Changes to polychlorinated biphenyl (PCB) signatures and enantiomer fractions across different tissue types in Guillemots

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Abstract

Two Guillemot carcases were dissected, each providing 12 discrete tissue samples and 3 samples of partially digested food. One hundred and five PCBs from the 209 PCBs determined by GCxGC-ToFMS were detected. The relative proportions of individual PCBs did not vary greatly within tissue types, although the PCB profile from undigested food could be distinguished. Enantiomer fractions (EFs) were determined for CB-95, CB-136 and CB-149 by GC-HRqToFMS. EFs in the partially digested food were near racemic, with high levels of enrichment for E1 CB-95 in the kidneys and liver (EF of 0.80 and 0.84 respectively). This provides some of the clearest evidence to date that fractionation takes place in the organs where metabolic biotransformation and elimination of PCBs occurs. Our findings also confirm the ability of non-lethal sampling techniques, such as collection of small (<1 g) blood samples, to provide PCB signatures that are representative of an individual organism.

Key Words

Polychlorinated biphenyl (PCBs); Guillemot (*Uria aalge*); North Atlantic; Tissue; Comprehensive two-dimensional gas chromatography (GCxGC); Enantiomer fraction

1 Introduction

Polychlorinated biphenyls (PCBs) predominantly enter animals through ingestion of contaminated food. This can result in the accumulation of PCBs, with higher PCB concentrations usually associated with lipid rich tissues (Maervoet et al., 2005, Karjalainen et al., 2006). Biomagnification can lead to elevated PCB concentrations in top predators (Hansen, 1999, Muir et al., 1988). In most cases the PCB signature in animals can be largely explained by their food source (Jaspers et al., 2013), although human induced changes in land use can also influence the signature (Fernie et al., 2008). Once incorporated, biotransformation and elimination of PCBs can vary from species to species. For example, animals such as bears and humans have been shown to be capable of metabolising some PCBs, while the equivalent capacity has not been observed in predatory birds (Jaspers et al., 2013).

Some studies have provided evidence that the relative proportions of PCBs varies between different tissue types as a result of the preferential accumulation of, for example, ortho-chlorinated PCBs in the brain of rats (Kodavanti et al., 1998). PCBs are highly soluble in lipids and therefore accumulate in tissues and organs according to their respective Kow–dependent release rates (Karjalainen et al., 2006). The accumulation of PCBs from digested food occurs as PCBs partition across the membrane lining the gastrointestinal tract into the bloodstream. The blood flow in different tissues initially drives the distribution of PCBs until an equilibrium is reached which is primarily driven by the tissue lipid content (Karjalainen et al., 2006). However, despite the variable relative accumulation rates of PCBs, highly similar PCB distributions have been previously measured in birds (Boumphrey et al., 1993), specifically in the gut, heart, liver and stomach tissues from a Leach's storm petrel (Megson et al., 2014). Relatively little is known about the relative proportions of individual PCBs within an organism as a function of the observed non-uniform accumulation, indicating the necessity for further work.

There are 19 out of 209 PCBs that are predicted to exist as stable atropisomers (Oki, 1983). In commercial mixtures both enantiomers are produced in equal proportions and thus are racemic. In animals, metabolic processes such as enzyme mediated oxidation have been proven to preferentially target one stereoisomer, resulting in atropisomeric enrichment (Harrad et al., 2006, Wong et al., 2002, Wu et al., 2014). The degree of enrichment is species specific and can vary for the different enantiomers measured. There is currently little information on how the enantiomer fractions vary in different organs in animals. Chu et al. (2003) showed that PCBs 95, 132 and 149 are near racemic in human muscle, brain and kidney tissue, whereas Kania-Korwel et al. (2010) identified enrichment of CB-95 in the blood, adipose tissue, brain and kidneys of mice.

