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# Juvenile harbor porpoises in the UK are exposed to a more neurotoxic mixture of polychlorinated biphenyls than adults

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## Abstract

Polychlorinated biphenyls (PCBs) are a group of 209 persistent and bio-accumulative toxic pollutants present as complex mixtures in human and animal tissues. Harbor porpoises accumulate some of the highest levels of PCBs because they are long-lived mammals that feed at a high trophic level. Studies typically use the sum of a suite of individual chlorobiphenyl congeners (CBs) to investigate PCBs in wildlife. However, toxic effects and thresholds of CB congeners differ, therefore population health risks of exposure may be under or over-estimated dependent on the congener profiles present. In this study, we found congener profiles varied with age, sex and location, particularly between adult females and juveniles. We found that adult females had the highest proportions of *octa*-chlorinated congeners whilst juveniles had the highest proportions of *tri*- and *tetra*-chlorinated congeners. This is likely to be a consequence of pollutant offloading between mothers and calves during lactation. Analysis of the individual congener toxicities found that juveniles were exposed to a more neurotoxic CB mixture at a time when they were most vulnerable to its effects. These findings are an important contribution towards our understanding of variation in congener profiles and the potential effects and threats of PCB exposure in cetaceans.

## 1. Introduction

Polychlorinated biphenyls (PCBs) are a large group of chemically related persistent and bio-accumulative pollutants known to cause neurotoxic and endocrine disrupting health effects in humans and wildlife (Faroon et al., 2003; Folland et al., 2016). Despite the European ban on PCBs in the mid-1980s, vast quantities still remain in stockpiles or in contaminated materials stored in landfills (DEFRA, 2013; Stuart-Smith and Jepson, 2017). Such legacy PCBs continue to enter the marine environment via several mechanisms such as terrestrial run off, dredging

and atmospheric transport (Jartun, 2011; Minh et al., 2006). Additionally, despite the ban, there are still PCBs in ‘open application’, such as in paints and sealants, that are also being released into the environment (Stuart-Smith and Jepson, 2017). Hence, PCBs are found globally throughout the terrestrial and marine environment and typically occur in the food chain as complex mixtures. The highest levels are found in long-lived odontocetes that feed at a high trophic level, where they have been associated with suppression of the immune and reproductive systems and are thought to contribute to population declines in several species (Desforges et al., 2018; Hall et al., 2006a; Murphy et al., 2015).

The mechanisms of toxicity and potencies of individual congeners vary according to differences in chemical structure (Hansen, 1998). All 209 chlorinated biphenyl (CB) congeners have the same basic structure: two connected phenolic rings with between one and ten chlorine molecules at different positions around the rings. Dioxin-like (DL) PCBs (similar in structure to dioxin) bind to the aryl hydrocarbon receptor (AhR) and induce the cytochrome P450 monooxygenase enzyme (CYP) 1A subfamily. DL PCBs were classically thought to be the most toxic and anti-estrogenic congeners due to their persistent and high-level stimulation of the AhR (Kodavanti and Loganathan, 2014). However, numerous toxic effects have been reported for nondioxin-like (NDL) PCBs that act independently of the AhR (Hansen, 1998; Pessah et al., 2019). A review of several neurotoxicity studies concluded that NDL lower chlorinated (*di-* and *tri-*) congeners were the most neurotoxic (Hansen, 1998). NDL *tetra-*chlorinated congeners (e.g. CB47) and less chlorinated mixtures have also been shown to cause greater disruption to thyroid homeostasis than highly chlorinated congeners (e.g. CB99, CB153) (Hansen, 1998). Whilst, NDL lower chlorinated PCBs have been shown to cause greater oxidative stress in cerebellar neurons than DL PCBs (Pessah et al., 2019). Hence, studies that estimate PCB mixture toxicities based on dioxin-like congeners and toxic equivalent factors (TEFs) may be inadequate.

To standardise and simplify monitoring of PCBs in human and animal tissues, the sum of concentrations of certain CB congeners are traditionally used to determine PCB body burdens. Risk assessments for these PCB exposures in marine mammals are carried out using toxicity thresholds for summed concentrations of PCBs derived by combining results from laboratory studies and field studies on otters, mink and seals (*Lutra lutra*, *Mustela vison*, *Phoca vitulina*) (Kannan et al., 2000a). Unfortunately, as these thresholds were based on summed congener concentrations, the CB congeners and their ratios in the mixtures varied between the studies and were not published in full. As a consequence, the total toxicity of PCB burdens in marine mammals may be more or less toxic than predicted using current approaches, dependent on the complete profile of PCB congeners present (Peñín et al., 2018). This may be particularly important during pregnancy and lactation where energetic demands cause the mobilization of contaminants into circulation and some of the maternal toxicant burden to be transferred to the calf via lactation, which has been associated with reduced calf survival in cetaceans (Schwacke et al., 2002). Variation in congener profiles between mothers and their offspring, as well as differences in the proportion of highly chlorinated congeners transferred during gestation compared with lactation, could profoundly affect PCB toxicity (Hamers et al., 2011; Hansen, 1998; Iwata et al., 2004; Pěnčíková et al., 2018). Therefore, to understand whether calves are at a different risk to adults and whether current toxicity thresholds are adequate, it is important to determine not just the total burden, but also the congener profile of the PCB mixture to which they are exposed.

To date no studies have addressed this question in cetaceans at population level. To investigate this question, we used the largest available cetacean toxicology dataset in the world, to obtain the blubber PCB congener concentrations of harbor porpoises stranded between 1992 and 2015. We used the PCB blubber concentrations to determine whether the congener profiles of individuals differ by age class and sex, in response to possible differences in pollutant

offloading from mothers to calves. We also considered whether the congener profiles of individuals vary according to their stranding location, possibly resulting from different chemical compositions of polluting sources, timings of release and atmospheric transport.

## **2. Materials and Methods**

### **2.1 Sampling**

Between 1992 and 2015 blubber congener concentrations were determined for 696 UK stranded harbor porpoises. Detailed and standardised necropsies were carried out according to standard procedures for cetaceans (Law et al., 2006). Blubber samples were taken from the left side of the body, at the caudal insertion of the dorsal fin. To minimise the impact of changes in pollutant levels and tissue dispersion with body decomposition, toxicological analysis was prioritised for blubber samples from freshly dead cases as defined in the necropsy protocol (Law, 1994; Law et al., 2006). Animals chosen for toxicological analysis were otherwise assumed to be a random sample of all the strandings that occurred over the study period.

### **2.2 PCB Analysis**

Blubber samples for contaminant analysis were taken and preserved at  $-20^{\circ}\text{C}$  using established protocols (Law, 1994). The concentrations of 25 PCB congeners, were determined (on a  $\text{mg kg}^{-1}$  wet weight basis) by the CEFAS laboratory (Lowestoft) using a method that followed the recommendations of the International Council for the Exploration of the Sea (ICES) and had been validated following participation in the QUASIMEME laboratory proficiency scheme (de Boer and Law, 2003; de Boer and Wells, 1997; ICES, 1998; Webster et al., 2013). In cases where the congener/isomer concentrations were below the limit of quantification ( $<0.0003$  or  $<0.0004$   $\text{mg kg}^{-1}$  wet weight), concentrations were set to zero. The numbers of the International Union of Pure and Applied Chemistry CB 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. These congeners were chosen for analysis based on their relatively high concentrations in commercial PCB mixtures and their wide range of chlorination. The individual congener concentrations were calculated and normalized to a lipid basis ( $\text{mg kg}^{-1}$  lipid) by solvent extracting lipids from the blubber and calculating the hexane extractable lipid content.

For Quality Assurance and Quality Control the CEFAS laboratory (Lowestoft) participates biannually in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency testing scheme. All analyses were carried out under full analytical quality control procedures, which included the analysis of a blank sample and the analysis of a certified reference material with every batch of 10 samples to assess the performance of the methods. Blanks for individual PCBs were always below the limit of quantitation. Where the levels of target analytes were beyond the range of the instrument calibration, we diluted and re-analysed the extract. We used the reference material BCR349 (cod liver oil; European Bureau of Community reference) and for each compound we plotted the reference material results as Shewhart quality control charts. The charts were created previously from repeated analysis of the reference material using the North West Analytical Quality Analyst software™ (Northwest Analytical Inc., USA). The warning and control limits for the charts were defined as  $2\sigma$  and  $3\sigma - 2x$  and  $3x$  the standard deviation from the mean for each compound. For each of the samples analysed the certified reference materials were within the limits set by the control charts. Therefore, all results were deemed to be valid. The expanded uncertainty MU (calculated as  $2 \times$  standard deviation of the control charts for the BCR349 reference material from the last 10 years) for the ICES7 PCBs ranges from 11.9% for CB153 to 17.9% for CB28, which is well within the requirement to be  $<50\%$  (SANCO, 2011).



