Levels of polychlorinated biphenyls are still associated with toxic effects in harbour porpoises (*Phocoena phocoena*) despite having fallen below proposed toxicity thresholds

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Abstract

Polychlorinated biphenyls (PCBs) are toxic, persistent and lipophilic chemical compounds that accumulate to high levels in harbour porpoises (*Phocoena phocoena*) and other cetaceans. It is important to monitor PCBs in wildlife, particularly in highly exposed populations, to understand if concentrations are declining and how levels relate to toxicological thresholds and indices of health like infectious disease mortality. Here we show, using generalised additive models and tissue samples of 814 UK-stranded harbour porpoises collected between 1990 and 2017, that mean blubber PCB concentrations have fallen below the proposed thresholds for toxic effects. However, we found they are still associated with increased rates of infectious disease mortality such that an increase in PCB blubber concentrations of 1 mg kg^{-1} lipid corresponds with a 5% increase in risk of infectious disease mortality. Moreover, rates of decline and levels varied geographically, and the overall rate of decline is slow in comparison to other pollutants. We believe this is evidence of long-term preservation in the population and continued environmental contamination from diffuse sources. Our findings have serious implications for the management of PCB contamination in the UK and reinforce the need to prevent PCBs entering the marine environment to ensure that levels continue to decline.

Keywords: Harbour porpoise; polychlorinated biphenyls; pollution; infectious disease; temporal trend; generalised additive models

1. Introduction

The impact of chemical pollution on long-lived marine apex predators has concerned scientists for some time.[1] For decades, industrial chemicals and pesticides were released into the environment and often ended up in the sea. Persistent organic pollutants (POPs) are a group of typically halogenated organic compounds that are of particular concern because of their resistance to environmental degradation and their significant impacts on the health of humans and wildlife [2,3]. The chemical properties and environmental impacts of POPs vary widely across the group. The more persistent polychlorinated biphenyls (PCBs) have been shown to induce reproductive and immuno-toxicity and cause the greatest concern in the marine environment in Europe[3–6].

Despite the European ban on PCBs in the mid-1980s, large amounts still require disposal.[4,7] Legacy PCBs continue to enter the marine environment via several mechanisms such as terrestrial run off, dredging and atmospheric transport and deposition.[8,9] In Swedish waters, many wildlife populations such as otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and the white-tailed eagle (*Haliaeetus albicilla*), have experienced population recoveries that coincide with a decrease of PCB concentrations in their tissues.[10] However, trends in the concentrations of PCBs in cetaceans in the United Kingdom (UK) have not been analysed since 2012, whereby it was reported that concentrations in harbour porpoises (*Phocoena phocoena*) had stabilised in the year 1998, at levels still deemed to be a toxicological threat.[11]

Determining the toxicological threat from PCBs is a challenging task. Whilst there are well-established dose-response relationships for many terrestrial species, which can be studied in laboratories, the direct impact PCBs have on marine apex predators remains uncertain.[12] Direct evidence of immune system impairment in marine mammals in captivity has been demonstrated

in a limited number of cases. Immune function tests both *in vitro* and *in vivo* in captive harbour seals (*Phoca vitulina*) showed seals exposed to higher levels of dietary organochlorines (including PCBs) experienced a reduction in host defence against viral infections.[13] Indirect evidence of the link between high PCB tissue burdens and immune system impairment has also been demonstrated by multiple epizootic outbreaks of morbillivirus in harbour seals and striped dolphins (*Stenella coeruleoalba)* in European waters.[14–16] Cumulative pathological investigations suggest that exposure to high concentrations of organochlorines (including PCBs) is a key factor in reducing host resistance.[17–19] Thus, observed adverse effects of PCB exposure in cetaceans are consistent with effects reported from laboratory studies on other mammals.[20]

While it is ethically and economically unviable to carry out controlled captive exposure experiments on marine mammals, the risks associated with pollutant exposure have been estimated in human health and wildlife epidemiology using logistic regression modelling. In 2006 it was estimated that there was an increased risk of infectious disease mortality in harbour porpoises of 2% associated with each mg kg^{-1} lipid increase in PCB blubber concentrations.[17] However, no studies have assessed whether this risk assessment is still appropriate for the UK population, given that this analysis was carried out fifteen years ago.

It is important, therefore, to reassess the current trends and levels of PCBs in UK cetaceans and understand how these relate to infectious disease mortality. In this study, we used the largest cetacean toxicology strandings dataset available to investigate the relationship between PCB concentrations and infectious disease mortality to quantify the change in risk at current exposure levels. We determined the temporal trends and current levels of PCBs in the blubber of UK-stranded/necropsied harbour porpoises using data collected between 1990-2017, which incorporated unpublished data from 2012-2017 with historical published data. We believe that

previously published analysis may have been confounded by the high proportion of underweight animals, present at the beginning of the study, as nutritional stress may have affected the concentrations of PCB in their blubber.[21] Hence, it is important to determine the trend using a larger dataset and controlling for confounding factors to understand the effectiveness of remediation strategies and the level of threat now posed to the population.