Here we present the results for the determination of all 209 PCB congeners and three atropisomers in 30 tissue samples obtained from two common guillemot (*Uria aalge*) birds. This study examines potential changes to the PCB signature and enantiomer fraction that may occur in different organs. The results are discussed in the context of providing evidence for the adoption of ethical, non-fatal sampling techniques, such as blood collection, to provide a reliable indicator of the PCB signature in future studies.

2 Materials and Methods

For this study we use the common guillemot (*Uria aalge*) to investigate if the relative proportions of PCBs and enantiomer fractions change within an individual organism. The common guillemot is the most abundant seabird breeding in the UK, with an estimated 1 million breeding pairs (Harris and Wanless, 2004). Guillemots are colonial, cliff-nesting seabirds that spend a large proportion of their time at sea foraging for food. The guillemot's diet is primarily comprised of benthic fish from the *Ammodytidae*, *Gadidae* and *Clupeidae* families but also includes a wide variety of invertebrates such as crustaceans, annelids and molluscs (Anderson et al., 2014, Bradstreet and Brown, 1985). The guillemot's diet is known to vary considerably due to the availability of prey.

2.1 Sample collection and preparation

Two guillemot carcases were collected from the south coast of the UK. The carcases were in good condition, with minimal degradation or damage, which enabled detailed dissections yielding samples from 12 different tissue types including: the kidney, heart, breast muscle, intestines, leg muscle, liver, blood, brain, pancreas, proventriculus, duodenum and gonads from each bird. Three samples were also obtained from partially digested food from within the gastrointestinal tract including the proventriculus contents, duodenum contents and intestine contents.

PCB signatures were determined by comprehensive two-dimensional gas chromatography coupled with time of flight mass spectrometry (GCxGC-ToFMS) and interpreted using principal component analysis to compare the relative proportions of 74 common PCB congeners in the different tissue types. Enantiomer fractions were determined using gas chromatography coupled with high resolution time of flight mass spectrometry.

2.2 Extraction procedure

Sample extraction was undertaken following the established method for PCB extraction in tissues reported by Megson et al. (2013) and outlined in Brown et al. (2013). All samples were freeze-dried (-45 ˚C; 0.2 mbar; 72 h) and ground into a powder. Samples were saponified in a methanolic potassium hydroxide solution (~ 4 mL H_2O:MeOH, 1:9; 20% KOH) for 60 min (80 ºC). Hexane (3 x 4 mL) was added to the saponified solutions, which were then vortexed (1 min) and centrifuged (1 min; 2,000 rpm). Supernatant solutions containing non-saponifiable lipids (NSLs) were transferred to clean vials with glass pipettes and dried using nitrogen to remove traces of H_2O/MeOH. NSLs were then re-suspended in hexane (0.5 mL) and fractionated (5 mL hexane) using column chromatography (SiO_2; 0.5 g). Samples were evaporated to incipient dryness and reconstituted with 10 µL of an internal standard comprising $^{13}C_{12}$ PCBs 60, 127 and 159 at a concentration of 10 ng mL^{-1} (CIL-EC-5370 EN-1948-4 PCB sampling standard, LGC) and 90 µL of hexane prior to analysis.

2.3 PCB signature analysis (GCxGC-ToFMS)

2.3.1 Analytical procedure

Samples were analysed to determine the presence of all 209 PCBs using the methods described by Megson et al. (2013) using a time-of-flight mass spectrometer (LECO, St.

Joseph, MI Pegasus 4D) coupled to a two dimensional gas chromatograph (Agilent Technologies 7890A) equipped with a thermal modulator (LECO, St. Joseph, MI). The gas chromatograph was installed with a Rtx-PCB (60 m x 0.18 mm x 0.18 µm) ^{1}D column and a Rxi-17 (1.5 m x 0.1 mm x 0.1 µm) ^{2}D column. A sample volume of 1 µL was injected in splitless mode. All data files were processed using ChromaTOF software set to identify 10,000 peaks with a signal-to-noise ratio of > 10:1.

