Environmental
Science
Processes & Impacts

PAPER

Cite this: *Environ. Sci.: Processes Impacts*, 2014, **16**, 2774

# Organochlorines in harbour porpoises (*Phocoena phocoena*) stranded along the southern North Sea between 2010 and 2013

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Received 15th September 2014
Accepted 6th October 2014

DOI: 10.1039/c4em00490f

rsc.li/process-impacts

7 polychlorinated biphenyls (PCBs), 6 dichlorodiphenyltrichloroethanes (DDXs) and 8 polybrominated diphenyl ethers (PBDEs) were measured in the blubber of 20 harbour porpoises stranded on the coasts of the southern North Sea between 2010 and 2013. The results showed that porpoises that died from infectious diseases displayed significantly higher levels of PCBs in their blubber compared to healthy porpoises that died from physical trauma. $\sum$7CBs and $\sum$DDXs were higher in juvenile porpoises compared to adult females. Except for three individuals, PBDE concentrations were below the limit of quantification in the blubber samples treated. In general, levels of PCBs and DDXs obtained in the blubber of porpoises from this study were in the same order of magnitude or even lower than those obtained in the blubber of porpoises stranded along the North East Atlantic Ocean and the Black Sea over the period 1987 and 2013. The results of the present study suggest that even if the status of marine pollution has been improved, a continuous long-term contamination by toxic organochlorines over many generations may be observed.

### Environmental impact

This paper investigated organochlorine concentrations and profiles in the blubber of stranded harbour porpoises (*Phocoena phocoena*) along the southern North Sea between 2010 and 2013. In the last decade, porpoise stranding has increased in French, Belgian and Dutch coastal waters. Since organochlorines are known to exert immunotoxicity in harbour porpoise individuals, special interest may be accorded to the contaminant levels in their organs and tissues. Based on the results, the paper relates polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) to the maturity status and the gender of stranded porpoises, along with the cause of death of porpoises whether it was a natural mortality or a death due to infectious diseases.

## 1. Introduction

The harbour porpoise (*Phocoena phocoena*) is a representative top predator species for the North Sea ecosystem. This long-lived species feeds at a high trophic level, thus it can accumulate relatively high levels of contaminants and is vulnerable to the effects of environmental changes.[1–3] In the past few years, an increased number of stranded porpoises in the southern part of the North Sea[4,5] has generated special interest toward this species. Concern has also been expressed about other potential threats such as food depletion[6] and pollutants.[7–9]

Exposure to persistent organochlorines such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) and their related compounds has caused abnormalities in higher trophic feeding animals from the North Sea, UK and various seas.[1–3,10] PCBs have been synthesized for industrial use and DDXs as agrochemicals.[11] The production of these organochlorines has been banned in Europe since the end of the 1970s generating the EU directive (79/117/EEC) for DDXs and the council directive (96/59/EC) for disposal of PCBs. However a continuous long-term contamination by toxic organochlorines over many generations may be expected.[2] POPs are lipophilic compounds and can bioaccumulate and magnify in the food chain; therefore their impact on top predator species is of particular concern.[11] Organic pollutants may have possible adverse effects on marine mammal populations. It has been demonstrated that thymic atrophy and splenic depletion in harbour porpoises from German North and Baltic Seas were significantly correlated with increased PCB and polybrominated diphenyl ether (PBDE) levels.[12]

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Compared to seals, birds and terrestrial mammals, harbour porpoises are suggested to have lower capacity to metabolize organochlorine compounds.[1,2,13,14] Since organochlorines are known to exert immunotoxicity in harbour porpoise individuals, special interest may be shown to the contaminant levels in their organs and tissues. A previous study on the metallic contaminants in livers and kidneys of harbour porpoises stranded along the southern North Sea did not reject the hypothesis that chemical contaminants may influence the health of harbour porpoises and contribute to the increased level of stranding seen during the last decade for the population in this area.[9] Therefore, the aims of the present study were (1) to relate organochlorine concentrations and profiles to the maturity status and the gender of stranded porpoises, (2) to investigate potential associations between organic contaminants (PCBs and pesticides) and the cause of death (traumatic or infectious) of porpoises and (3) to compare the contaminant levels in porpoises from this study to other porpoises stranded along European waters (North East Atlantic Ocean and the Black Sea) in order to assess the current contamination status of harbour porpoises in the study area.