### 2.3 Statistical Analysis

All analyses were carried out using the statistical computer programme R (version 3.4.3) (R Core Team, 2016). We used a subset of the harbor porpoise stranding data (n=696), which only included trauma cases with complete age class data (n=347). This was to control against any confounding influence from the remobilisation of PCBs that may occur in sick and underweight animals (n=349). We expressed the congener concentrations as fractions of the sum of the 25 congeners that were measured ( $\Sigma 25$  CBs). We investigated any differences due to age class and sex by categorising all animals by age class and the adults by sex. Individuals were categorized into age classes according to their body length and sexual maturity. We determined sexual maturity using gonadal appearance and by looking for histological evidence of spermatogenesis in male testes. To ensure that the method of classification was reliable over the extended time period of the study we established that there was no temporal trend in body length. Individuals with body lengths greater than 90cm that were sexually mature were classified as adults, juveniles were classified as individuals with body lengths greater than 90cm that were sexually immature and individuals with body lengths less than 90cm (Jepson, 2003). Exact age was determined for a subset of individuals (n=236) by quantification of growth layer groups from analyses of decalcified tooth sections using the methods outlined by Rogan et al., (2004) and Lockyer et al., (1995). The pooled standard deviation associated with this technique has been shown to be <0.5 years therefore, associated errors should not impact age class determination (Bjoerge et al., 1995). We did not categorise the juveniles by sex as we wanted to investigate the possible effect of pollutant offloading from mothers to calves. Levels of PCBs in females accumulate until they reach sexual maturity and successfully reproduce and offload some of their PCBs to their calves, after which their body burdens decline

following each successful pregnancy. Females that do not successfully reproduce and males continue to accumulate PCBs over their lifetime via prey ingestion (Tanabe et al., 1981). Therefore, differences in mean PCB burdens between the sexes only occurs in sexually mature animals. We conducted analysis that verified that there were no significant differences between male and female juveniles (See Supplementary Information Tables S4 & S5). Thus, it was only relevant to categorise adult animals by sex. We were unable to categorise females according to whether or not they had successfully reproduced however, if congener variation is associated with maternal transfer of pollutants, the large sample size should ensure this is still apparent even if some non-reproducing females are present in the data.

This led to three groups being investigated (juveniles, adult males and adult females) whereby we compared adult females with adult males and adult females with juveniles to test whether there was evidence of selective transfer of congeners via lactation. To investigate geographical variation, we categorized the stranding locations into three geographic areas that were previously defined in a study assessing temporal trends of contaminants in stranded cetaceans (Law et al., 2012). The three areas were Scotland, the West (defined as the west coast of England and Wales including the south-west coast up until Dorset and the Isle of Man) and East England (defined as the east coast of England and the south-east coast up to and including Dorset). We chose to categorise the locations in this way because the division of the east and west coasts in England and Wales allowed for any longitudinal differences to be observed. While the division of Scotland from England and Wales allowed us to observe any latitudinal differences.

To determine whether the degree of chlorination and the metabolic pathway of congeners affected the PCB profiles we carried out analyses using two separate classifications (Table 1). First, we grouped the congeners according to their degree of chlorination. This method of

classification allowed every assayed congener to be assigned a group and has been proposed in the literature as the most suitable classification method (Moysich et al., 1999; Warner et al., 2012). Furthermore, increasing degree of chlorination corresponds with increasing lipophilicity and can therefore highlight certain accumulation patterns that may not be apparent when analysing congeners individually (Safe and Hutzinger, 1984). Second, we classified the congeners according to their metabolic pathway/structural activity group (SAG). This classification scheme was proposed in a study that suggested cetaceans have reduced capability of metabolising nondioxin-like PCBs, in comparison to other mammals, because the activity of their CYP2B enzyme is lower (Boon et al., 1997).

To investigate congener profile variation, we used principal component analysis (PCA) to examine the data before grouping any congeners. We then grouped the congeners by degree of chlorination and carried out PCA to further assess variation between age class and sex. We fitted linear models and carried out Tukey's Honestly Significant Difference (HSD) tests to identify which differences were statistically significant. We investigated the association between the metabolic pathway of the congeners and congener profiles by conducting PCA on the congeners grouped by their structural activity group (SAG). To explore the differences further the five SAGs were grouped into two further groups according to their reported persistence in cetaceans (highly persistent and less persistent) (Boon et al., 1997). SAGs 1, 2 & 5 which are not readily metabolised in cetaceans were grouped together and SAGs 3 and 4, which are metabolised by CYP-450, were grouped together. SAGs 1, 2 & 5 are dominated by the more highly chlorinated *hexa*- and *hepta*-chlorinated congeners whilst SAGs 3 & 4 are dominated by the less chlorinated *tri*- and *tetra*-chlorinated congeners. Where age data was available (n=236) we fitted linear regression models to the data where the proportion of  $\Sigma 25$  CBs in each group was the dependent variable and age was the predictor variable. The proportion of  $\Sigma 25$  CBs was log transformed to reduce variance and meet the assumption of

normality. We selected the best models by comparing models with different variables and forms and chose the models with the fewest predictors whereby the difference in AIC (Akaike's Information Criterion) relative to the minimum AIC was  $<2$  (Akaike, 1973).

To ensure there was not an overrepresentation of animals from certain locations, time-periods, age classes or sexes, which could affect the results, we calculated the proportions in each category to assess the likelihood of bias. We found that there were no large overrepresentations (See Supplementary Information Table S7 & S8). The relatively large sample size should also help to mitigate this risk of bias. We also carried out separate PCAs, for age class and sex, using subsets of the data. We subsetted the data spatially and temporally to ensure our conclusions were consistent and not confounded by overrepresentation (See Supplementary Information Figures S5, S6 & S7).

For all of the analyses detailed above we used the mean proportion of the  $\Sigma 25$ CBs made up by each congener or group of congeners. For all of the PCAs the congeners were zero centred and scaled to have unit variance. For each analysis we plotted the first two components of the PCA and calculated the 70% confidence ellipses for the variables that we were investigating. Ellipse overlap between groups was estimated as per Jackson et al., (2011).

### **3. Results**

#### **3.1 Variation of Individual Congeners Profiles Between Age Class and Sex**

We found that 25 components were required to describe the variation in congener profiles. The first two components accounted for 25% and 19% of the variance respectively and revealed some clustering by age class and sex (Figure 1A). We found relatively little overlap between the groups (overlap: adult-females/juveniles = 0.19, adult-females/adult-males = 0.14, adult-males/juveniles=0.28). Therefore, it is reasonable to conclude that age class and sex drove

some of the variation in congener profiles between individuals. The congener specific loadings across the first two components (PC1 & PC2) are shown in the Supplementary Information Figure S10. CBs 170, 180, 183, 187 and 194 had high positive scores across both components. The other congeners had positive scores in PC2 and negative scores in PC1 with the exceptions of CB 138 and 153, which had positive scores for PC1, and CBs 52, 66, 138, 149, 151 and 153, which had negative scores for PC2.

The loadings arrows show that adult females had a greater proportion of the highly chlorinated congeners while juveniles had a greater proportion of the less chlorinated congeners. We tested the differences for significance between adult males and adult females and also between adult females and juveniles. We found adult males had significantly higher proportions of CBs 52, 138, 149, 151 and 153 when compared with adult females ( $p < 0.05$ ). We found adult females had significantly higher proportions of CBs 156, 170, 180, 183, 187 and 194 when compared with juveniles and juveniles had significantly higher proportions of CBs 52, 101, 118, 138, 149, 151, 153 when compared with adult females ( $p < 0.05$ ). All of the statistical results can be found in the Supplementary Information Table S1.

### **3.2 Variation of Congener Chlorination Profiles Between Age Class and Sex**

We found that five components were required to describe the variation in congener profiles (when grouped by their degree of chlorination). The first two components accounted for 53% and 29% of the variance respectively and also revealed clustering by age class and sex (Supplementary Information Figure S1). We found relatively little overlap between the groups (overlap: adult-females/juveniles = 0.20, adult-females/adult-males = 0.16, adult-males/juveniles=0.29). Analysis of the mean proportions of congeners (classified by degree of chlorination) found that adult males had significantly higher proportions of *hexa*-chlorinated congeners than adult females ( $p < 0.05$ ). When we compared adult females with juveniles, we

found juveniles had significantly higher proportions of *tri*-, *tetra*- and *penta*-chlorinated congeners whilst adult females had significantly higher proportions of *hepta*- and *octa*-chlorinated congeners ( $p < 0.05$ ) (Figure 2). All of the statistical results can be found in the Supplementary Information Table S6.

### 3.3 Variation of Congener Structural Activity Groups (SAGs) Between Age Class and Sex

When the congeners were grouped by their structural activity group (SAG) we found that five components were required to describe the variation in congener profiles. The first two components accounted for 57% and 21% of the variance respectively. The overlap between the ellipses was greater compared with the ellipses in the PCA plot for the individual congeners, particularly between adult males and females, suggesting less variation (overlap: adult-females/juveniles = 0.27, adult-females/adult-males = 0.64, adult-males/juveniles=0.29) (Figure 1B). The loadings for the first two components (PC1 & PC2) are shown in the Supplementary Information Figure S11. SAGs 1 & 2 had high positive scores in PC1 whilst SAGs 3, 4 & 5 had negative scores on PC1. SAGS 2,3, & 4 had positive scores on PC2 whilst SAGs 1 & 5 had negative scores on PC2. We found adult males had significantly higher proportions of SAG5 than adult females ( $p < 0.05$ ). We found juveniles had significantly lower proportions of SAG1 and significantly higher proportions of SAGs 3, 4 and 5 when compared with adult females ( $p < 0.05$ ). The complete statistical results can be found in the Supplementary Information Table S2.