2.3.2 Data Quality

Analytical blanks were run with each batch of approximately 10 samples. All samples were spiked with a $^{13}C_{12}$ internal standard (CB-60, CB-127, CB-159) which was used to quantify PCB concentrations by isotope dilution. Concentrations were normalised to dry weight tissue mass and are therefore reported as µg g^{-1}. As samples were originally extracted for the analysis of other lipids, PCB recovery could not be accurately determined for each sample; therefore reported concentrations were not corrected based on sample recovery or lipid content. PCBs are located within the lipid fraction, which was quantitatively extracted, therefore any bias should not be significant. Furthermore, because results for enantiomer fractions are relative these values are not biased and recovery correction is not necessary. Limits of detection (LOD) for individual PCBs were in the range 0.1 - 5 ng g^{-1} (dry weight). Accuracy and precision were measured for the sum of the European Union 7 indicator congeners (EC7) (CB-28, CB-52, CB-101, CB-118, CB-138, CB-153, CB-180) by analysing a 10 mg L^{-1} Aroclor 1248 standard three times. The sum of the EC7 congeners for the three samples was 105 ± 0.9 % (1 σ).

2.4 Chiral analysis (GC-HRqToFMS)

2.4.1 Analytical procedure

The Enantiomeric Fractions (EFs) of CBs 95, 136 and 149 were analysed based on the gas chromatography conditions specified by Robson and Harrad (2004). Samples were analysed using an Agilent 7890 Gas Chromatograph coupled to a Waters Xevo G2-XS qTOF based on the conditions specified in Megson et al. (2016) The corona voltage was set at 5 mAu, the cone gas at a flow rate of 175 L h^{-1}, and the desolvation gas flow set at 175 L h^{-1}. Ionization was undertaken using an atmospheric pressure chemical ionization source at 150 °C with the detector run in full scan mode using two target enhanced functions on masses 326 and 360. The two most abundant isotopes of each enantiomer were recorded with a mass accuracy of <1ppm.

2.4.2 Data Quality

The chromatographic performance of the method was assessed prior to each run of 10 samples by analysing a 1:1:1 mixture of Aroclors 1248, 1252 and 1260. Enantiomeric fractions were calculated as per Harner et al. (2000), whereby EF = E1/(E1+E2), E1 is the first eluting or the (+) enantiomer and E2 is the second eluting enantiomer. Samples where only accepted for quantitation if; the enantiomeric fractions of the three atropisomers studied were 0.50 (±0.01) in the Aroclor mixture; the least abundant enantiomer of the pair had a signal to noise (S:N) ratio greater than 10:1; and the isotope ratios were within 20% of their theoretical values. The instrumental LODs were calculated by analysing a standard mixture

of CB-95 and CB-149; LODs were established at a concentration of 0.1 pg μL^{-1} per enantiomer. Procedural blanks were prepared for each batch of 10 samples; no chiral PCBs were detected in the blanks above the LODs.

2.5 Statistical analysis

Exploratory data analysis was undertaken using principal component analysis (PCA) performed using PRIMER 6 software. PCA is a statistical technique that is often used to simplify complex datasets as it reduces the dimensionality of the dataset by transforming it to a set of new uncorrelated eigenvectors called principal components (Johnson et al., 2002).

Where a PCB was not detected it was included in the dataset as a '0'. As part of the data quality check, other values were substituted for '0', including the smallest integrated peak area and the smallest integrated peak area divided by 2, but these had no observable effect on the data output and so the '0's were retained. To reduce any bias from a high proportion of non-detects for a specific congener, PCBs that were not detected in over 60% of samples (i.e. PCBs present in less than 18 out of the 30 samples) were removed from the analysis following the guidance of Helsel (2006). This resulted in a data set containing 30 samples and 74 PCBs. Before performing PCA the data were normalised by transformation to a percent metric to remove concentration/dilution effects. The data were then mean centred and scaled using a Z-transform (autoscale transform) to prevent high concentration variables from dominating the analysis (Johnson et al., 2007).