## 2. Materials and methods

### 2.1. Sampling and data collection

Harbour porpoises stranded in the southern North Sea along the northern France and Belgian coasts between 2010 and 2013 were collected for POP analyses (Fig. 1). Due to their lipophilic nature, POPs are known to accumulate in the fatty tissues, hence in the blubber of cetaceans.[2,15] Therefore blubber was sampled from the cranial insertion of the dorsal fin and stored wrapped in aluminum foil at −20 °C. All washed ashore carcasses were either freshly dead or slightly decomposed. Post-mortem investigations were performed according to the protocol from Kuiken and Hartmann[16] and Jauniaux *et al.*[17] The length of individuals was used to determine age groups. Porpoises with lengths ranging from 91 to 130 cm were considered as juveniles and animals greater than 130 cm were considered as adults.[18] According to the blubber thickness measured at the cranial insertion of the dorsal fin, the nutritional status of animals was evaluated. All animals were divided into 4 groups according to the cause of death. Harbour porpoises that died from infectious diseases including parasitic, bacterial, mycotic and viral infections and those that died from lung edema, pneumonia and emaciations represented the first group. Porpoises that died from physical trauma associated with suffocation, traumatic injuries and entanglement in fishing nets represented the second group. The third group represented porpoises that died of other causes (tumor, starvation, …) or whose cause of death could not be determined. Finally porpoises that died from seal predations represented the fourth group.

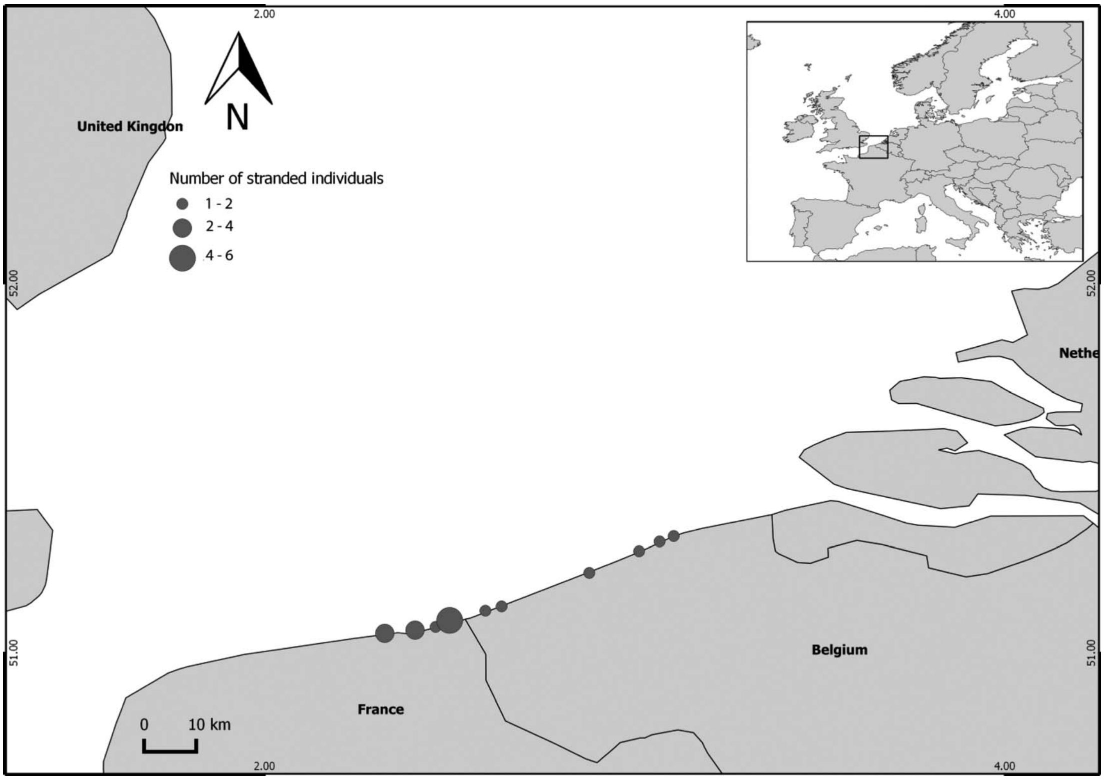

Fig. 1 Harbour porpoise stranding locations and numbers along the southern North Sea analyzed in this study (2010–2013).