After fitting linear models to the SAGs grouped by their reported persistence in cetaceans, (highly persistent [SAGs 1,2&5] and less persistent [SAGs 3&4]) we found there was a significant relationship with age ( $p < 0.05$ ). For highly persistent congeners the best fitting model included sex, location, year of stranding and age (Figure 3). However, for the less

persistent congeners sex was not included in the final model. The full model results are available in the Supporting Information Table S9. We found that proportions of the less persistent congeners declined with age and the proportions of the highly persistent congeners increased with age. Similarly, the proportion of highly persistent congeners increased over time (year of stranding) and the proportion of less persistent congeners decreased. Geographically, the proportion of highly persistent congeners was significantly higher in the West than Scotland and East England and vice versa for the less persistent congeners ( $p < 0.05$ ) (Supporting Information Table S3).

### 3.4 Geographical Variation of Congener Chlorination Profiles

As discussed in section 3.2 we found that five components were required to describe the variation in congener profiles (when grouped by their degree of chlorination). The first two components of the PCA revealed clustering by geographic area (Figure 4). The ellipses show that the profiles of individuals that stranded on the east coast of England had the greatest variance and their profiles overlap with animals that stranded in Scotland and the West (overlap: West/Scotland = 0.31, West/East = 0.69, East/Scotland=0.46). The data points and ellipses for the West and Scotland overlap the least suggesting greater variation between the profiles of individuals that stranded in these areas (Figure 4). The loadings arrows indicate that animals that stranded in the West had higher proportions of highly chlorinated congeners whilst animals that stranded in Scotland had higher proportions of less chlorinated congeners.

Analysis of the mean proportions of congeners (classified by degree of chlorination) found that animals that stranded in Scotland had significantly higher proportions of the less chlorinated (*tri-*, *tetra-*, *penta-* and *hexa-*) congeners than the West ( $p < 0.05$ ). While, animals that stranded on the West coast had the highest proportion of highly chlorinated (*hepta-* and

*octa-*) congeners ( $p < 0.05$ ). The full statistical results are in the Supporting Information Table S4.

Analysis of the mean proportions of congeners (classified by their persistence as defined by their SAG) found that animals that stranded in Scotland had the lowest proportion of highly persistent congeners and the highest proportion of less persistent congeners (Figure 5). While, animals that stranded in the West had the highest proportion of highly persistent congeners and the lowest proportion of less persistent congeners.

#### **4. Discussion**

Variation in congener profiles is likely to be driven by a number of factors such as differences in prey profiles, differences in the quantities and congener profiles of initial PCB contamination sources, differences in prey choices as well as differences in the complex physiochemical and physiological processes that occur between individuals. However, maternal offloading of pollutants to calves is the most plausible explanation for the variation we found between adult females and juveniles. The higher proportions of highly chlorinated congeners found in adult females and higher proportions of less chlorinated congeners found in juveniles suggest that the congener profile of the PCB mixture transferred from mother to calf is partially driven by the degree of chlorination because lower degrees of chlorination correspond to lower lipid solubility. However, the dynamics of PCB offloading in cetaceans are poorly understood.

Studies on humans and wildlife have found variation between the congener profiles of mothers, their milk and their offspring (Aguilar and Borrell, 1994; Debier et al., 2003; Fångström et al., 2005; Ramos et al., 1997). There are several factors driving this variation, including the molecular structure of congeners affecting tissue distribution and accumulation, age-related metabolic differences, foraging differences, milk fat content, lactation duration and reproductive histories (Addison and Brodie, 1987; Boon et al., 1997; Hall et al., 2006b; Reddy



et al., 2001; Yordy et al., 2010). Contaminant transfer rates between mothers and calves have been shown to decrease with each successful pregnancy as the first calf receives the highest pollutant load. Therefore, the dynamics of maternal offloading are heavily influenced by progeny number (Wells et al., 2005). Environmental factors such as the regional variation of contaminant concentrations, known to occur in the UK, are also likely to affect the rate and dynamics of maternal offloading (Law et al., 2012). Investigation of the contaminant profiles of stranded long-finned pilot whales (*Globicephala melas*) showed that the ratio of highly chlorinated congeners transferred between four mother and foetus pairs increased over the gestation period. Following pregnancy, higher proportions of less chlorinated congeners were transferred during lactation than during gestation (Weijs et al., 2013). In grey seals (*Halichoerus grypus*) the proportion of highly chlorinated congeners in mothers and pups has been shown to increase with lactation duration. However, the congener profile of the milk remained constant suggesting highly chlorinated congeners are selectively adsorbed (Debieer et al., 2003). The differences between these two marine mammal species further illustrate the complexity of pollutant transfer and accumulation dynamics.

The variation between adult females and males that is indicative of maternal offloading was greater when the congeners were grouped by chlorination or analysed individually than when the congeners were grouped by their structural activity group. Classification by structural activity group is based on cetaceans' capability to metabolise certain congeners more easily than others and so is not directly associated with lipid solubility. Therefore, it is likely that variation is influenced more heavily by age than sex.

For the congeners that were highly persistent and difficult to metabolise sex was a significant predictor, of the proportion of  $\Sigma 25\text{CBs}$ , in the model. Cetaceans cannot metabolise these congeners easily and so they accumulate high amounts (Boon et al., 1997). Adult females are

able to offload some of their burden to their calves. However, adult males continue to accumulate these congeners throughout their life and therefore tend to have higher proportions of these congeners than adult females because they do not detoxify through reproduction. We found that proportions of the highly persistent congeners increased with age. This was expected as these congeners are difficult to metabolise and excrete and so levels continue to build up over an animal's lifetime.

For the congeners that were less persistent and more easily metabolised sex was not included in the final model. It is likely that individuals across all age classes and sexes accumulate much lower levels of these congeners. Therefore, relatively small amounts will be transferred from mother to calf causing there to be no significant difference between levels in adult males and females. We found that proportions of the less persistent congeners declined with age. This is consistent with the congener metabolic pathway classification as the congeners are not resistant to biotransformation in cetaceans and so do not accumulate with age (Boon et al., 1997).

Year of stranding was a significant predictor in both models implying that congener profiles vary temporally, and animals are exposed to different PCB mixtures at different points in time. This is consistent with findings that showed that at the point of source congener profiles shift from being similar to the profile of the mixture released to a profile containing heavier congeners as lighter congeners are mobilised or metabolised (Saba and Boehm, 2011). Our results show that animals that stranded more recently are likely to have a higher proportion of more persistent PCBs because the less persistent PCBs take less time to be broken down. This suggests that the rate of PCB inputs into the environment is likely to be declining. The most recent Marine Strategy Framework Directive (MSFD) assessment of PCB concentrations in biota and sediment corroborates this finding. The assessment found that concentrations of the less persistent congeners (CB52, CB101) had decreased while all other congeners measured

showed no change. Moreover, concentrations of the less persistent congeners (CB52, CB101 and CB118) in biota had decreased in three of the four UK regions investigated while concentrations of the more persistent CB180 had only decreased in one of the regions (Maes et al., 2018; OSPAR, 2015).

Part of the reason for the temporal shift in congener profiles is that lighter congeners are dispersed more readily (Harner and Bidleman, 1996). Our study found geographical differences between the congener profiles when they were grouped by degree of chlorination and when they were grouped by metabolic pathway. Dispersal dynamics are governed by several factors including the congener's chemical properties, the presence of other compounds and environmental conditions. Hence, the toxicity of a PCB mixture at a specific geographic location is a function of both total concentration and the congener profile (Gioia et al., 2013). The geographical variation that we identified could be explained by differences in the congener profiles of the initial PCB sources and the timings of the releases. There may also be regional differences in diets which cause variation because different prey species may have different abilities to metabolise PCB congeners (Santos et al., 2004).

We found that the most significant differences in congener profiles, grouped by degree of chlorination and by structural activity group, were between Scotland and the West. PCBs were traditionally produced on the more heavily industrialised West coast (Harrad et al., 1994; Robin, 2010). In the UK it has been shown that PCB mixtures in air are dominated by *tri*- and *tetra*-congeners and that relative atmospheric concentrations of *tetra*-chlorinated PCBs increase with increasing latitude or decreasing temperature (Halsall et al., 1995; Ockenden et al., 1998). Therefore, the higher proportions of less chlorinated congeners in Scotland could be evidence of atmospheric transport whereby, the less chlorinated congeners are transported more readily than the highly chlorinated congeners. The variation could also be partly

explained by the metabolic pathway of the congeners whereby, the less persistent PCBs are bio-transformed over time. Therefore, higher proportions of more persistent congeners remain at the PCB source, as we see in animals that stranded in the West. The significant geographic variation of congener profiles further demonstrates that a single toxicity threshold for the sum of PCB congeners may not be adequate to determine population risk for UK harbor porpoises.

