

Please cite the Published Version

Megson, D, Brown, TA, O'Sullivan, G, Robson, M, Ortiz, X, Worsfold, PJ, Comber, S, Lohan, MC and Reiner, EJ (2018) Changes to polychlorinated biphenyl (PCB) signatures and enantiomer fractions across different tissue types in Guillemots. *Marine Pollution Bulletin*, 131. pp. 174-179. ISSN 0025-326X

DOI: <https://doi.org/10.1016/j.marpolbul.2018.04.014>

Publisher: Elsevier

Downloaded from: <https://e-space.mmu.ac.uk/620548/>

Usage rights:  [Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0](https://creativecommons.org/licenses/by-nc-nd/4.0/)

Additional Information: This is an Author Accepted Manuscript of a paper accepted for publication in *Marine Pollution Bulletin*, published by and copyright Elsevier.

Enquiries:

If you have questions about this document, contact openresearch@mmu.ac.uk. Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

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

3 David Megson ^{a b*}, Thomas A. Brown ^{b c}, Gwen O'Sullivan ^d, Matthew Robson ^e, Xavier Ortiz
4 ^e Paul J. Worsfold ^b, Sean Comber ^b, Maeve C. Lohan ^b, Eric J. Reiner ^e.

5 ^a. School of Science and the Environment, Manchester Metropolitan University, Manchester
6 M1 5GD, UK.

7 ^b. Biogeochemistry Research Centre, SoGEES, Plymouth University, Plymouth, Devon, PL4
8 8AA, UK

9 ^c Marine Ecology and Chemistry, Scottish Association for Marine Science, Oban, PA37 1QA,
10 UK

11 ^d. Department of Earth & Environmental Science, Mount Royal University, 4825 Mount Royal
12 Gate SW, Calgary, Alberta, T3E 6K6, Canada

13 ^e. Ontario Ministry of the Environment and Climate Change, 125 Resources Road, Toronto,
14 Ontario, M9P 3V6, Canada

15

16 ***Corresponding Author**

17 David Megson
18 School of Science and the Environment,
19 Manchester Metropolitan University,
20 Manchester, M1 5GD, UK

21 E-mail: dpmegson@hotmail.co.uk

22

23 **Abstract**

24 Two Guillemot carcasses were dissected, each providing 12 discrete tissue samples and 3
25 samples of partially digested food. One hundred and five PCBs from the 209 PCBs
26 determined by GCxGC-ToFMS were detected. The relative proportions of individual PCBs
27 did not vary greatly within tissue types, although the PCB profile from undigested food could
28 be distinguished. Enantiomer fractions (EFs) were determined for CB-95, CB-136 and CB-
29 149 by GC-HRqToFMS. EFs in the partially digested food were near racemic, with high
30 levels of enrichment for E1 CB-95 in the kidneys and liver (EF of 0.80 and 0.84 respectively).
31 This provides some of the clearest evidence to date that fractionation takes place in the
32 organs where metabolic biotransformation and elimination of PCBs occurs. Our findings also
33 confirm the ability of non-lethal sampling techniques, such as collection of small (<1 g) blood
34 samples, to provide PCB signatures that are representative of an individual organism.

35 **Key Words**

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

38 1 Introduction

39 Polychlorinated biphenyls (PCBs) predominantly enter animals through ingestion of
40 contaminated food. This can result in the accumulation of PCBs, with higher PCB
41 concentrations usually associated with lipid rich tissues (Maervoet et al., 2005, Karjalainen et
42 al., 2006). Biomagnification can lead to elevated PCB concentrations in top predators
43 (Hansen, 1999, Muir et al., 1988). In most cases the PCB signature in animals can be largely
44 explained by their food source (Jaspers et al., 2013), although human induced changes in
45 land use can also influence the signature (Fernie et al., 2008). Once incorporated,
46 biotransformation and elimination of PCBs can vary from species to species. For example,
47 animals such as bears and humans have been shown to be capable of metabolising some
48 PCBs, while the equivalent capacity has not been observed in predatory birds (Jaspers et al.,
49 2013).