2. Materials and Methods

2.1 Sampling

Between the years 1990 and 2017 we determined blubber PCB concentrations for 814 UK-harbour porpoises from necropsies carried out according to standard cetacean post-mortem procedures.[22] The animals that were necropsied had stranded on land around the UK coast, typically on beaches, and so were opportunistically sampled. As part of these investigations individuals' length, weight, girth, sex, age class and the latitude and longitude of the stranding location were recorded. Toxicological analysis was only conducted on blubber samples from animals that had undergone minimal to moderate levels of decomposition, according to the condition scoring guide outlined in the post-mortem protocol.[22] This was to minimise the impact of changes in pollutant tissue dispersion and levels associated with decomposition.[23] The animals that were analysed for PCBs were otherwise assumed to be a random sample of the strandings that occurred over the study period. However, it should be noted that by prioritising fresher carcasses the sampling may be biased towards animals that died closer to shore, which may be skewed towards certain causes of death. We tested whether this was significant by fitting a linear model to the toxicological strandings dataset (n=814) and the overall strandings dataset (n=6734) and used

cause of death and the dataset as predictor variables. We found there was no statistical difference between the proportions of each cause of death in the datasets (F-value = 15.914, p-value > 0.05).

2.2 PCB Analysis

A standardised methodology was used, over the entire sampling period, to extract and preserve the blubber samples for contaminant analysis.[23] The CEFAS laboratory (Lowestoft) determined the concentrations of ∑25 CB congeners (on a mg kg^{-1} wet weight basis) using a method that was validated by continuing participation in the QUASIMEME laboratory proficiency scheme and followed the recommendations of the International Council for the Exploration of the Sea (ICES).[24–27] In cases where the congener/isomer concentrations were below the limit of quantification (<0.0003 or <0.0004 mg kg^{-1} wet weight), concentrations were set at half the limit, as per Law et al. (2012). The numbers of the International Union of Pure and Applied Chemistry CBs congeners analysed were: 18, 28, 31, 44, 47, 49, 52, 66, 101, 105, 110, 118, 128, 138, 141, 149, 151, 153, 156, 158, 170, 180, 183, 187, 194. We chose to analyse these congeners because they were relatively abundant in commercial PCB mixtures and have a broad range of chlorination. They also incorporate the seven PCBs prioritised for international monitoring by ICES. The sum of the individual congener concentrations was calculated and normalized to a lipid basis (mg kg^{-1} lipid) by solvent extracting lipids from the blubber and calculating the hexane extractable lipid content.[27]

2.3 Statistical Analysis

As part of the pathological investigations certain biological attributes were recorded including information on weight, length, age and sex. For smaller cetaceans like the harbour porpoise, a basic index of weight to length ratio is thought to be the most appropriate metric of body condition and is widely acknowledged as a good predictor of fitness in marine mammals.[28–30] The weight and length data variable for the individuals in this study followed a power relationship and so a power regression model was fitted to obtain a metric that could be used as a proxy for body condition. The residuals from the best-fit regression line were extracted and used for further modelling whereby, values above the model fit represented cases in good nutrition and individuals below the line represented cases in poor nutritional condition. Body length and sexual maturity were used to categorise the individuals into age classes. Neonates were defined as individuals with a body length less than 90cm, juveniles were defined as individuals with a body length greater than 90cm that were sexually immature and adults were defined as individuals with a body length greater than 90cm that were sexually mature.[31] For the purposes of this study the neonates and juveniles were grouped together and classed as subadults. Cause of death was divided into three categories: "trauma", "infectious disease" and "other" (including not established, starvation, neoplasia and live strandings). Date of stranding was used to categorise strandings into seasons (Dec-Feb "Winter", Mar-May "Spring", Jun-Aug "Summer", Sept-Nov "Autumn"). The latitude and longitude of the stranding location of each animal was collected and used to investigate geographical variation. We used the coordinates to categorise the individuals into three geographic areas, Scotland, West England & Wales and East England (Figure 1), that were previously defined in a study of contaminants in stranded cetaceans in the UK.[19]