### 2.2. POP analysis

POPs were determined in 20 samples of blubber from harbour porpoises stranded along the southern North Sea between 2010 and 2013. 10 to 20 g of blubber was freeze-dried and its water content was determined by the difference of weights before and after lyophilization. Samples were extracted for 8 hours using a Soxhlet apparatus with a mixture of nonpolar solvents cyclohexane–toluene (1/1; v/v). 10 to 40 % of lipids were dissolved in 5 ml of hexane. 1 ml of sulfuric acid (96%) was added in order to precipitate lipids. From the extract, separate aliquots were taken for PCB, DDX and PBDE analyses.

Seven PCB congeners (whose IUPAC numbers are: CB 28, 52, 101, 118, 153, 138 and 180) recommended by the International Council for the exploration of the Sea (ICES) were considered. PCBs were measured with an Agilent 6890N gas chromatograph coupled to a 5973 Network MSD (GC-MS). The injector temperature was initially 80 °C and after 1 min the temperature was elevated by 20 °C $\text{min}^{-1}$ up to 130 °C, thereafter the temperature was elevated by 7 °C $\text{min}^{-1}$ up to 270 °C and kept for 6 min. The $^{13}C_{12}$-labeled PCB congeners 28, 52, 101, 138, 153, and 180 were used as internal standards. Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 80%. The limit of quantification (LOQ, according to the "Norme Française" EN 1528) was 0.01 μg $g^{-1}$ lipids.

Six DDXs (*o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT and *p,p′*-DDE) were measured with an Agilent 6890A gas chromatograph coupled with a 5973 Network MSD (GC-MS). The GC was equipped to an Rxi XLB 30 m × 0.25 mm × 0.25 μm silica column. The injector temperature was initially 100 °C and after 3 min the temperature was elevated by 12 °C $\text{min}^{-1}$ up to 180 °C, thereafter the temperature was elevated by 5 °C $\text{min}^{-1}$ up to 300 °C and kept for 6 min. The internal standard used was $^{13}C_{12}$-labeled *p,p′*-DDE. Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 80%. The LOQ (according to the "Norme Française" EN 1528) was 0.01 μg $g^{-1}$ lipids.

Polybrominated diphenyl ether (PBDE) congeners (BDE 28, 47, 99, 100, 153, 154, 183 and 209) were measured with an Agilent 7890A gas chromatograph coupled to a mass spectrometer system (GS/MS/MS Quattro Micro Waters). The GC was equipped with an Rtx 1614, 15 m × 0.25 mm × 0.1 μm silica column. The injector temperature was initially 250 °C and after 2 min the temperature was elevated by 20 °C $\text{min}^{-1}$ up to 310 °C at which it was maintained for 4 min. The carrier gas was helium with a constant flow rate (3 ml $\text{min}^{-1}$). The internal standards added were: $^{13}C_{12}$-labeled BDE congeners 28, 47 and 99 (50 ng $\text{ml}^{-1}$), $^{13}C_{12}$-labeled BDE congeners 153, 154 and 189 (100 ng $\text{ml}^{-1}$) and $^{13}C_{12}$-labeled BDE 209 (250 ng $\text{ml}^{-1}$). Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 70%. The LOQ was 0.05 μg $g^{-1}$ lipids.

### 2.3. Data treatment

Data analysis was performed using "XLSTAT – Pro" 2013 (Addinsoft). The level of significance was set at $\alpha = 0.05$. When values were below the limit of quantification, half the limit of quantification was assigned for statistical analyses. To assess the differences in POP concentrations between porpoises that died from infectious diseases and those that died from physical trauma, a Mann–Whitney *U* test or a Student's *t*-test was used when the necessary assumptions of normality and homogeneity of variances for parametric statistics were satisfied. Moreover, to compare POP concentrations between juveniles and adults (males and females) in the blubber of porpoises from the southern North Sea, a Kruskal–Wallis test followed by the Dunn test for multiple comparisons were used to check for pairwise differences. Finally, correlations between POPs in the blubber were tested using the Spearman coefficient.