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

64 There are 19 out of 209 PCBs that are predicted to exist as stable atropisomers (Oki, 1983).
65 In commercial mixtures both enantiomers are produced in equal proportions and thus are
66 racemic. In animals, metabolic processes such as enzyme mediated oxidation have been
67 proven to preferentially target one stereoisomer, resulting in atropisomeric enrichment
68 (Harrad et al., 2006, Wong et al., 2002, Wu et al., 2014). The degree of enrichment is
69 species specific and can vary for the different enantiomers measured. There is currently little
70 information on how the enantiomer fractions vary in different organs in animals. Chu et al.
71 (2003) showed that PCBs 95, 132 and 149 are near racemic in human muscle, brain and
72 kidney tissue, whereas Kania-Korwel et al. (2010) identified enrichment of CB-95 in the
73 blood, adipose tissue, brain and kidneys of mice.

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

80 **2 Materials and Methods**

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

90 **2.1 Sample collection and preparation**

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

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

103 **2.2 Extraction procedure**

104 Sample extraction was undertaken following the established method for PCB extraction in
105 tissues reported by Megson et al. (2013) and outlined in Brown et al. (2013). All samples
106 were freeze-dried (-45 °C; 0.2 mbar; 72 h) and ground into a powder. Samples were
107 saponified in a methanolic potassium hydroxide solution (~ 4 mL H₂O:MeOH, 1:9; 20% KOH)
108 for 60 min (80 °C). Hexane (3 x 4 mL) was added to the saponified solutions, which were
109 then vortexed (1 min) and centrifuged (1 min; 2,000 rpm). Supernatant solutions containing
110 non-saponifiable lipids (NSLs) were transferred to clean vials with glass pipettes and dried
111 using nitrogen to remove traces of H₂O/MeOH. NSLs were then re-suspended in hexane
112 (0.5 mL) and fractionated (5 mL hexane) using column chromatography (SiO₂; 0.5 g).
113 Samples were evaporated to incipient dryness and reconstituted with 10 µL of an internal
114 standard comprising ¹³C₁₂ PCBs 60, 127 and 159 at a concentration of 10 ng mL⁻¹ (CIL-EC-
115 5370 EN-1948-4 PCB sampling standard, LGC) and 90 µL of hexane prior to analysis.

116 **2.3 PCB signature analysis (GCxGC-ToFMS)**

117 *2.3.1 Analytical procedure*

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

120 Joseph, MI Pegasus 4D) coupled to a two dimensional gas chromatograph (Agilent
121 Technologies 7890A) equipped with a thermal modulator (LECO, St. Joseph, MI). The gas
122 chromatograph was installed with a Rtx-PCB (60 m x 0.18 mm x 0.18 μm) ^1D column and a
123 Rxi-17 (1.5 m x 0.1 mm x 0.1 μm) ^2D column. A sample volume of 1 μL was injected in
124 splitless mode. All data files were processed using ChromaTOF software set to identify
125 10,000 peaks with a signal-to-noise ratio of $> 10:1$.

126 2.3.2 Data Quality

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

141 2.4 Chiral analysis (GC-HRqToFMS)

142 2.4.1 Analytical procedure

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

152 2.4.2 Data Quality

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

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

164 **2.5 Statistical analysis**

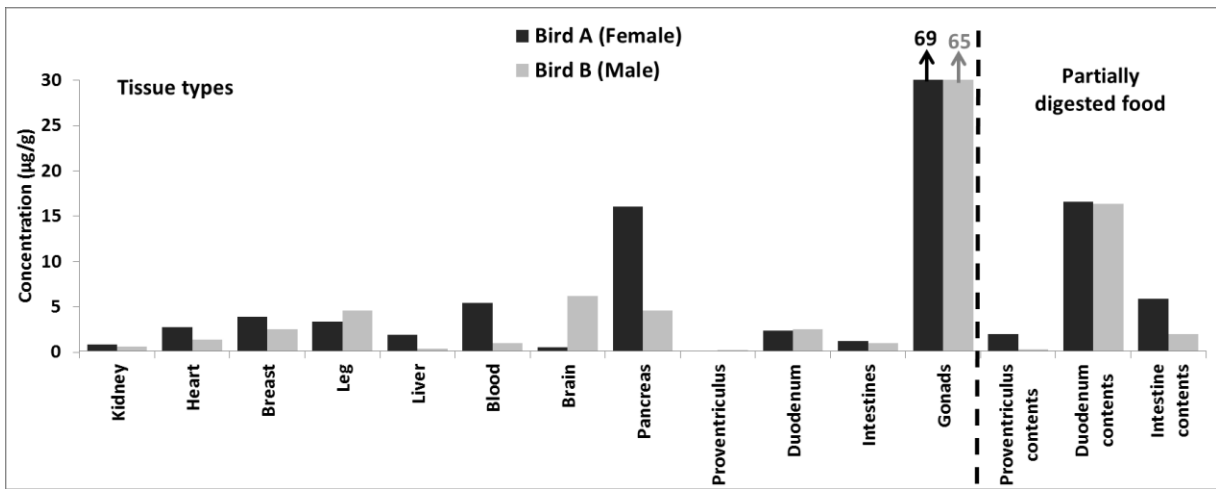
165 Exploratory data analysis was undertaken using principal component analysis (PCA)
166 performed using PRIMER 6 software. PCA is a statistical technique that is often used to
167 simplify complex datasets as it reduces the dimensionality of the dataset by transforming it to
168 a set of new uncorrelated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