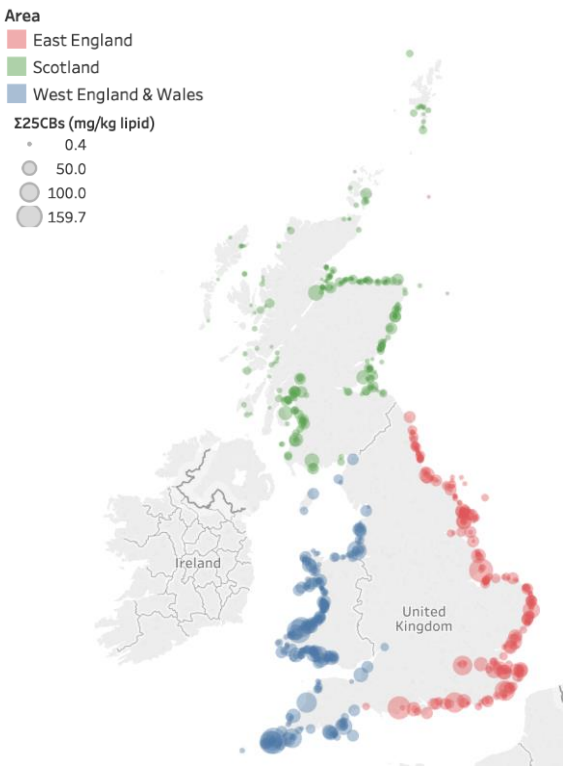

Figure 1: Geographic locations and area classifications of the individuals that stranded and were analysed to obtain blubber concentrations for the sum of 25 selected congeners of polychlorinated biphenyls (Σ25CBs). The colours of the dots represent the area classification and the dots are sized by the blubber concentrations of Σ25CBs.

2.3.1 Temporal and spatial trends

We carried out all of the analyses using the statistical software R (version 3.4.3).[32] Prior to model fitting we carried out extensive data exploration to identify collinearity between the variables (listed in the Sampling section), detect outliers and remove individuals with missing values. This resulted in a subset of 777 individuals being included in the analysis.

Previous analyses have shown that ∑25 CB concentrations are heavily influenced by factors such as nutritional condition, age class and sex that may confound temporal trends.[33,34] To account for this, we modelled ∑25 CBs with covariates, which were selected because there was existing evidence that they could affect ∑25 CBs concentrations, and used the model residuals for our

158 temporal analysis. Following extensive data exploration, we established that there was a linear
159 relationship between ∑25 CBs and other covariates. Therefore, we fitted several multiple linear
160 regression models to the variables, which could explain the variability in the data using ∑25 CBs
161 as the response variable. The variables included in the full model were nutritional condition, sex,
162 age class, cause of death, latitude, longitude and an interaction term between age class and sex.
163 We tested all possible variable combinations to obtain several candidate models which were ranked
164 according to their AIC (Akaike's Information Criterion) values. We selected the model with the
165 fewest predictors whereby the difference in AIC relative to the minimum AIC was <4.[35] We
166 performed model validation by assessing the diagnostic plots and plotting the model residuals
167 against selected variables to assess the variance.
168 To model temporal trends in ∑25 CB concentrations, generalised additive models (GAMs), with
169 an identity link function were fitted, to the smoothed number of days since the 1st of January 1990
170 and the residuals of the model fitted to ∑25 CBs and confounding covariates, using the *gam*
171 function available within the R library *mcgv* and *nlme*.[32,36] Thin plate regression splines were
172 applied to smooth the number of days to prevent over fitting of the model.
173 To investigate geographical variation, we fitted GAMs to a subset of the data for each
174 geographical area. The GAMs were fitted to the residuals of the same linear regression model,
175 between ∑25CBs and selected covariates, which was used for the whole of the UK. We chose to
176 subset the data rather than add area as a variable to the model because of limited data availability
177 in Scotland between 1990 and 1993. As a consequence, the trend for Scotland was modelled from
178 1994 onwards, data from previous years were excluded (n=8).
179 For all GAMs, the basis dimension to determine the degree of smoothing was determined using
180 the integrated smoothness estimation within *mgcv*.[32,36] This was validated using generalised cross

validation and visual assessment of the smoothing splines to assess whether the value of the smoothing dimension was appropriate.[36] To ensure the models were not over fitted the smoothing penalty term was set at 1.4 as per Kim and Gu (2004). Diagnostic plots were used to assess the models' assumptions of normality, heterogeneity and independence and the variances of residuals were examined for further model validation.

2.3.2 Infectious disease mortality

To calculate the PCB exposure odds ratio of infectious disease mortality, a subset of 641 of the 814 harbour porpoises were chosen for analysis (based on cause of death). Animals that died from neoplasia, live stranding, not established and starvation were excluded (n=126). Individuals were also excluded if their body weight, girth or length data were missing (n=47) to ensure the effect of nutritional status could be investigated. We used a case-controlled approach to compare animals that died of infectious disease (cases) with animals that died of trauma (controls) to investigate whether there was a relationship between high concentrations of PCBs and infectious disease mortality. There were 267 individuals in the infectious disease category and 374 individuals in the trauma category. The complete classification of cases and controls and detailed causes of death are shown in Supplementary Information Table S1.