## 3. POP results

The results of POP analysis in the blubber of 20 harbour porpoises stranded in the southern North Sea between 2010 and 2013 are presented in Table 1. PCB concentrations (sum of 7 congeners) varied widely between individuals reporting an average of 18 ± 25 μg $g^{-1}$ lipids and ranging between 0.6 and 110 μg $g^{-1}$ lipids. The CB profiles in the blubber of all harbour porpoises analyzed were dominant by the recalcitrant congener CB 153 with proportions more than 40% of total CB. In the descending order, the levels are: CB 153, CB 138, CB 180, CB 101, CB 118, CB 52 and CB 28. The results for CB 153 are also shown in Table 1 in order to compare with other studies. Similarly, DDX concentrations varied largely between individuals with an average of 15 ± 20 μg $g^{-1}$ lipids. The smallest and largest values ranged between 0.7 and 96 μg $g^{-1}$ lipids. The DDX profiles were dominant by *p,p′*-DDE and *p,p′*-DDD contributing more than 80% to the sum of DDXs. In the descending order, the levels are: *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDE, *o,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT.

All PBDE concentrations were below the limit of quantification (0.05 μg $g^{-1}$ lipids) in the blubber samples treated except for three juvenile harbour porpoises. The congener BDE 47 was the most concentrated with values of 1.06 μg $g^{-1}$ lipids (juveniles that died from infectious diseases) and 0.19 and 0.15 μg $g^{-1}$ lipids (juveniles that died from physical trauma). Moreover, two other congeners (BDE 99 and BDE 153) were also detected in the juvenile that died from infectious diseases with concentrations of 0.64 and 0.18 μg $g^{-1}$ lipids, respectively. The harbour porpoises that displayed the maximum level of PBDE (sum of the 3 BDEs: 1.89 μg $g^{-1}$ lipids) also exhibited the maximum level of $\sum$7CBs (110 μg $g^{-1}$ lipids).

A Spearman rank correlation matrix was established in order to track the correlation between POPs in the blubber. A significant positive correlation was observed between $\sum$7CBs and $\sum$6DDXs ($p < 0.05$).

### 3.1. POPs and maturity status

Since only one adult male was analyzed for POPs, values were excluded for the rest of the statistical tests. Juveniles exhibited higher $\sum$7CB and $\sum$DDXs levels than adult females (Table 1). More specifically, juvenile males were the most contaminated

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**Table 1** Mean concentrations of the sum of PCBs, CB 153 and DDXs (μg g$^{-1}$ lipids) in blubber of harbour porpoises from different regions of the North East Atlantic Ocean and the Black Sea. Years in brackets refer to the date of stranding. A: Adults; J: Juveniles; AM: Adult males; AF: Adult females; JM: Juvenile males; JF: Juvenile females; *n*: number of samples. * median; ** ∑7CBs