3 Results and discussion

3.1 PCB concentrations and signatures in Guillemot tissues

A total of 105 different PCBs were detected in the samples. PCBs present in the highest concentrations included PCBs 118, 146, 153, 163 and 187. These congeners are regularly detected in the environment as they were present in high proportions in Aroclor mixtures (Frame et al., 1996). However, it was interesting to note that the non-Aroclor PCBs 11 and 209 were also detected in the samples (estimated at approximately 0.01% and 0.05% respectively of total PCBs). Their presence in these samples provides more evidence to show that they are now ubiquitous contaminants in the environment (Hu et al., 2011, King et al., 2002, Hu et al., 2008, Rodenburg et al., 2010). PCB concentrations were calculated for the EC7 congeners and ranged from 0.19 μg g^{-1} to 69 μg g^{-1}. These values were greater than those reported in common terns (*Sterna hirundo*) from Ireland (0.035 μg g^{-1}) (Acampora et al., 2017), and comparable to levels reported in harbour porpoises (*Phocoena phocoena*) in UK waters (0.4 to 160 μg g^{-1}) (Jepson et al., 2016). However, concentrations were approximately one order of magnitude lower than several other marine mammals from European waters where levels of over 100 μg g^{-1} were regularly detected in bottlenose dolphins (*Tursiops truncates*), striped dolphins (*Stenella coeruleoalba*) and killer whales (*Orcinus orca*) (Jepson et al., 2016). While total EC7 PCB concentrations were generally similar between comparable tissues of the two birds, concentrations did differ greatly between particular tissue types (Figure 1). For example, the highest EC7 PCB concentrations were identified in the lipid-rich gonads, where concentrations were approximately one order of magnitude greater than those measured in other tissues (Figure 1).

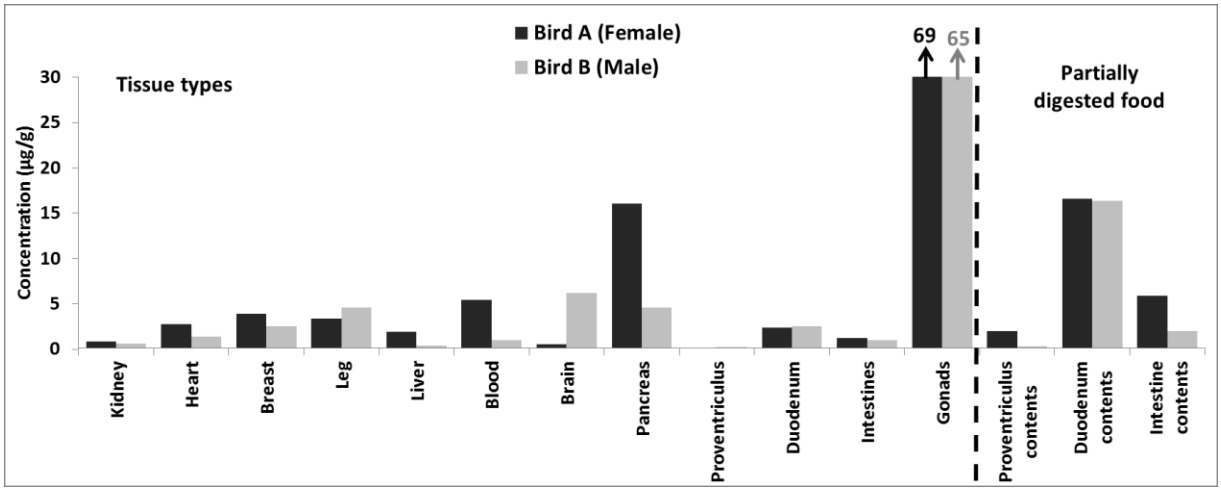

Figure 1. EC7 PCB concentrations in different tissue types and partially digested food from the two Guillemot birds sampled.

