHINS *(Tursiops truncatus)* FROM THE CANARY ISLANDS

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