To gain a better understanding of the significance of these findings the toxicities of the profiles need to be quantified. The vast range of mechanisms, toxicities, and synergistic and antagonistic interactions make this a difficult task. Toxicity equivalent factors (TEFs) can be summed to quantify the overall toxicity of a mixture (Van den Berg et al., 2006). However, TEFs only exist for dioxin-like PCBs and so ignore a large number of modes of toxicity. Furthermore, of the congeners analysed in this study only three of them are dioxin-like. However, the literature can provide some insight into how chlorination affects toxicity. A review of congener toxicities collated four studies that determined the congener specific potencies for four different neurochemical end points and found that in every study lower chlorinated congeners were the most toxic (Hansen, 1998). The same review also looked at thyroid homeostasis and found the *tetra*-chlorinated congener CB47 and lower chlorinated mixtures decreased serum T4 more than highly chlorinated congeners. Lightly chlorinated PCBs have also been shown to impair dopamine signalling, promote tumor growth and cause increased oxidative stress when compared with dioxin-like congeners (Pěňčíková et al., 2018; Pessah et al., 2019). These results are concerning as we found the highest proportions of less chlorinated congeners in juveniles.

Dioxin-like (DL) congeners cause toxicity by binding to the aryl hydrocarbon receptor (AhR). The AhR affects a number of regulatory proteins and so DL congeners can cause numerous toxic effects including immunotoxicity and endocrine disruption (Kodavanti and

Loganathan, 2014). The AhR has also been shown to mediate neuro and vascular genesis, which are particularly important processes during development (Lahvis et al., 2000; Larigot et al., 2018). Of the three dioxin-like congeners measured in this study juveniles had significantly higher levels than adult females for CB 118 (Supporting Information Table S1 & Figure S8). As neurotoxicity, thyroid and endocrine disruption and enzyme induction are critical end points of toxicity particularly during development, finding higher levels of congeners, which cause these effects, in UK juvenile harbor porpoises is a great concern. Furthermore, studies have shown that levels of exposure that cause no effects in adults can cause biological effects in developing animals (Baumann et al., 1983; Vitalone et al., 2010).

We have shown that congener profiles vary by age class and sex in harbor porpoises and that there is a large weight of evidence to suggest different congeners have different toxic thresholds and modes of action. However, when discussing toxicity, it is important to consider this variation in the context of overall concentrations. In the absence of congener specific toxicity thresholds for cetaceans we compared congener concentrations with the Environmental Assessment Criteria (EAC) set out by the UK Marine Strategy Framework Directive used to assess congeners (28, 101, 105, 118, 153 and 180) in fish and shellfish. We found that for CBs 101, 118, 153 and 180 all juveniles exceeded the EAC. And for CBs 28 and 105, 60% and 81% of juveniles exceeded the EAC respectively (Maes et al., 2018; OSPAR, 2015). Furthermore, several of the juveniles in this study were found to have PCB concentrations that exceed the most widely used toxicity threshold in cetaceans of  $9 \text{ mg kg}^{-1}$  lipid (Supplementary Information Figure S12) (Kannan et al., 2000b). Therefore, it is reasonable to conclude that juveniles in this study were exposed to toxic concentrations of PCBs.

This is the first study to investigate possible drivers of PCB congener profile variation in a UK cetacean and we have shown that profiles vary with age class, sex and geographical

location. It should be noted that due to the nature of strandings data there may be some biases present. Congener profiles may be altered by decomposition and therefore profiles of stranded individuals may vary from those of live animals. Similarly, causes of death such as infectious disease or those that cause loss of blubber mass may affect the congener profile (Borrell and Aguilar, 1990). It is also important to note that animal movement is a limitation of the location stranding data. Some individuals may move over their lifespan or die and be carried in ocean currents and strand in a different location causing them to accrue contaminants in a different location to where they strand. However, this effect should be minimised by selecting freshly dead carcasses because this increases the likelihood that an animal died close to where they stranded. It is also important to note that conclusions around the different toxicities of mixtures are limited as this study has only considered variation of a restricted number of CB congeners. Much of the cetacean toxicology work in the UK has focussed on PCBs because levels in cetaceans are much higher than other measured pollutants. Furthermore, levels have not declined at the same rate as other pollutants and analyses have shown that levels may have stabilised (Law et al., 2012, 2008, 2003). Despite this, it is still important to consider that harbor porpoises are exposed to a wide range of other legacy and emerging contaminants that will also affect the toxicity of pollutant burdens (Andresen et al., 2007; Galatius et al., 2013). Therefore, it is important for future work to take a mixture toxicology approach to identify the emerging substances these animals are exposed to and evaluate the toxicology of the complete pollutant burden to estimate risk.

## **5. Conclusion**

Our results show that congener profiles of harbor porpoises in the UK vary with age class, sex and location. Despite the ban on the production and use of PCBs in Europe in the late 1980s PCBs continue to enter the environment and blubber concentrations in cetaceans remain high

(Jepson et al., 2016). Large variations in congener profiles are likely to equate to large variations in the overall toxicities of the congener mixtures and therefore it may no longer be sufficient to just look at overall burden. Our analysis has shown that calves are likely to be exposed to a more neurotoxic PCB mixture than adults. This is particularly concerning as developmental effects of PCBs have been shown to occur at lower levels of exposure (Baumann et al., 1983; Vitalone et al., 2010). Therefore, using toxicity thresholds that assume the same congener profiles across all age classes may result in an over or under estimation of risk. This work is an important contribution towards understanding more about the variation in congener profiles, the drivers of juvenile mortality and the potential effects and threats of pollutant exposure in cetaceans. However, the problem appears to be more complex than first thought. Further work is required to understand how toxic effects vary with different congener mixtures and at different life stages and how diet factors play a role so that we can better understand how to mitigate the risk of legacy pollutants in the marine environment.

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### **Supplementary information**

**Table S1:** Results of analysis of variance and Tukey's HSD tests between the mean proportions of each congener for each age class and sex

**Table S2:** Results of analysis of variance and Tukey's HSD tests between the mean proportions of each structural activity group for each age class and sex

**Table S3:** Results of analysis of variance and Tukey's HSD tests between the mean proportions of each structural activity group grouped by persistence for each age area

**Table S4:** Results of analysis of variance and Tukey's HSD tests between the mean proportions of each congener grouped by degree of chlorination for each age area

**Table S5:** Results of analysis of variance between male and female juveniles for mean proportions of each congener

**Table S6:** Results of analysis of variance and Tukey's HSD tests between the mean proportions of each congener grouped by degree of chlorination for each age class and sex

**Table S7:** Relative frequencies of age class and sex for each area

**Table S8:** Relative frequencies of age class and sex for each five-year interval

**Table S9:** Summaries of the linear regression models to the congeners grouped by their persistence in cetaceans

**Table S12:** Summary concentration data for each congener by age class and sex

**Table S13:** Summary concentration data for the dioxin like CBs by age class and sex

**Figure S1:** Principle Component 1 plotted against Principal Component 2 for the PCA carried out on the congener grouped by their degree of chlorination, coloured by age class and sex.

**Figure S2:** Congener proportions of the sum of 25 congeners ( $\Sigma 25\text{CBs}$ ) (grouped by their structural activity group) for each area.

**Figure S3:** Congener proportions plotted against age for congeners grouped by the persistence of their structural activity group. (A) Highly persistent congeners in East England (B) Highly persistent congeners in the West coast of England & Wales (C) Highly persistent congeners in Scotland (D) Less persistent congeners in East England (E) Less persistent congeners in the West coast of England & Wales (F) Less persistent congeners in Scotland

**Figure S4:** Principle Component 1 plotted against Principal Component 2 for the PCA carried out on the individual congeners, coloured by age class and sex (neonates grouped separately).



**Figure S5:** Principle Component 1 plotted against Principal Component 2 for the PCA carried out on congeners grouped by their degree of chlorination. (A) Data from the whole of the UK (B) Data from Scotland (C) Data from the East coast of England (D) Data from the West coast of England & Wales

**Figure S6:** Principle Component 1 plotted against Principal Component 2 for the PCA carried out on the individual congeners, coloured by time intervals of five years

**Figure S7:** Principle Component 1 plotted against Principal Component 2 for the PCA carried out on the individual congeners, coloured by time intervals of five years. (A) Data from the whole of the UK (B) Data from the UK between 2000 and 2004 d (C) Data from the West coast of England & Wales between 2000 and 2004 (D) Data from the West coast of England & Wales

**Figure S8:** Dioxin like congener concentrations for each age class and sex.

**Figure S9:** Representations (% of total records) of each age class and sex from each location separated by five-year time period.

**Figure S10:** The loadings of each congener for principal component 1 (PC1) and principal component 2 (PC2) from the principal component analysis carried out for each congener

**Figure S11:** The loadings of each congener for principal component 1 (PC1) and principal component 2 (PC2) from the principal component analysis carried out for the congeners grouped by their structural activity group.

**Figure S12:** Concentrations of the sum of the 25 chlorinated biphenyl congeners measured for each age class and sex. The red horizontal line represents the widely used toxicity threshold for cetaceans of 9mg/ kg lipid weight.

## References

- Addison, R.F., Brodie, P.F., 1987. Transfer of organochlorine residues from blubber through the circulatory system to milk in the lactating grey seal *Halichoerus grypus*. *Can. J. Fish. Aquat. Sci.* 44, 782–786.
- Aguilar, A., Borrell, A., 1994. Assessment of organochlorine pollutants in cetaceans by means

of skin and hypodermic biopsies. Nondestruct. biomarkers Vertebr.