In an effort to identify a more suitable means of comparing PCBs between individuals the relative proportion of PCBs (referred to here as a 'signature') obtained from the 12 tissue types and 3 samples of partially digested food were compared (individual signatures are presented in Supplementary information 1). The PCB signatures were comprised of 74 PCBs and were visually similar for each tissue type within and between birds (Figure 2). This is consistent with findings reported in Jaspers et al. (2013) who also identified that PCB signatures are highly influenced by food source.

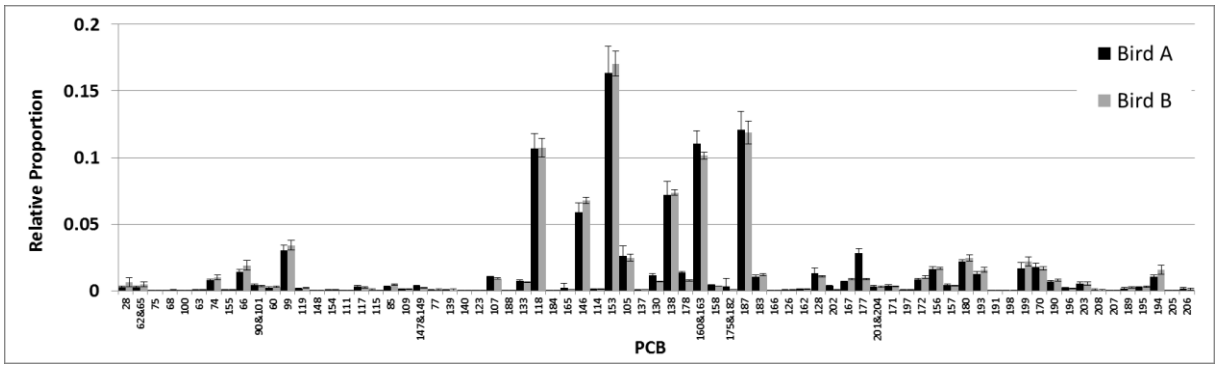

Figure 2. Average signature in 12 tissue samples from each bird for 74 PCBs, error bars represent +/- 1 standard deviation.

Despite both birds operating in the same foraging area it was still possible to identify subtle differences between samples from each bird using principal component analysis (Figure 3). Within Figure 3 it can be observed that organs from each bird can be differentiated based on the influence of principal component 2. We suspect that this is likely to be due to the different sexes of the two birds, rather than geographical differences in foraging. However, it could also be explained by an age difference since Bird A had slightly higher total PCB concentrations with higher proportions of the more chlorinated PCBs which are generally more resistant to biotransformation and elimination (Hansen, 1999). Although samples were obtained from only two individuals, the results clearly indicate that PCB signatures remain constant between the different tissue types analysed in each bird, which is an important

finding that is consistent with previous studies undertaken on fewer organs (Boumphrey et al., 1993, Megson et al., 2014)

Figure 3 also highlights the variation in PCB signature in partially digested food and tissue. The PCB signature derived from partially digested food items, in particular from the proventriculus (first part of the birds stomach), differed from those measured in the respective host bird (Supplementary Information 1) and strongly influenced principal component 1 of the scores plot (Figure 3). Closer examination of the individual PCBs revealed that this was primarily due to higher proportions of the less chlorinated PCBs, along with CB-118, CB-99 & 101, CB-105 and CB-147 & 149, in the partially digested food (Figure 4; Supplementary Information 1). Uniquely, eight PCBs that were absent from the tissue samples were recorded in all of the partially digested food samples (CB-44, CB-49, CB-64, CB-71, CB-87, CB-88&95, and CB-179). The majority of these PCBs contained either -25 or -236 substitution patterns. Many of the congeners present in high proportions in the undigested food have also previously been reported in high proportions in members of the *Gadidae* family (Megson et al., 2013) from the southwest coast of Great Britain, which guillemots are known to feed on (Anderson et al., 2014). Since both samples from the proventriculus contents grouped in a similar area in the scores plot (Figure 3), we suggest that both birds had recently consumed a similar prey type.