PCB blubber concentrations are influenced by an individual's nutritional condition and blubber mass to the extent that large variation in condition and blubber mass can make concentrations incomparable.[38] To minimise the impact of this variation on our results we standardised the PCB concentrations, of nutritionally stressed individuals, according to an individual's blubber mass and condition as per the method defined by Hall et al. (2006a). The body mass and length power regression model was used to predict a body mass for each individual. We then used the

203 individual's actual body mass to calculate an estimated total blubber mass using a linear regression
204 model that relates blubber mass to body mass, length and girth measurements. We calculated a
205 standardised blubber mass by subtracting the individual's actual body mass from the predicted
206 mass and adding the estimated blubber mass. The adjusted PCB concentrations were estimated by
207 multiplying the PCB concentrations by the ratio of estimated blubber mass to the standardised
208 blubber mass. Concentrations were only adjusted for animals deemed to be nutritionally stressed
209 whereby their predicted body mass was greater than their actual body mass. This method is
210 outlined in Appendix I of the Supplementary Information and described in more detail by Hall et
211 al. (2006a).

212 We investigated the relationship between PCB blubber concentrations and infectious disease
213 mortality by fitting generalised linear models with binomial distributions and logit link functions.
214 Cause of death was used as the response variable, PCB concentrations and other selected covariates
215 were used as potential predictors. The potential predictors were selected according to biological
216 rationale that they could impact cause of death. The variables included in the full model were
217 nutritional condition, sex, age class, latitude, longitude, season, year of stranding with interaction
218 terms between age class and sex and between season and year. We used the same approach,
219 described in the temporal and spatial trends methods section, to extract a set of plausible models
220 from the candidate models. Our final prediction model was obtained by averaging the set of
221 plausible models. To validate the model, we plotted the residuals of the model against other
222 variables and assessed the variance. We assessed the model for over dispersion using the ratio of
223 deviance and residual deviance (1.051) and the value was within the proposed acceptable limits
224 outlined in the literature (<1.5).[39] Further model validation was carried out by conducting the
225 Hosmer Lemeshow Goodness of Fit test, which indicated a good fit.[40]

3. Results

3.1 Long term trends in blubber PCB concentrations in UK-stranded harbour porpoises

Our results clearly illustrate that in 2007 modelled mean PCB concentrations (∑25 CBs) in the blubber of harbour porpoises fell below the most widely used threshold for toxic effects (9 mg kg^{-1} lipid) derived by Jepson et al (2016), when the UK was treated as a single geographical region (Figure 2). However, 39% (n=15/38) of the individuals sampled in 2016 and 2017 exceeded this threshold. Moreover, when we modelled the sub-regions in the UK separately, we found geographic variation in PCB concentrations and rates of decline.

We show that at the beginning of the study period (1990-1998) blubber PCB concentrations appeared to be in decline (Figure 2). This decline appeared to stop around 1998 after which concentrations were stable until 2006. In the most recent years of the study PCB blubber concentrations have begun to decline again and, in 2007, fell below the established threshold for toxic effects in marine mammals.[12]