| | ∑PCBs | | | | CB 153 | | | ∑DDXs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Age/gender | Mean ± SD | Min–max | *n* | Mean ± SD | Min–max | *n* | Mean ± SD | Min–max | *n* | References |
| Danish and Norwegian waters (1987–1991) | M | 23.3 | (3.7–65) | 34 | | | | 16.39 | (3.2–45.1) | 34 | 21 |
| Baltic sea (1985–1993) | JM | 16 ± 8 | (2.9–32) | 13 | 6.6 ± 3.6 | (1.1–13) | 13 | 15 ± 18 | (1.5–59) | 11 | 22 |
| Baltic sea (1988–1989) | AM | 46 ± 29 | (14–78) | 4 | 20 ± 13 | (5.9–33) | 4 | 116 ± 134 | (20– 308) | 4 | |
| Kattegat–Skagerrak seas (1989–1990) | JM | 11 ± 5.0 | (2.2– 20) | 10 | 4.8 ± 2.5 | (1.0– 10) | 10 | 20 ± 13 | (5.7–36) | 8 | |
| Kattegat–Skagerrak seas (1988–1990) | AM | 13 ± 5.2 | (6.7–22) | 7 | 5.7 ± 2.3 | (3.0–9.5) | 7 | 25 ± 20 | (2.8–61) | 7 | |
| Kattegat–Skagerrak seas (1978–1981) | AM | 40 ± 22 | (17–67) | 5 | 19 ± 12 | (6.0–33) | 5 | 98 ± 43 | (35–154) | 5 | |
| West coast of Norway (1988–1990) | AM | 15 ± 11 | (7.2–33) | 8 | 5.6 ± 4.6 | (2.5–14) | 8 | 9.1 ± 7.4 | (3.1–22) | 6 | |
| Southern North Sea (2001–2003) | F | 15 ± 8.6 | | 19 | | | | | | | 26 |
| Scotland (2001–2003) | F | 10.5 ± 13.2 | | 31 | | | | | | | |
| Ireland (2001–2003) | F | 53.5 ± 48 | | 12 | | | | | | | |
| France (2001–2003) | F | 13.8 ± 11 | | 2 | | | | | | | |
| Galicia (2001–2003) | F | 53 ± 42 | | 3 | | | | | | | |
| Southern North Sea (1999–2004) | JF | 12.9 ± 11.9 | (1.3–39.3) | 9 | 3.7 ± 4.1 | (0.2–13.4) | 9 | | | | 19 |
| | JM | 15.4 ± 10.7 | (5.3–39.8) | 12 | 3.9 ± 3.0 | (1.2–11.5) | 12 | | | | |
| | AF | 7.3 ± 2.0 | (4.4–8.9) | 5 | 1.7 ± 0.6 | (1.0–2.3) | 5 | | | | |
| | AM | 82.9 ± 31.8 | (38.7–125.5) | 8 | 28.7 ± 12.0 | (11.6–46.0) | 8 | | | | |
| East England (1991–2005) | M | 11.6 ± 9.7 | | 23 | | | | | | | 35 |
| Southern North Sea (1991–2005) | M | 46.4 ± 30.7 | | 21 | | | | | | | |
| Black Sea (1998) | A | 13.2* | (8.8–24.9) | 11 | | | | 77.3* | (55–157) | 11 | 33 |
| | J | 7.0* | (4.9–13.7) | 9 | | | | 40.9* | (27.4–82) | 9 | |
| North Sea (1990–1999) | A | 81.5 | | 1 | | | | 22.9 | | 1 | 29 |
| North Sea (2000–2008) | A | 24.9 | (15.3–34.5) | 2 | | | | 3.4 | (1.2–1.4) | 2 | |
| North Sea (1990–1999) | J | 19.1 | | 1 | | | | 4.5 | | 1 | |
| North Sea (2000–2008) | J | 9.9 | (1.1–68.2) | 5 | | | | 1.7 | (0.4–6.4) | 5 | |
| North West Iberian Peninsula (2004–2008) | JF | 10.8 ± 2.8 | | 5 | 2.9 ± 0.8 | | 5 | | | | 23 |
| | JM | 9.4 ± 3 | | 3 | 2.8 ± 1 | | 3 | | | | |
| | AF | 37.5 ± 30.8 | | 3 | 12.0 ± 9.7 | | 3 | | | | |
| | AM | 50.8 | | 1 | 16.6 | | 1 | | | | |
| Southern North Sea (2010–2013) | JF | 32 ± 21** | (7.4–48) | 3 | 14 ± 10 | (3–22) | 3 | 16 ± 10 | (8–27) | 3 | Present study |
| | JM | 20 ± 31** | (0.6–110) | 12 | 9 ± 15 | (0.3–54) | 12 | 19 ± 25 | (2.4–96) | 12 | |
| | AF | 4 ± 1,8** | (2.5–7) | 4 | 1.8 ± 0.9 | (1–3) | 4 | 1.9 ± 1.3 | (0.7–3.5) | 4 | |
| | AM | 22** | — | 1 | 10 | — | 1 | 13 | — | 1 | |

individuals compared to juvenile females and adult females. Due to the low number of animals analyzed, statistical analysis did not show differences between maturity statuses for the CB compounds ($p > 0.05$), whereas for DDX levels, juveniles displayed significantly higher concentrations compared to adult females ($p < 0.05$). Consequently, juveniles will be considered as one group for further comparisons.

### 3.2. POPs and causes of death

From 20 porpoises analyzed for POPs, post-mortem investigations showed that 10 porpoises died from infectious diseases, 7 died from physical trauma, 2 whose cause of death could not be determined and one died from seal predation. The blubber of porpoises that died from infectious diseases displayed a significantly lower thickness than porpoises that died from physical trauma ($p < 0.05$). Fig. 2 shows that the mean $\sum$7CB level in the diseased group displayed higher concentrations than the trauma group, but these differences were not statistically significant. Both groups displayed nearly the same levels of $\sum$DDX (Fig. 2), hence no significant differences were found between both groups for DDX levels.