Akaike, H., 1973. Information theory as an extension of the maximum likelihood principle–In: Second International Symposium on Information Theory (Eds) BN Petrov, F. Csaki. BNPBF Csaki. Budapest Acad. Kiado.

Andresen, J.A., Muir, D., Ueno, D., Darling, C., Theobald, N., Bester, K., 2007. Emerging pollutants in the north sea in comparison to Lake Ontario, Canada, data. Environ. Toxicol. Chem. 26, 1081–1089. <https://doi.org/10.1897/06-416R.1>

Baumann, M., Deml, E., Schäffer, E., Greim, H., 1983. Effects of polychlorinated biphenyls at low dose levels in rats. Arch. Environ. Contam. Toxicol. 12, 509–515. <https://doi.org/10.1007/BF01056545>

Bjoerge, A., Hohn, A.A., Kvam, T., Lockyer, C., Schweder, T., Aarefjord, H., 1995. Report of the harbour porpoise age determination workshop, Oslo, 21-23 May 1990. REPORTS-INTERNATIONAL Whal. Comm. Spec. ISSUES 16, 477–498.

Boon, J.P., Van der Meer, J., Allchin, C.R., Law, R.J., Klungsøyr, J., Leonards, P.E.G., Spliid, H., Storr-Hansen, E., Mckenzie, C., Wells, D.E., 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. Arch. Environ. Contam. Toxicol. 33, 298–311.

Borrell, A., Aguilar, A., 1990. Loss of organochlorine compounds in the tissues of a decomposing stranded dolphin. Bull. Environ. Contam. Toxicol. 45, 46–53.

de Boer, J., Law, R.J., 2003. Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatic hydrocarbons. J. Chromatogr. A 1000, 223–251.

- de Boer, J., Wells, D.E., 1997. Chlorobiphenyls and organochlorine pesticides in fish and sediments-three years of QUASIMEME laboratory performance studies. *Mar. Pollut. Bull.* 35, 52–63.
- Debier, C., Pomeroy, P.P., Dupont, C., Joiris, C., Comblin, V., Le Boulenge, E., Larondelle, Y., Thome, J.P., 2003. Dynamics of PCB transfer from mother to pup during lactation in UK grey seals *Halichoerus grypus*: differences in PCB profile between compartments of transfer and changes during the lactation period. *Mar. Ecol. Prog. Ser.* 247, 249–256. <https://doi.org/10.3354/meps247249>
- DEFRA, 2013. National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants – United Kingdom of Great Britain and Northern Ireland.
- Desforges, J.-P., Hall, A., McConnell, B., Rosing-Asvid, A., Barber, J.L., Brownlow, A., De Guise, S., Eulaers, I., Jepson, P.D., Letcher, R.J., 2018. Predicting global killer whale population collapse from PCB pollution. *Science* (80-. ). 361, 1373–1376.
- Fängström, B., Hovander, L., Bignert, A., Athanassiadis, I., Linderholm, L., Grandjean, P., Weihe, P., Bergman, Å., 2005. Concentrations of Polybrominated Diphenyl Ethers, Polychlorinated Biphenyls, and Polychlorobiphenyls in Serum from Pregnant Faroese Women and Their Children 7 Years Later. *Environ. Sci. Technol.* 39, 9457–9463. <https://doi.org/10.1021/es0513032>
- Faroon, O.M., Samuel Keith, L., Smith-Simon, C., De Rosa, C.T., Organization, W.H., 2003. Polychlorinated biphenyls: human health aspects.
- Folland, W.R., Newsted, J.L., Fitzgerald, S.D., Fuchsman, P.C., Bradley, P.W., Kern, J., Kannan, K., Remington, R.E., Zwiernik, M.J., 2016. Growth and reproductive effects from dietary exposure to Aroclor 1268 in mink (*Neovison vison*), a surrogate model for

- marine mammals. *Environ. Toxicol. Chem.* 35, 604–618.
- Galatius, A., Bossi, R., Sonne, C., Riget, F.F., Kinze, C.C., Lockyer, C., Teilmann, J., Dietz, R., 2013. PFAS profiles in three North Sea top predators: metabolic differences among species? *Environ. Sci. Pollut. Res.* 20, 8013–8020. <https://doi.org/10.1007/s11356-013-1633-x>
- Gioia, R., Dachs, J., Nizzetto, L., Lohmann, R., Jones, K.C., 2013. Atmospheric Transport, Cycling and Dynamics of Polychlorinated Biphenyls (PCBs) from Source Regions to Remote Oceanic Areas, in: *Occurrence, Fate and Impact of Atmospheric Pollutants on Environmental and Human Health*, ACS Symposium Series. American Chemical Society, pp. 1–3. <https://doi.org/doi:10.1021/bk-2013-1149.ch001>
- Hall, A.J., Hugunin, K., Deaville, R., Law, R.J., Allchin, C.R., Jepson, P.D., 2006a. The risk of infection from polychlorinated biphenyl exposure in the harbor porpoise (*Phocoena phocoena*): A case-control approach. *Environ. Health Perspect.* 114, 704–711. <https://doi.org/10.1289/ehp.8222>
- Hall, A.J., McConnell, B.J., Rowles, T.K., Aguilar, A., Borrell, A., Schwacke, L., Reijnders, P.J.H., Wells, R.S., 2006b. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. *Environ. Health Perspect.* 114, 60–64. <https://doi.org/10.1289/ehp.8053>
- Halsall, C.J., Lee, R.G.M., Coleman, P.J., Burnett, V., Harding-Jones, P., Jones, K.C., 1995. PCBs in UK urban air. *Environ. Sci. Technol.* 29, 2368–2376.
- Hamers, T., Kamstra, J.H., Cenijn, P.H., Pěnčíková, K., Simeckova, P., Vondracek, J., Andersson, P.L., Stenberg, M., Machala, M., 2011. In vitro toxicity profiling of ultrapure non-dioxin-like polychlorinated biphenyl (NDL-PCB) congeners and their relative toxic

contribution to PCB-mixtures in humans. *Toxicol. Sci.* kfr043.

Hansen, L.G., 1998. Stepping backward to improve assessment of PCB congener toxicities.

*Environ. Health Perspect.* 106, 171.

Harner, T., Bidleman, T.F., 1996. Measurements of Octanol–Air Partition Coefficients for

Polychlorinated Biphenyls. *J. Chem. Eng. Data* 41, 895–899.

<https://doi.org/10.1021/je960097y>

Harrad, S.J., Sewart, A.P., Alcock, R., Boumphrey, R., Burnett, V., Duarte-Davidson, R.,

Halsall, C., Sanders, G., Waterhouse, K., Wild, S.R., 1994. Polychlorinated biphenyls

(PCBs) in the British environment: sinks, sources and temporal trends. *Environ. Pollut.*

85, 131–146.

ICES, 1998. Report on the Results of the ICES/IOC/OSPARCOM Intercomparison

Programme on the Determination of Chlorobiphenyl Congeners in Marine Media-steps

3a, 3b, 4 and Assessment. International Council for the Exploration of the Sea.

Iwata, H., Watanabe, M., Okajima, Y., Tanabe, S., Amano, M., Miyazaki, N., Petrov, E.A.,

2004. Toxicokinetics of PCDD, PCDF, and coplanar PCB congeners in Baikal seals, *Pusa*

*sibirica*: age-related accumulation, maternal transfer, and hepatic sequestration. *Environ.*

*Sci. Technol.* 38, 3505–3513.

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths

among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *J. Anim.*

*Ecol.* 80, 595–602.

Jartun, M., 2011. Building Materials: An Important Source for Polychlorinated Biphenyls

(PCBs) in Urban Soils, in: *Mapping the Chemical Environment of Urban Areas*. John

Wiley & Sons, Ltd, pp. 128–133. <https://doi.org/10.1002/9780470670071.ch9>

Jepson, P.D., 2003. Pathology and toxicology of stranded harbour porpoises (*Phocoena phocoena*) in UK waters. R. Vet. Coll. Doctor of, 221.

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, À., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., Doeschate, M., Esteban, R., Penrose, R., Perkins, M.W., Smith, B., Stephanis, R. De, Tregenza, N., Verborgh, P., Fernández, A., Law, R.J., 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. *Sci. Rep.* 6, 18573. <https://doi.org/10.1038/srep18573>

Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000a. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum. Ecol. Risk Assess.* 6, 181–201.

Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000b. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum. Ecol. Risk Assess.*

Kodavanti, P.R.S., Loganathan, B.G., 2014. Chapter 25 - Polychlorinated biphenyls, polybrominated biphenyls, and brominated flame retardants, in: Gupta, R.C.B.T.-B. in T. (Ed.), *Biomarkers in Toxicology*. Academic Press, Boston, pp. 433–450. <https://doi.org/https://doi.org/10.1016/B978-0-12-404630-6.00025-7>

Lahvis, G.P., Lindell, S.L., Thomas, R.S., McCuskey, R.S., Murphy, C., Glover, E., Bentz, M., Southard, J., Bradfield, C.A., 2000. Portosystemic shunting and persistent fetal vascular structures in aryl hydrocarbon receptor-deficient mice. *Proc. Natl. Acad. Sci.* 97, 10442 LP – 10447. <https://doi.org/10.1073/pnas.190256997>

- Larigot, L., Juricek, L., Dairou, J., Coumoul, X., 2018. AhR signaling pathways and regulatory functions. *Biochim. open* 7, 1–9. <https://doi.org/10.1016/j.biopen.2018.05.001>
- Law, R.J., 1994. Collaborative UK marine mammal project: summary of data produced 1988–1992 (Fisheries Research Technical Report). MAFF Dir. Fish. Res. Lowestoft.
- Law, R.J., Alae, M., Allchin, C.R., Boon, J.P., Lebeuf, M., Lepom, P., Stern, G.A., 2003. Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife. *Environ. Int.* 29, 757–770.
- Law, R.J., Barry, J., Barber, J.L., Bersuder, P., Deaville, R., Reid, R.J., Brownlow, A., Penrose, R., Barnett, J., Loveridge, J., Smith, B., Jepson, P.D., 2012. Contaminants in cetaceans from UK waters: Status as assessed within the Cetacean Strandings Investigation Programme from 1990 to 2008. *Mar. Pollut. Bull.* 64, 1485–1494. <https://doi.org/10.1016/j.marpolbul.2012.05.024>
- Law, R.J., Bersuder, P., Mead, L.K., Jepson, P.D., 2008. PFOS and PFOA in the livers of harbour porpoises (*Phocoena phocoena*) stranded or bycaught around the UK. *Mar. Pollut. Bull.* 56, 792.
- Law, R.J., Jepson, P.D., Deaville, R., Reid, R.J., 2006. Collaborative UK Marine Mammals Strandings Project: summary of contaminant data for the period 1993-2001. *Sci. Ser. Tech. report. Cent. Environ. Fish. Aquac. Sci.* 131, 72.
- Lockyer, C., 1995. A review of factors involved in zonation in odontocete teeth, and an investigation of the likely impact of environmental factors and major life events on harbour porpoise tooth structure. *REPORTS-INTERNATIONAL Whal. Comm. Spec. ISSUES* 16, 511–530.

- Maes, T., Webster, L., Nicolaus, E.E.M., Fryer, R., 2018. Trends and status of polychlorinated biphenyls in UK biotaTitle [WWW Document]. UK Mar. Online Assess. Tool.
- Minh, N.H., Minh, T.B., Kajiwara, N., Kunisue, T., Subramanian, A., Iwata, H., Tana, T.S., Baburajendran, R., Karuppiah, S., Viet, P.H., Tuyen, B.C., Tanabe, S., 2006. Contamination by Persistent Organic Pollutants in Dumping Sites of Asian Developing Countries: Implication of Emerging Pollution Sources. *Arch. Environ. Contam. Toxicol.* 50, 474–481. <https://doi.org/10.1007/s00244-005-1087-3>
- Moysich, K.B., Mendola, P., Schisterman, E.F., Freudenheim, J.L., Ambrosone, C.B., Vena, J.E., Shields, P.G., Kostyniak, P., Greizerstein, H., Graham, S., Marshall, J.R., 1999. An evaluation of proposed frameworks for grouping polychlorinated biphenyl (PCB) congener data into meaningful analytic units. *Am. J. Ind. Med.* 35, 223–231. [https://doi.org/10.1002/\(SICI\)1097-0274\(199903\)35:3<223::AID-AJIM2>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-0274(199903)35:3<223::AID-AJIM2>3.0.CO;2-L)
- Murphy, S., Barber, J.L., Learmonth, J.A., Read, F.L., Deaville, R., Perkins, M.W., Brownlow, A., Davison, N., Penrose, R., Pierce, G.J., Law, R.J., Jepson, P.D., 2015. Reproductive failure in UK harbour porpoises *phocoena phocoena*: Legacy of pollutant exposure? *PLoS One* 10, 1–32. <https://doi.org/10.1371/journal.pone.0131085>
- Ockenden, W.A., Sweetman, A.J., Prest, H.F., Steinnes, E., Jones, K.C., 1998. Toward an understanding of the global atmospheric distribution of persistent organic pollutants: The use of semipermeable membrane devices as time-integrated passive samplers. *Environ. Sci. Technol.* 32, 2795–2803.
- OSPAR, 2015. Status and Trends of Polychlorinated Biphenyls (PCB) in Fish and Shellfish.
- Pěňčíková, K., Svrzkova, L., Strapacova, S., Neca, J., Bartonkova, I., Dvorak, Z., Hyzdalova, M., Pivnicka, J., Palkova, L., Lehmler, H.-J., Li, X., Vondracek, J., Machala, M., 2018.



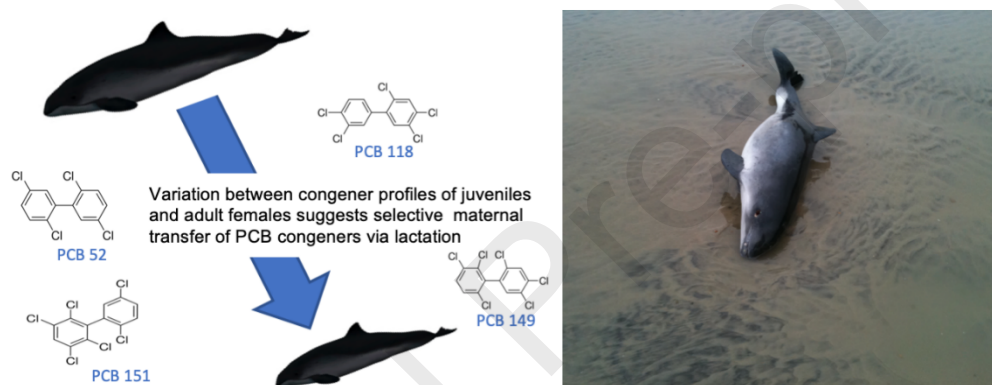
- In vitro profiling of toxic effects of prominent environmental lower-chlorinated PCB congeners linked with endocrine disruption and tumor promotion. *Environ. Pollut.* 237, 473–486. <https://doi.org/10.1016/j.envpol.2018.02.067>
- Peñín, I., Levin, M., Acevedo-Whitehouse, K., Jasperse, L., Gebhard, E., Gulland, F.M.D., De Guise, S., 2018. Effects of polychlorinated biphenyls (PCB) on California sea lion (*Zalophus californianus*) lymphocyte functions upon in vitro exposure. *Environ. Res.* 167, 708–717. <https://doi.org/10.1016/j.envres.2018.08.028>
- Pessah, I.N., Lein, P.J., Seegal, R.F., Sagiv, S.K., 2019. Neurotoxicity of polychlorinated biphenyls and related organohalogenes. *Acta Neuropathol.* 138, 363–387. <https://doi.org/10.1007/s00401-019-01978-1>
- R Core Team, 2016. R: A Language and Environment for Statistical Computing.
- Ramos, L., Hernandez, L.M., Gonzalez, M.J., 1997. Variation of PCB congener levels during lactation period and relationship to their molecular structure. *Arch. Environ. Contam. Toxicol.* 33, 97–103. <https://doi.org/10.1007/s002449900229>
- Reddy, M.L., Reif, J.S., Bachand, A., Ridgway, S.H., 2001. Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. *Sci. Total Environ.* 274, 171–182.
- Robin, M.-M., 2010. The world according to Monsanto: pollution, corruption, and the control of the world's food supply. The New Press.
- Rogan, E., Murphy, S., Learmonth, J.A., Gonzalez, A., Dabin, W., 2004. Age determination in small cetaceans from the NE Atlantic. *Bioaccumulation persistent Org. Pollut. small cetaceans Eur. waters Transp. pathways impact Reprod. Append.* 4, 34.

- Saba, T., Boehm, P.D., 2011. Congener-based analysis of the weathering of PCB Aroclor 1242 in paper mill sludge. *Chemosphere* 82, 1321–1328.
- Safe, S., Hutzinger, O., 1984. Polychlorinated Biphenyls (PCBs) and Polybrominated Biphenyls (PBBs): Biochemistry, Toxicology, and Mechanism of Action. *CRC Crit. Rev. Toxicol.* 13, 319–395. <https://doi.org/10.3109/10408448409023762>
- SANCO/12495/2011, 2011. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed.
- Santos, M.B., Pierce, G.J., Learmonth, J.A., Reid, R.J., Ross, H.M., Patterson, I.A.P., Reid, D.G., Beare, D., 2004. Variability in the diet of harbor porpoises (*Phocoena phocoena*) in Scottish waters 1992-2003. *Mar. Mammal Sci.* 20, 1–27. <https://doi.org/10.1111/j.1748-7692.2004.tb01138.x>
- Schwacke, L.H., Voit, E.O., Hansen, L.J., Wells, R.S., Mitchum, G.B., Hohn, A.A., Fair, P.A., 2002. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the Southeast United States Coast. *Environ. Toxicol. Chem.* 21, 2752–2764. <https://doi.org/10.1002/etc.5620211232>
- Stuart-Smith, S.J., Jepson, P.D., 2017. Persistent threats need persistent counteraction: Responding to PCB pollution in marine mammals. *Mar. Policy* 84, 69–75. <https://doi.org/10.1016/j.marpol.2017.06.033>
- Tanabe, S., Tanaka, H., Maruyama, K., Tatsukawa, R., 1981. Elimination of chlorinated hydrocarbons from mother striped dolphins (*Stenella coeruleoalba*) through parturition and lactation. *T. Fujiyama Stud. levels Organochlor. Compd. heavy Met. Mar. Org. Univ. Ryukyu, Japan* 115–121.

- Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. *Toxicol. Sci.* 93, 223–241.
- Vitalone, A., Catalani, A., Cinque, C., Fattori, V., Matteucci, P., Zuena, A.R., Costa, L.G., 2010. Long-term effects of developmental exposure to low doses of PCB 126 and methylmercury. *Toxicol. Lett.* 197, 38–45.  
<https://doi.org/https://doi.org/10.1016/j.toxlet.2010.04.024>
- Warner, J., Osuch, J.R., Karmaus, W., Landgraf, J.R., Taffe, B., O’Keefe, M., Mikucki, D., Haan, P., 2012. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. *Sci. Total Environ.* 414, 81–89.  
<https://doi.org/https://doi.org/10.1016/j.scitotenv.2011.10.044>
- Webster, L., Roose, P., Bersuder, P., Kotterman, M., Haarich, M., Vorkamp, K., 2013. Determination of polychlorinated biphenyls (PCBs) in sediment and biota.
- Weijs, L., Tibax, D., Roach, A.C., Manning, T.M., Chapman, J.C., Edge, K., Blust, R., Covaci, A., 2013. Assessing levels of halogenated organic compounds in mass-stranded long-finned pilot whales (*Globicephala melas*) from Australia. *Sci. Total Environ.* 461–462, 117–125. <https://doi.org/https://doi.org/10.1016/j.scitotenv.2013.04.090>
- Wells, R.S., Tornero, V., Borrell, A., Aguilar, A., Rowles, T.K., Rhinehart, H.L., Hofmann, S., Jarman, W.M., Hohn, A.A., Sweeney, J.C., 2005. Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. *Sci. Total Environ.* 349,

106–119. <https://doi.org/http://dx.doi.org/10.1016/j.scitotenv.2005.01.010>

Yordy, J.E., Wells, R.S., Balmer, B.C., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., 2010. Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (*Tursiops truncatus*) resident to Sarasota Bay, FL. *Sci. Total Environ.* 408, 2163–2172.



## Highlights

- 347 harbor porpoise PCB congener profiles were analysed
- Juveniles had higher proportions of less chlorinated congeners than adults
- Individuals that stranded on the West coast of England and Wales had higher proportions of congeners that are known to be more persistent in cetaceans
- To assess risk the toxicity of different congener profiles at different life stages needs to be quantified

**2 column fitting table:**

Table 1: Congener classification schemes. Dioxin like congeners are marked with an asterisk (\*)

<b><u>Structural activity group (SAG)</u></b>	<b><u>IUPAC Congener Number</u></b>	<b><u>Definition</u></b>	<b><u>Persistence grouping</u></b>
<b>1</b>	153, 180, 183, 187, 194	No vicinal hydrogen atoms, more than 2 <i>ortho</i> -chlorines	Highly persistent
<b>2</b>	128, 138, 170	Only vicinal hydrogen atoms in <i>ortho</i> and <i>meta</i> positions, 2 or more <i>ortho</i> -chlorines	Highly persistent
<b>3</b>	66, 105*, 118*	Only vicinal hydrogen atoms in <i>ortho</i> and <i>meta</i> positions, less than 2 <i>ortho</i> -chlorines	Less persistent
<b>4</b>	18, 44, 49, 52, 101	Only vicinal hydrogen atoms in <i>meta</i> and <i>para</i> positions, 2 or less <i>ortho</i> -chlorines	Less persistent
<b>5</b>	149, 151	Only vicinal hydrogen atoms in <i>meta</i> and <i>para</i> positions, more than 2 <i>ortho</i> -chlorines	Highly persistent
<b><u>Degree of chlorination</u></b>	<b><u>IUPAC Congener Numbers</u></b>	<b><u>Definition</u></b>	
<b><i>Tri-/Tetra-</i></b>	18, 28, 31, 44, 47, 49, 52, 66	Congeners contain 3 or 4 chlorine atoms	
<b><i>Penta-</i></b>	101, 105*, 110, 118*	Congeners contain 5 chlorine atoms	
<b><i>Hexa-</i></b>	128, 138, 141, 149, 151, 153, 156*, 158	Congeners contain 6 chlorinate atoms	
<b><i>Hepta-</i></b>	170, 180, 183, 187	Congeners contain 7 chlorine atoms	

Octa-

194

Congeners contain 8 chlorine atoms

## 2 column fitting figure:

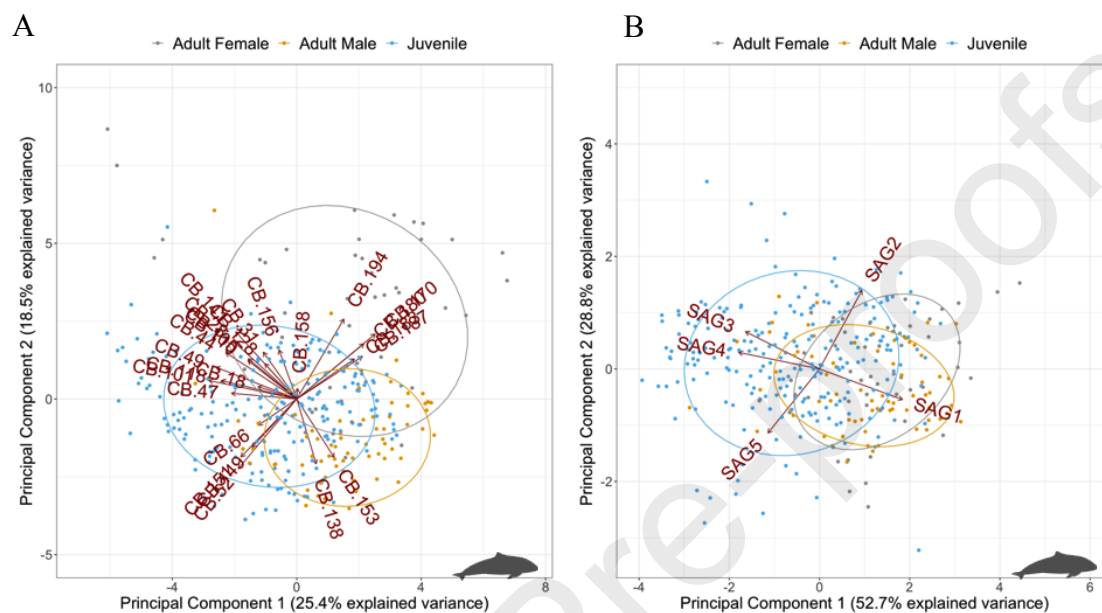


Figure 1(A) Principle Component 1 plotted against Principle Component 2 for the PCA carried out on the individual congeners, coloured by age class and sex. The sizing of the confidence ellipses was set at 70%. Figure 1(B) Principle Component 1 plotted against Principle Component 2 for the PCA carried out on the congeners grouped by their by their structural activity group. The sizing of the confidence ellipses was set at 70%.