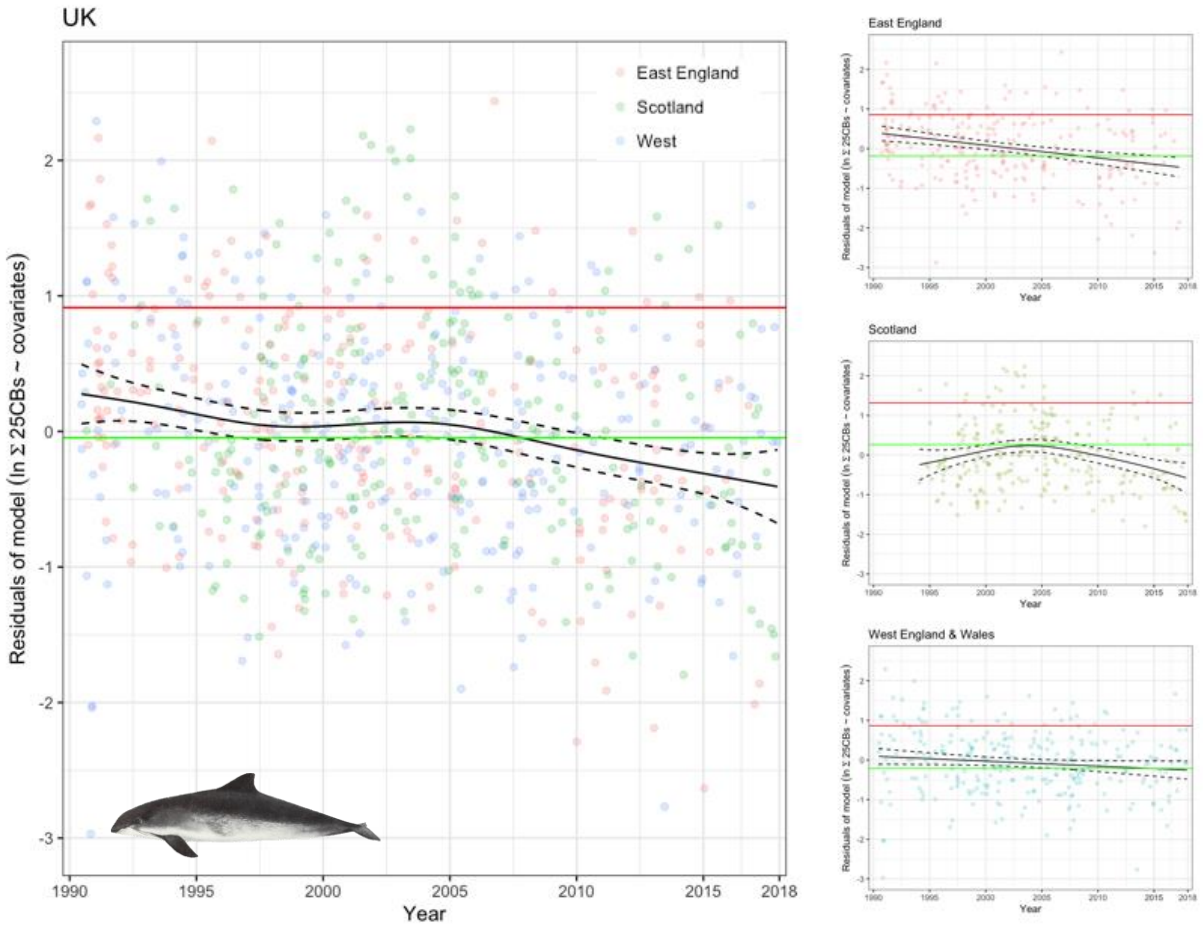

Figure 2: The smoothing splines from the generalised additive models fitted to the residuals of the linear regression model (Equation 1) against number of days since the 1st of January 1990 for the UK and three sub-regions. The solid line represents the smoothed trend and the dashed lines represent twice the standard error. The green lines represent the most widely used proposed threshold for toxicological effects of polychlorinated biphenyls in cetaceans (9 mg kg^{-1} lipid) [12]. The red lines represent the highest proposed threshold for toxicological effects (41 mg kg^{-1} lipid) [41]. For clarity points less than -3 were removed (UK n=3, East England n=2, Scotland n=0, West England & Wales n=1).

When we modelled the geographic sub-regions separately, we found inter-regional variation as well as variation between the regions and the whole of the UK (Figure 2). We found that East England and West England and Wales showed a steady decline over the entire period, however,

the rate of decline was greatest in East England. Levels in East England appear to have fallen below the most widely used threshold for toxic effects (9 mg kg^{-1} lipid) in 2007, approximately two years later than the UK as a whole. Levels in West England and Wales appear to have fallen below the threshold in 2017 however, the standard errors span the threshold. We found that, unlike the other two areas, modelled mean concentrations in Scotland did not experience a continuous decline over the study period. We found that PCB blubber concentrations increased at the beginning of the study period and peaked around 2004 after which they declined steadily. We found that modelled mean concentrations were higher on the west coast of Scotland than the east coast (see Supplementary Info Figures S1 & S2).

The final form of the model used to extract residuals that were fitted to the GAMs was linear and included nutritional condition, latitude and an interaction term between sex and age class as explanatory variables (Equation 1). Summary statistics have been included in the Supplementary Information Table S2.

$$\ln \sum 25CBs \sim \beta_0 + \beta_1 Nutritional\ condition + \beta_2 Sex + \beta_3 Age\ Class + \beta_4 Latitude + \beta_5 Sex * Age\ Class$$

Equation 1: The final form of the model used to extract residuals that were fitted to the GAMs.

3.2 Association between PCB exposure and infectious disease mortality

Our results clearly show that PCB exposure is associated with an increased risk of infectious disease mortality in harbour porpoises (Table 1). We found that the exposure odds ratio for ∑25 CBs blubber concentrations and infectious disease mortality was 1.05 (97.5% CI: 1.03-1.07). Hence, for a 1mg kg^{-1} lipid increase in ∑25 CBs blubber concentrations there is an increased risk of death from infectious disease of 5%. We found that nutritional condition was the biggest

predictor of death from infectious disease (Table 1). Subadults were predicted to have a lower risk of death from infectious disease than adults and males were predicted to have a lower risk than females. Animals that strand in winter were also predicted to have a significantly higher risk of infectious disease mortality.