## 4. Discussion

### 4.1. PCB levels

Unlike some essential trace elements, persistent organic pollutants are non-essential for survival. They have a strong affinity to lipid-rich tissues and organs because of their lipophilicity and hence they are retained mainly in the blubber of cetaceans.[2,15] The biotransformation capacity of PCBs and DDXs is known to be lower in small cetaceans compared to seals, birds and terrestrial mammals.[1,2,13,14]

The wide range between the minimum and the maximum for PCB concentrations (Table 1) may underline the involvement of numerous biological factors for instance age, gender, diet, body conditions and metabolic capacity to degrade toxic contaminants regarding PCB lipid accumulation.[1,2,19,20] It has been documented that CB 153 levels are higher in the majority of the samples from aquatic mammals.[21,13] The levels of CB 153 in juvenile harbour porpoises from this study (Table 1) ranged between 0.3 and 54 $\mu$g g$^{-1}$ lipids. These levels were generally higher than those reported in immature male porpoises (1985–1990) from the Baltic Sea (1.1–13 $\mu$g g$^{-1}$ lipids) and the Kattegat–Skagerrak Seas (1.0–10 $\mu$g g$^{-1}$ lipids).[22] Similarly, juvenile porpoises stranded in our study exhibited higher CB 153 contents compared to juvenile porpoises from the southern North Sea stranded between 1994 and 2004 (0.2–13.4 $\mu$g g$^{-1}$ lipids)[19] and those from the North West Iberian Peninsula stranded between 2004 and 2008 (2.9 ± 0.8 $\mu$g g$^{-1}$ lipids)[23] (Table 1). However, caution should be taken in interpreting the findings of the present study since the total sample size of 20 individuals may be relatively small for temporal comparisons. A significant accumulation of PCBs with age is apparent in male porpoises from the Scandinavian Waters[21] and from the southern North Sea[19] as well as in male fin whales from the coasts of Spain.[15] Unfortunately such accumulation with age could not be verified for our study due to the fact that we were not able to analyze more than one adult male harbour porpoise (Table 1). Juveniles had higher PCB levels than adult females with similar trends for the congener CB 153. It has been reported that adult females have decreasing levels of organic contaminants explained by the transfer of organochlorines to their offsprings during gestation and lactation.[2,10,15,24] Such findings may explain variations in PCB levels between adult females and juvenile porpoises stranded in the southern North Sea.

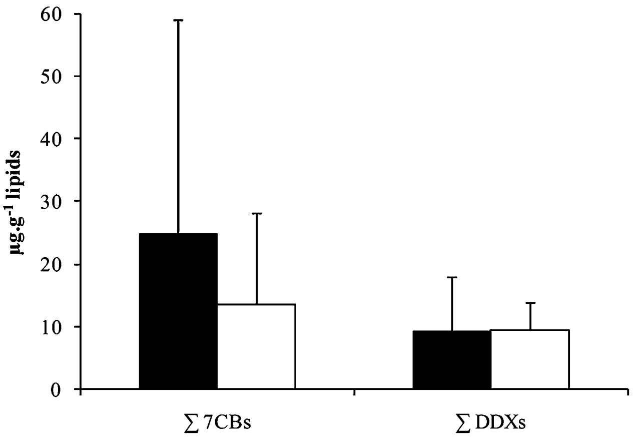

Fig. 2 Mean blubber concentrations ($\mu$g g$^{-1}$ lipids) of the $\sum$7PCBs and $\sum$DDXs of harbour porpoises stranded in the Southern North Sea between 2010 and 2013 for animals that died from infectious diseases ($n = 10$; black bars) and physical trauma ($n = 7$; white bars).

Furthermore, porpoises that died from infectious diseases exhibited higher PCB levels (25 ± 34 $\mu$g g$^{-1}$ lipids) compared to those that died from physical trauma (14 ± 15 $\mu$g g$^{-1}$ lipids) (Fig. 2). Same trends were found in other studies with a more representative sampling for porpoises stranded in the United Kingdom[10,25] and western European seas.[26] Jepson *et al.*[10,25] suggested that pre-existing disease processes may cause mobilization and metabolic breakdown of blubber lipid stores which lead to highlighting levels of PCBs in harbour porpoise blubber and support a causal relationship between PCB exposure and infectious disease mortality. Moreover, porpoises that died from physical trauma ($n = 7$) displayed thicker blubber compared to porpoises that died from infectious diseases ($n = 10$) ($p < 0.05$). It has been suggested that pollutants may be diluted in a thicker blubber layer.[21] This leads us to the fact that porpoises could be exposed to the same levels of organochlorines in the environment, but concentrations of pollutants may be more pronounced in diseased porpoises due to emaciation processes.