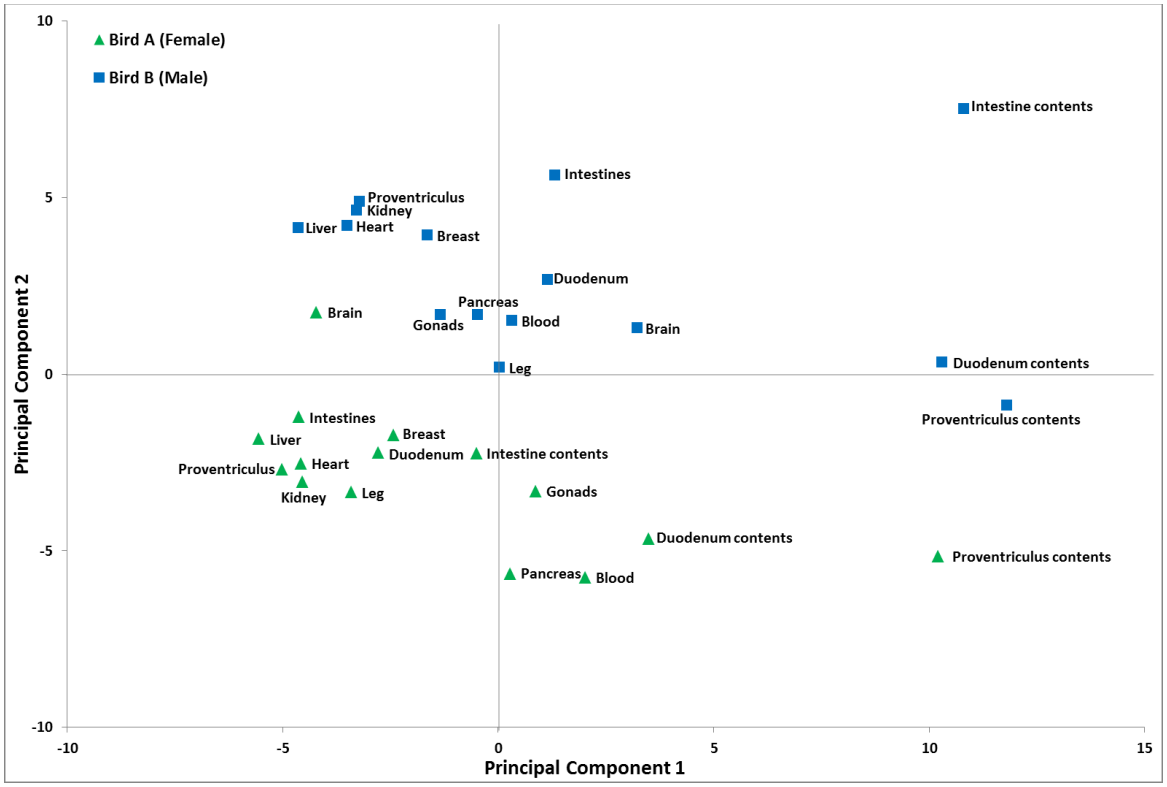

Figure 3. Scores plot of PC1 and PC2 showing the difference in PCB signature between the two birds, along with a different PCB signature in the partially digested food samples.