*Table 1: Summary statistics of the logistic regression model fitted to the data where cases were defined as animals that died of infectious disease and controls were defined as animals that died from trauma. All continuous variables were centered and scaled. (The coefficient estimates were calculated in relation to a female adult that stranded in Autumn). * indicates statistical significance*

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)
(Intercept)	0.321	0.280	0.281	1.142	0.253
Subadult	-1.414	0.318	0.319	4.440	0.000*
Nutritional condition	-1.153	0.119	0.119	9.695	0.000*
Latitude	0.216	0.119	0.119	1.815	0.070
Longitude	0.166	0.120	0.120	1.388	0.165
Spring	0.375	0.282	0.283	1.327	0.184
Summer	-0.256	0.293	0.294	0.873	0.383
Winter	1.232	0.289	0.289	4.261	0.000*
Male	-1.097	0.355	0.355	3.088	0.002*
∑25 CBs	0.669	0.134	0.135	4.970	0.000*
Subadult Male	0.721	0.496	0.496	1.452	0.147
Year	0.036	0.076	0.076	0.469	0.639

To investigate the variation in mortality risk over different ranges of blubber PCB concentrations the increase in risk was calculated across various concentration differences (Figure 3). We found that at the population mean adjusted concentration of 11.5 mg kg^{-1} lipid there is an increased risk of death from infectious disease of 59% (97.5% CI:36%-82%). If we take the population mean concentration from the final year of the study (8.09 mg kg^{-1} lipid) there is an increase in risk of 41% (97.5% CI:25%-58%).

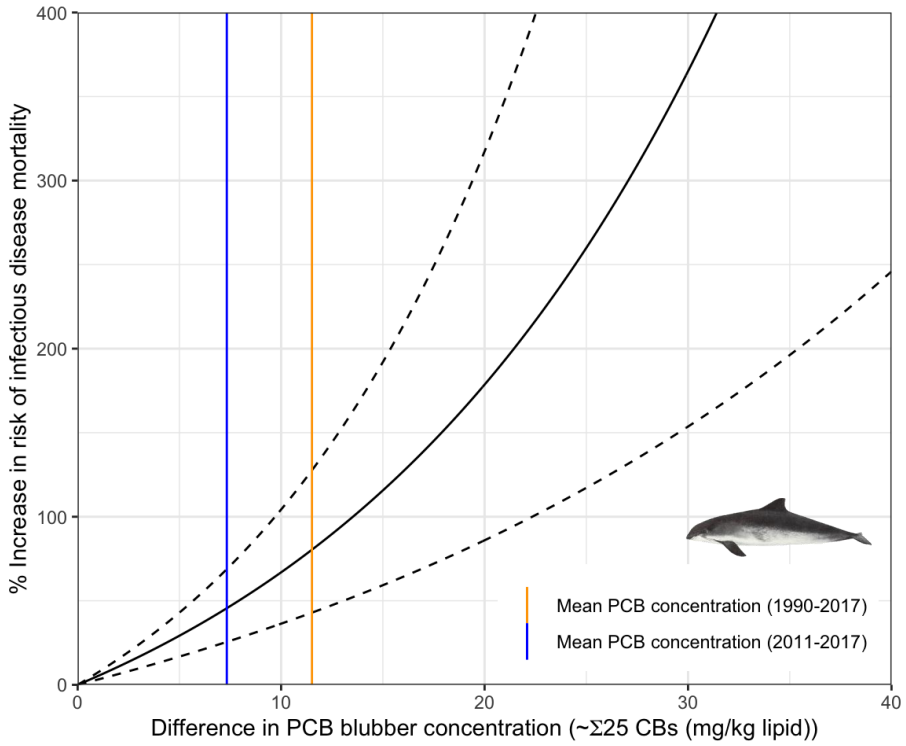

Figure 3: The increased risk of death by infectious disease against the adjusted sum of 25 chlorobiphenyl congeners (∑25 CBs (mg kg^{-1} lipid)) blubber concentrations as predicted by the logistic regression model. The population mean over the entire study period (orange line) and over the last five years of the study (blue line) have been included for reference. The dashed lines represent the 97.5% confidence intervals.

4. Discussion

Here we show that modelled mean PCB concentrations in the blubber of harbour porpoises in the UK have fallen below the established threshold for toxic effects (9 mg kg^{-1} lipid).[12] However, we found concentrations are still associated with increased rates of infectious disease mortality. Moreover, the inclusion of fifteen years of additional data in our epidemiological analysis, has more than doubled the previous estimate of the increase in risk associated with a 1mg kg^{-1} lipid increase in PCB concentrations, from 2% to 5%.[42] Moreover, when we compare the trend for PCBs with other persistent organic pollutants (POPs) in marine mammals (e.g.

hexabromadecadane, brominated diphenyl ethers, hexachlorobenzene, hexachloro-cyclohexanes, dieldrin, dichlorodiphenyltrichloroethane) we see that concentrations of other POPs have declined much more rapidly, despite legislation to control the production and disposal of PCBs being implemented at a similar time to other POPs.[19,43] The slower rate of decline in comparison to other POPs is likely to be a combination of higher initial levels of contamination, greater persistence of PCBs and the continued release of PCBs into the marine environment via diffuse inputs.[4]