A toxic threshold concentration in the liver (17 $\mu$g g$^{-1}$ lipids) for total PCBs determined for adverse health effects in marine mammals is proposed.[27] In order to compare the levels of PCBs in the blubber of porpoises from the present study with the proposed limit, PCB concentrations had to be converted given that the threshold is based on the commercial PCB mixture Aroclor 1254. The conversion factor, from the seven ICES congeners (CB 28, 52, 101, 118, 153 and 180) to total PCBs, may be obtained by multiplying the sum of the seven congeners by three. According to the equation: total PCB concentration (as Aroclor 1254) = 3.0 × sum of the seven ICES congeners (lipid weight).[25] In the present study, 60% of the animals analyzed exceeded this threshold. In the UK, in the porpoises with total PCB levels that exceeded the threshold (17 $\mu g\ g^{-1}$ lipids), total PCB levels were significantly higher in porpoises that died due to infectious diseases compared to healthy porpoises that died due to physical trauma.[25] Moreover, 74% of harbour porpoises from the southern North Sea[25] and 75% of the harbour porpoises from the North West Iberian Peninsula exceeded this threshold.[23] However, caution should be taken when applying this threshold, since this value was derived from the liver of laboratory aquatic mammals (seals, European otters and minks) that were fed with field food items. The extrapolation of this threshold level to the blubber of stranded harbour porpoises that were feeding on a variety of prey species may be questionable.

### 4.2. DDX levels

Because of the different chemical natures of organochlorines, the distribution patterns of DDXs in different tissues and organs of animals are generally more variable than those of the CBs.[1] In the present study, $p,p'$-DDE and $p,p'$-DDD had the largest contribution to the sum of DDX (more than 80%) which is in agreement with previous studies.[1,22,28,29] Marine mammals have induced levels of cytochrome P450-1A and 2B that are capable of metabolizing $p,p'$-DDT.[13] Thus, relatively high concentrations of DDT metabolites ($p,p'$-DDE and DDD) in marine mammal tissues were related to the higher metabolism of DDT in marine mammals along with the bioaccumulation of DDT metabolites through their life span.[30] In addition, ratios of $p,p'$-DDE/$\sum$DDXs may indicate whether a new source of DDT is entering the environment. Therefore, a ratio greater than 0.6 implies a stable system indicating that there is no new or recent input of DDXs in the environment.[31,28] The mean ratio in the blubber of porpoises from the southern North Sea was 0.6 which may indicate that there is no new input of DDXs in this region. The level of the sum of DDX in porpoises from this study was 15 ± 20 (0.7–96) $\mu g\ g^{-1}$ lipids (Table 1), higher than those reported in livers of porpoises stranded between 1997 and 2000 (3.4 ± 2.3; 0.3–44.3 $\mu g\ g^{-1}$ lipids)[32] and in the blubber of juvenile porpoises stranded between 2000 and 2008 (1.7; 0.6–6.4 $\mu g\ g^{-1}$ lipids)[29] along the southern North Sea. Male porpoises (16.4; 3–45 $\mu g\ g^{-1}$ lipids) from Scandinavian waters stranded between 1987 and 1991[21] and juvenile porpoises from the Baltic Sea (15 ± 18; 1.5–59 $\mu g\ g^{-1}$ lipids) and Kattegat–Skagerrak seas (20 ± 13; 5.7–36 $\mu g\ g^{-1}$ lipids) stranded between 1985 and 1993[22] showed almost the same concentrations of DDXs compared to porpoises from our study, whereas porpoises from the Black Sea stranded in 1993 and 1998[28,33] had higher concentrations (Table 1). Juveniles displayed significantly higher DDX levels compared to adult females ($p < 0.005$). As mentioned previously, this could be explained by the transfer of organochlorines from adult females to their offsprings. Furthermore, the great differences in organochlorine concentrations between the adult males analyzed and females were related to the lactational period. This sexual difference is more pronounced in harbour porpoises compared to other cetaceans due to their longer lactation period.[28] An age-dependent accumulation was also found for all DDT residues in porpoises from various regions.[15,21,28,29]

Unlike PCB trends, porpoises that died from infectious diseases (9.3 ± 9 $\mu g\ g^{-1}$ lipids) showed almost the same levels of DDXs compared to healthy porpoises that died from physical trauma (9.5 ± 4 $\mu g\ g^{-1}$ lipids) (Fig. 2). A significant correlation was observed between DDXs and PCBs for the 20 animals analyzed ($p < 0.001$). This finding was in agreement with the study of Jepson[34] showing significant correlation between $\sum$25CBs and DDTs ($p < 0.001$) for a more representative sampling ($n = 169$) of porpoises stranded on UK coasts between 1989 and 2001.