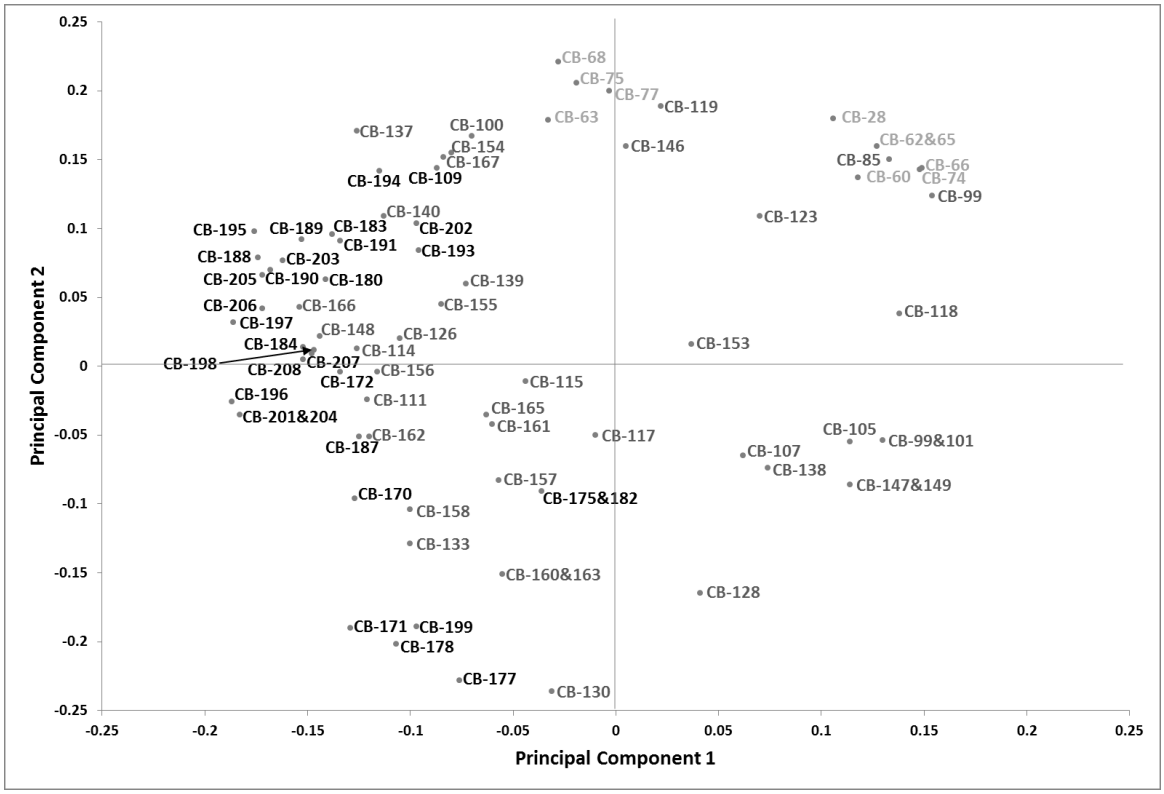

Figure 4. Loadings plot of PC1 and PC2 showing higher proportions of the less chlorinated biphenyls in the partially digested food. PCBs with 1 to 4 chlorines are coloured in **light grey**, PCBs with 5 and 6 chlorines are in **dark grey**, and PCBs with 7 to 10 chlorines are in **black.**

The ability of principal component analysis to distinguish samples recovered from the two birds indicates a strong degree of perpetuation of the PCB signature in different tissues. This finding is consistent with previous studies undertaken on fewer tissue types, which have shown that blood flow in different tissues is effective at distributing PCBs around the body until an equilibrium is reached which is primarily driven by the tissue lipid content (Karjalainen et al., 2006). Here we show that in the guillemot this redistribution does not appear to cause fractionation of more lipophilic PCBs to more lipid rich tissues. Instead the blood flow appears to distribute PCBs relatively evenly, resulting in comparable PCB signatures for blood and all analysed tissues. The consistency of PCB signatures in the different tissue types within each bird indicates that a small mass of blood (<1g) can be used to represent the PCB signature of the organism as a whole. This would correspond to approximately 1 mL of blood which could be sourced from most birds without any detrimental effects.