Our results are in agreement with some studies that have investigated the temporal trends of PCB concentrations in fish, soil and the atmosphere in the UK and globally, which observed downward trends.[44–46] However, the most recent Marine Strategy Framework Directive (MSFD) assessment of mussels and four species of fish in the UK found evidence of nuanced geographical and taxonomical trends with declines occurring in some but not all populations.[47,48] Despite our finding that levels in the UK harbour porpoise are declining we observed a slower rate of decline in harbour porpoises when compared with overall trends in fish in the UK, which is likely to be the result of a combination of their high trophic feeding position and relatively long-life span causing a lag in any decline.[48]

Despite finding that blubber PCB concentrations for the UK have fallen below the most widely used toxicity threshold we have shown that there are still individuals that are above this threshold. We also found distinct geographical differences in the trends and overall levels. We found that levels in West England and Wales are experiencing a slower decline than the rest of the UK and may still be above the toxicity threshold. This variation may be explained by spatial ecology for example, individuals in different geographical areas may have different feeding ecologies, which could affect PCB accumulation rates. However, we believe the most likely explanation is that PCBs are continuing to enter the environment at a higher rate in West England and Wales as this

is where PCBs were traditionally produced, therefore there may be higher amounts of legacy PCBs in the region.[49] Indeed, the most recent OSPAR assessment of sediment concentrations found that there was no significant downward trend in the Irish and Scottish West Coast and that mean concentrations were higher in the Irish Sea than the Northern North Sea and the Irish and Scottish West Coast.[50] We observed that there was a period where levels increased in Scotland that corresponded with levels decreasing in the other areas. PCB concentrations are dependent on a number of factors including the eutrophication of systems, the variability of sinks such as degradation and the variability of volatizasation rates and run-off from land.[49,51,52] However, we believe it is likely that the differences in concentrations between Scotland and the West coast of England and Wales were partially driven by the dispersal of PCBs over time from areas where they were produced to previously uncontaminated areas.[49,53] This phenomenon has been well documented in the Northern Hemisphere whereby, PCBs are transported from midlatitudes, where they were manufactured, to the Artic.[54] A hypothesis for the mechanisms of PCB dispersal known as the "differential removal hypothesis" states that the dispersal of PCBs is primarily driven by a gradient of contamination levels whilst, a previous hypothesis states that dispersal is driven by latitudinal temperature gradients.[55,56] Both of these explanations would fit with the differences observed between the trends in Scotland and West England and Wales. Therefore, the higher amounts of PCBs entering the environment in West England and Wales may be transported to Scotland, via environmental transport or animal movements, causing the increase in PCB concentrations in Scotland between 1994-2005. Hence, it is vital to carry out remediation work in the UK to prevent PCBs entering the environment and ensure levels remain below the toxicity threshold.

Thresholds for the toxic effects of PCBs in harbour porpoises are typically derived from toxicological data on other species for a variety of end points and should therefore be interpreted as an approximation in the absence of more accurate toxicology data. The significant increase in risk of infectious disease mortality associated with PCB blubber concentrations supports previous findings that PCBs *in vivo* and *in vitro* can cause immunosuppression in marine mammals.[5,13,57] When we compared our results with a previous study we found a higher exposure odds ratio of 1.05 compared with the previous study's odds ratio of 1.02.[58] Despite levels now being below established toxicological thresholds, we found mean PCB concentrations, in the most recent year of the study, were associated with a 41% increase in risk of infectious disease mortality. This suggests that PCB contamination may still be causing an increase in the number of deaths from infectious disease.[12]

In addition to the increase in risk associated with PCB concentrations we also found that age class, season, nutritional condition and sex had a significant effect on the risk of infectious disease mortality. The higher mortality risk found in adults may be due to adults being exposed to a greater number of pathogens, as a result of differences in prey choice, and subadults being more vulnerable than adults to other causes of death including starvation and bycatch.[59,60] It is also possible that our model was unable to differentiate between the effects of age class and PCB concentrations. Hence, the effect of age class was confounded by PCB concentrations and so increased PCB levels in adults were the cause of the higher mortality risk. The higher risk of infectious disease mortality found in females could be caused by a possible weakening of the immune system, during reproduction and lactation, causing them to be more susceptible to infectious disease. Seasonal differences in pathogen types and abundance may explain why infectious disease mortality was

greatest in winter.[61] Moreover, animals' immune systems may be more likely to be compromised in winter because of colder water and reduced prey availability.[62]

We found that nutritional condition has a large effect on the odds ratio of infectious disease mortality. The relationship between nutritional condition and death by infectious disease are, however, intrinsically linked as nutritional stress can inhibit the immune system whilst infectious disease can cause nutritional stress. This stress can trigger blubber loss as the animal uses energy stores and this in turn can cause PCBs held in the blubber and other fat-rich body tissues to mobilise into the bloodstream where they are more toxic and can increase the likelihood of the animal contracting and dying from an infectious disease.[21,38] Therefore, in an attempt to control for changes in PCB concentrations in nutritionally stressed animals the PCB concentrations were standardised according to nutritional condition. Whilst there are still levels of uncertainty in this approach it is still reasonable to conclude that the increased risk of infectious disease mortality is because of higher PCB exposure in the cases than the controls as opposed to nutritional stress causing increased PCB concentrations.