## 1.5 column fitting figure:

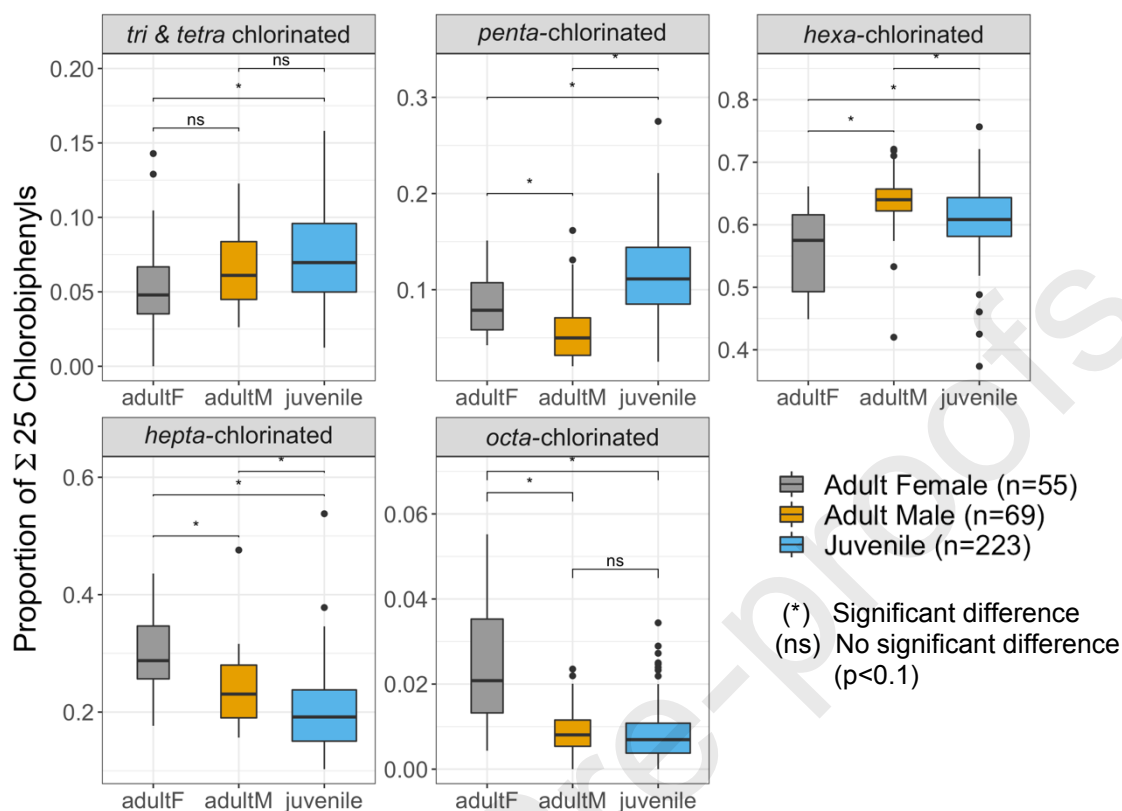


Figure 2: Congener proportions (grouped by degree of chlorination) for each age class and sex. The width of the boxes is proportional to the sample size. The horizontal lines represent the median value. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the upper hinge to the largest value unless the largest value is greater than 1.5 times the interquartile range (IQR) in which case the upper whisker is limited at  $1.5 \times \text{IQR}$ . The lower whisker extends from the lower hinge to the smallest value unless the smallest value is greater than  $1.5 \times \text{IQR}$  in which case the lower whisker is limited at  $1.5 \times \text{IQR}$ . Data beyond the end of the whiskers are outliers and are plotted individually.

## 2 column fitting figure:

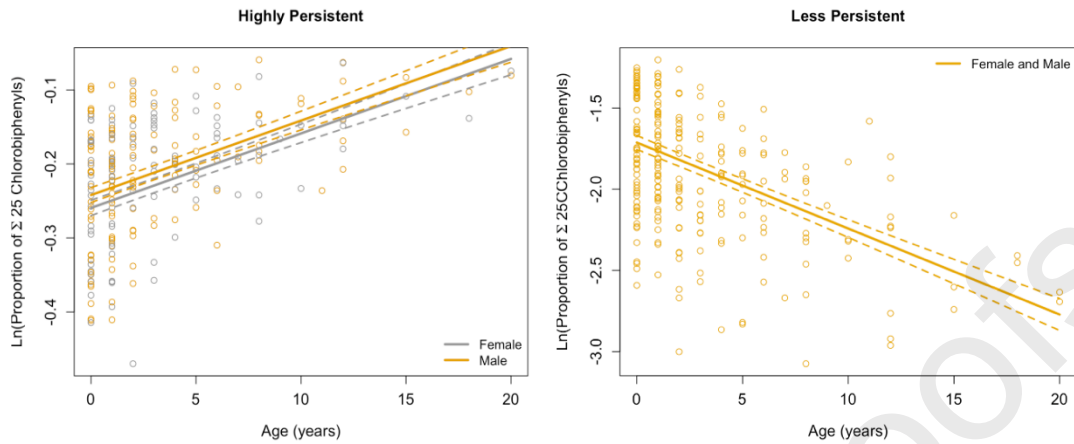


Figure 3: Proportion of the sum of 25 chlorobiphenyl congeners ( $\Sigma 25\text{CBs}$ ) against age for congeners grouped by the persistence of their structural activity group (SAG). (A) represents the highly persistent congeners (SAGs 1, 2 & 5). (B) represents the less persistent congeners (SAGs 3 & 4). The solid line represents the linear regression model fitted to the data ( $p < 1e^{-12}$ ). The solid line shows the trend for males and females in the median year of the study period (2001) in East England, the figures for the other areas are included in the supplementary information Figure S3. This area was chosen as it was the mid-point between the 3 areas studied. The dashed lines are 95% confidence intervals.

## 1 column fitting figure:

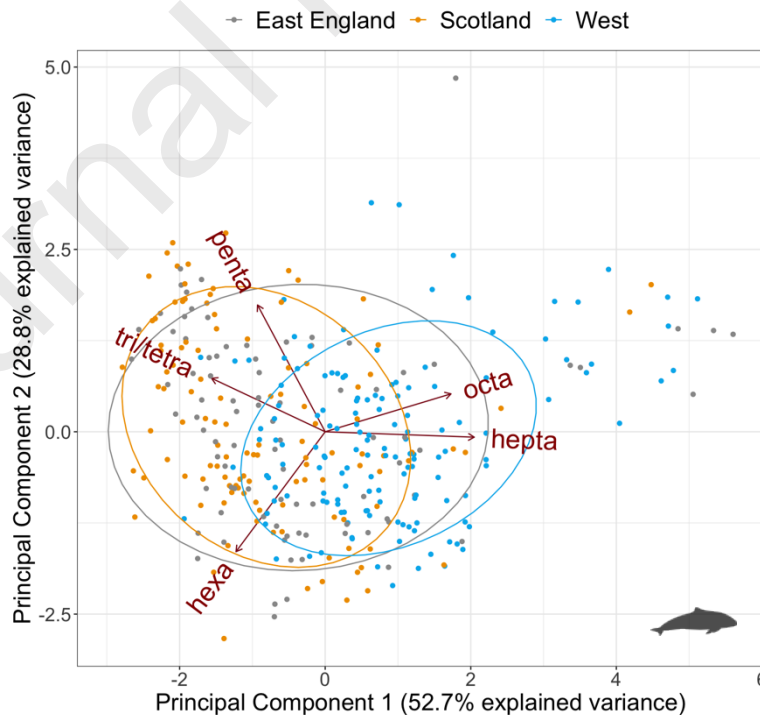




Figure 4: Principle Component 1 plotted against Principal Component 2 for the PCA carried out on the congeners grouped by degree of chlorination, coloured by geographical area. The sizing of the confidence ellipses was set at 70%.

2 column fitting figure:

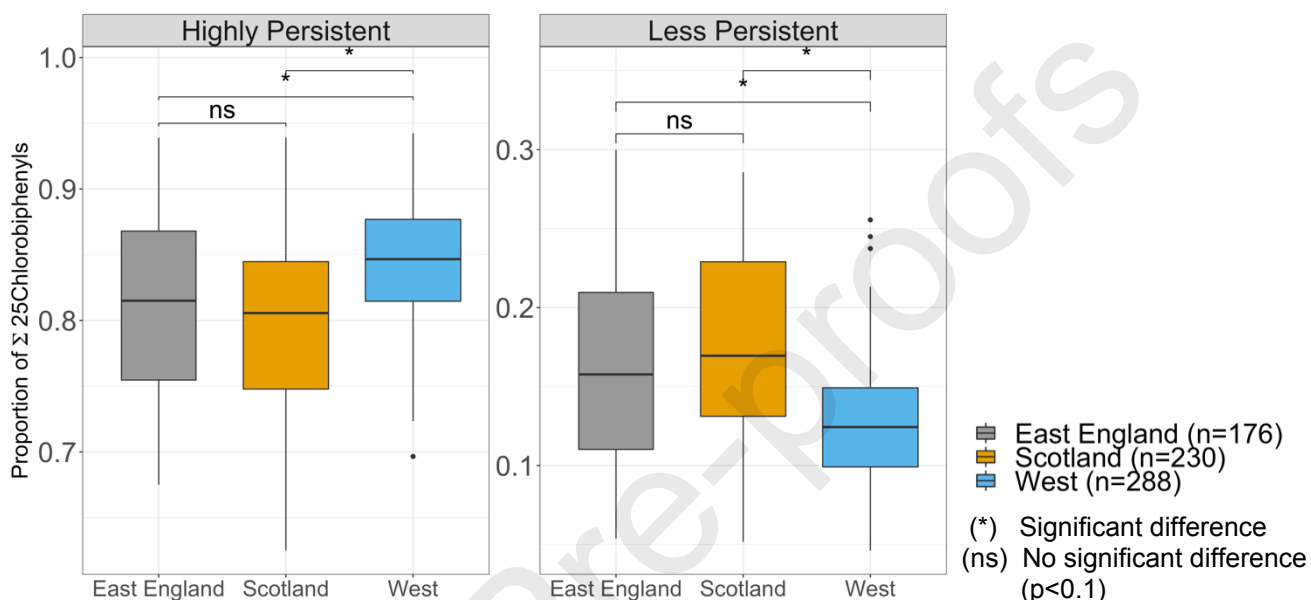


Figure 5: Congener proportions of the sum of 25 congeners ( $\Sigma$ 25CBs) (grouped by their persistence as defined by their SAG) for each area. The horizontal lines represent the median value. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the upper hinge to the largest value unless the largest value is greater than 1.5 times the interquartile range (IQR) in which case the upper whisker is limited at 1.5 x IQR. The lower whisker extends from the lower hinge to the smallest value unless the smallest value is greater than 1.5 times the interquartile range (IQR) in which case the lower whisker is limited at 1.5 x IQR. Data beyond the end of the whiskers are outliers and are plotted individually.

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<i>Octa-</i>	194	Congeners contain 8 chlorine atoms	

## Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
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