3.2 Chiral PCBs in different tissue types

Chiral PCBs have previously been monitored in wildlife and results to date indicate that enantioselective processing can occur for several species (Wong et al., 2002, Buckman et al., 2006, Warner et al., 2009, Kania-Korwel et al., 2010, Kania-Korwel et al., 2008a, Kania-Korwel et al., 2007, Kania-Korwel et al., 2008b, Kania-Korwel and Lehmler, 2016). Despite the relatively large number of studies on chiral PCBs in animals it is currently unclear if atropselective metabolism at the site of absorption or in other, extrahepatic tissues contributes to the atropisomeric enrichment of chiral PCBs (Kania-Korwel and Lehmler,

2016). This study aims to help address this knowledge gap through the analysis of 12 different tissue samples and 3 partially digested food samples in two birds (Supplementary Information 2).

Concentrations of chiral PCBs were below the analytical limits of detection (0.1 pg μL^{-1}) in many of the tissue samples analysed. CB-149 was not detected during this study, although both CB-95 and CB-136 were identified in several samples. The available results indicate some degree of fractionation in the different tissue samples. This differed from the PCB signature data which showed a strong degree of perpetuation in the different tissue types. The highest levels of enantiomer enrichment for E1 were recorded for CB-95 in the liver of Bird A (0.84) and kidney of Bird B (0.80). High levels of enrichment were also recorded for CB-95 in the breast tissue of Bird A (0.69) and Bird B (0.76).

The contents of the proventriculus, duodenum and intestine all had a near racemic enantiomer fraction (mean = 0.50 ± 0.03 (1 standard deviation)). Without wanting to over interpret this dataset, the results provide some insight as to where enantioselective processing occurs. The food ingested contained near racemic enantiomer fractions which can be used as a baseline for comparison. The process of absorption of PCBs into the proventiculus, duodenum and intestines does not appear to result in significant fractionation. However this is not surprising considering that absorption of PCBs in the gastrointestinal tract is a passive transport process so makes no contribution to the atropisomeric enrichment of chiral PCBs (Kania-Korwel and Lehmler, 2016). The data reported here provides some of the clearest evidence to date to suggest that fractionation occurs in the organs where metabolic biotransformation and elimination of PCBs occurs (kidney and liver). This fractionation is also recorded in muscle tissue in the breast. The results also indicate that the enantiomer fraction is not consistent within different organs from the same individual. This finding warrants further investigation to establish how and where enantioselective fractionation occurs.

The results indicate that CB-95 is much more susceptible to fractionation than CB-136. This trend has also been identified by Megson et al. (2015) in humans. There are currently few studies that assess changes to the enantiomer fractions in different tissues. Chu et al. (2003) identified that PCBs 95, 132 and 149 are near racemic in human muscle, brain and kidney tissue whereas enrichment of CB-136 was recorded in rainbow trout (Wong et al., 2002, Buckman et al., 2006), mice (Warner et al., 2009) and rats (Kania-Korwel et al., 2010, Kania-Korwel et al., 2008a, Kania-Korwel et al., 2007, Kania-Korwel et al., 2008b).

4 Conclusions

Over 100 different PCBs were detected in guillemot tissue, with concentrations of the i7 PCBs in the low $\mu g\ g^{-1}$ range. Samples were obtained from 12 different tissue types and partially digested food samples obtained from three different points within the gastrointestinal tract. The highest PCB concentrations were identified in the lipid-rich gonads at concentrations of approximately one order of magnitude greater than those measured in other tissues. Whilst PCB concentrations varied in different organs the relative proportions of PCBs were consistent between the different tissue types. This represents a significant development in PCB signature analysis in animals since it negates the inherent difficulties

associated with comparing concentration data. The results show that the collection of only 1 mL of blood represents a useful, ethically sound, analytical method.

This study also highlights the ability to measure individual enantiomers with femtogram detection limits. The results provide some of the clearest evidence to date to suggest that enantioselective metabolism does occur in the kidneys and liver which results in enrichment of the E1 stereoisomer of CB-95 in these organs.

Finally, with the growing demand for ethical, non-lethal sampling strategies, this study illustrates that 1 mL samples of blood (<1 g) can be used to provide representative and detailed congener specific PCB data.

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