Our findings make an important contribution to understanding more about the temporal trends of PCBs and possible drivers of infectious disease mortality in cetaceans. However, the scope of our study did not include whether risk varies according to different pathogens or parasites and our analysis does not include non-fatal infections. We also cannot completely rule out that selection bias in the controls may have impacted our findings. While we attempted to select the cases and controls independently of PCB exposure there is a possibility that animals that died of physical trauma had a higher or lower mean PCB exposure than the general population, which could result in an under or over estimation of the odds ratio. However, we suspect that this is likely to have a minimal effect as there is no evidence to suggest that animals that die from physical trauma have

altered PCB concentrations. It is also important to note that animal movement and carcass drift may have affected our results. Very little is known about the home range size of harbour porpoises in the UK. Therefore, large home ranges or the movement of carcasses in ocean currents may cause individuals to accrue contaminants in a different location to where they strand. However, a tracking study of harbour porpoises in the Bay of Fundy and Gulf of Maine, Canada, found that none of the tracked individuals left the Gulf of Maine during the 66 day tracking period.[63] Hence, if UK porpoise movements are similar to those in the Gulf of Maine then the geographical boundaries that we have used should be large enough to minimise the impact of animal movement on our results. Moreover, the effect of carcass drift should be minimised by selecting recently deceased carcasses because this increases the likelihood that an animal died close to where they stranded, as only decomposed, gas-filled carcasses can float and drift long distances.[64]

The association between PCB exposure and infectious disease mortality in cetaceans is well documented in the literature.[5] Moreover, there are several epidemiological studies that add to the weight of evidence.[13,18] Our study confirms this association and further indicates an increased risk of mortality from exposure at lower levels than have been previously suggested.[12,42]

However, it is important to consider that logistic regression modelling attributes a measure of risk for each unit increase in concentration. Yet, toxicity thresholds are typically based on a fixed level at which negative effects occur. If this is the case with PCBs and immunosuppression, then there may be no increased risk below a certain concentration. However, a number of cetacean studies both *in vivo* and *in vitro* have demonstrated that PCBs cause immunosuppression in a dose-dependent manner.[5,13,57] Further effort is required to understand whether risk increases from zero or whether there are safe limits whereby no negative effects occur. Nonetheless we have found a significant association between PCB blubber concentrations and infectious disease mortality,

which is particularly important in the context of other cetacean species. Specifically, in very coastal species such as bottlenose dolphins, which have been shown to have high PCB concentrations, in the UK and in some enclosed Mediterranean areas.[11] Similarly, killer whales have been shown to accumulate the highest concentrations of PCBs in cetaceans, and populations in the UK and Strait of Gibraltar face an immediate threat of extinction from exposure at population level.[65]

This is the first epidemiological study to show that PCBs are still a threat to harbour porpoises in the UK despite mean concentrations having fallen below established levels of toxicological concern (9 mg kg^{-1} lipid).[12] We have shown for the first time that levels of PCBs in UK harbour porpoises are declining. However, concentrations still appear to be associated with an increased risk of infectious disease mortality and the rate of decline of PCBs appears to be slow when compared with studies on other pollutants. In addition, we found considerable variation in concentrations and rates of decline between the sub-regions, which suggests PCBs are continuing to enter the environment. Our findings have serious management implications as we suggest that more remediation action is required to reduce or prevent further discharges to ensure that levels continue to decline and remain below the thresholds for toxic effects. We also suggest that the risk of contamination from secondary sources should be mitigated against via strict international compliance with the Stockholm Convention.[2] This study makes an important contribution towards understanding the trends of pollutant exposure in cetaceans and assessing associated risks; however, further research is required to quantify more robust toxic thresholds for chronic exposure to PCBs.

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Supporting information

Appendix I: Method description for standardizing PCB concentrations to adjust for nutritional stress

Appendix II: Supplementary Figures and Tables

Table S1: Detailed cause of death for cases and controls

Table S2: Summary statistics of the linear model fitted to the PCB blubber concentrations and selected covariates.

Figure S1: The smoothing splines from the generalised additive models fitted to the residuals of the linear regression model (Equation 1) against number of days since the 1st of January 1990 for the West coast of Scotland.

Figure S2: The smoothing splines from the generalised additive models fitted to the residuals of the linear regression model (Equation 1) against number of days since the 1st of January 1990 for the East coast of Scotland.

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