In the present study, the levels of BDE congeners were detected in only three juvenile porpoises (>LOQ = 0.05 $\mu g\ g^{-1}$ lipids). The concentrations obtained seem to be in the same order of magnitude as harbour porpoises stranded along the southern North Sea between 1999 and 2004 (range 0.22–5.93 $\mu g\ g^{-1}$ lipids),[19] between 2001 and 2003 (1.06–0.8 $\mu g\ g^{-1}$ lipids)[26] and more recently between 2000 and 2008 (range 0.28–1.83 $\mu g\ g^{-1}$ lipids).[29] Similar to PCBs,[35] Law *et al.*[36] observed also a decline for $\sum$9BDE concentrations in the blubber of harbour porpoises stranded or bycaught from the UK during the period 1992–2008. However, $\sum$BDE concentrations in stranded porpoises dying due to infectious diseases were higher than levels in bycaught animals.[36]

It has been suggested that after the ban in 1970s and 1980s, organochlorines in biota were in continuous decline.[37] For instance, Berggrena *et al.*[22] found a temporal decline in $\sum$DDT and $\sum$PCB levels between porpoises collected in 1978–81 compared to those from 1988–90 in the Kattegat–Skagerrak seas. A decline in organochlorines has been documented for harbour porpoises in Danish waters[38] and in the Bay of Fundy, Canada.[24] A temporal variation was also observed in $\sum$25CB levels between 1989 and 2001 for porpoises from the United Kingdom demonstrating a gradual decline from the early 1990s to 2001.[25] A recent study by Law *et al.*[35] showed quite a slow decline for CB concentrations in UK porpoises stranded from 1991 to 1998 and then reached a plateau thereafter until 2009. Tanabe *et al.*[2] suggested that a high transmission rate of organochlorines is pronounced in cetaceans. Thus, even if the status of marine pollution has been improved, a continuous long-term contamination by toxic organochlorines over many generations may be observed.

## 5. Conclusion

The present study provides an assessment of some persistent organic pollutant levels in the blubber of harbour porpoises stranded along the southern North Sea between 2010 and 2013. Levels of PCBs were significantly higher in porpoises that died from infectious diseases compared to healthy porpoises that died from physical trauma. Furthermore, the sum of PCBs and DDXs was higher in juvenile porpoises compared to adult females, which is in agreement with previous studies. According to the ratio $p,p'$-DDE/$\sum$DDXs, our results suggest that there is no new input of DDXs in this region. In addition, levels of PCBs and DDXs obtained in the blubber of porpoises from this study were in the same order of magnitude or even lower than porpoises stranded along the North East Atlantic Ocean and the Black Sea over the period 1987 and 2013. We believe that even though the levels of organochlorines are slowly declining in the marine environment, they are still high enough in harbour porpoises and still capable of causing negative effects. Moreover, a threshold level is proposed only for total PCBs (17 μg g$^{-1}$ lipids) above which there are health effects in mammals. Along with the fact that this threshold is questionable, no such threshold is proposed in the literature for other organochlorines such as DDXs and PBDEs. Therefore, it is important to keep monitoring the levels of these compounds in the top predator harbour porpoise in the North Sea.

## Acknowledgements

We would like to thank the French Stranding Network for their efforts in collecting the samples, especially "Centre de Recherche sur les Mammiferes Marins" (CRMM) and "Observatoire pour la Conservation et l'Etude des Animaux et Milieux Marins" (OCEAMM). The "Grand Port Maritime de Dunkerque" (France) is acknowledged for financial support of this study. Mahfouz Celine is financially supported by a PhD fellowship from the National Council for Scientific Research (Lebanon) and Université du Littoral Côte d'Opale (France). We would like to thank two anonymous reviewers for their helpful comments on a previous version of this manuscript.

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