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

180 **3 Results and discussion**

181 **3.1 PCB concentrations and signatures in Guillemot tissues**

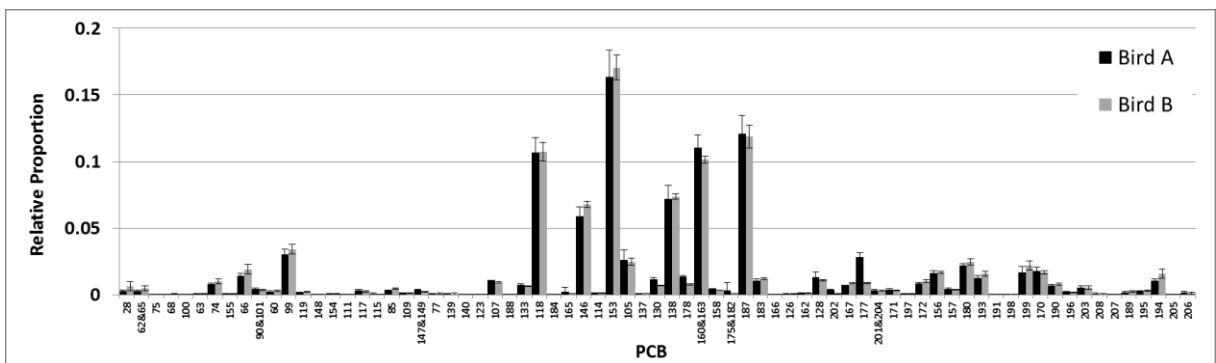
182 A total of 105 different PCBs were detected in the samples. PCBs present in the highest
183 concentrations included PCBs 118, 146, 153, 163 and 187. These congeners are regularly
184 detected in the environment as they were present in high proportions in Aroclor mixtures
185 (Frame et al., 1996). However, it was interesting to note that the non-Aroclor PCBs 11 and
186 209 were also detected in the samples (estimated at approximately 0.01% and 0.05%
187 respectively of total PCBs). Their presence in these samples provides more evidence to
188 show that they are now ubiquitous contaminants in the environment (Hu et al., 2011, King et
189 al., 2002, Hu et al., 2008, Rodenburg et al., 2010). PCB concentrations were calculated for
190 the EC7 congeners and ranged from 0.19 $\mu\text{g g}^{-1}$ to 69 $\mu\text{g g}^{-1}$. These values were greater
191 than those reported in common terns (*Sterna hirundo*) from Ireland (0.035 $\mu\text{g g}^{-1}$) (Acampora
192 et al., 2017), and comparable to levels reported in harbour porpoises (*Phocoena phocoena*)
193 in UK waters (0.4 to 160 $\mu\text{g g}^{-1}$) (Jepson et al., 2016). However, concentrations were
194 approximately one order of magnitude lower than several other marine mammals from
195 European waters where levels of over 100 $\mu\text{g g}^{-1}$ were regularly detected in bottlenose
196 dolphins (*Tursiops truncatus*), striped dolphins (*Stenella coeruleoalba*) and killer whales
197 (*Orcinus orca*) (Jepson et al., 2016). While total EC7 PCB concentrations were generally
198 similar between comparable tissues of the two birds, concentrations did differ greatly
199 between particular tissue types (Figure 1). For example, the highest EC7 PCB
200 concentrations were identified in the lipid-rich gonads, where concentrations were
201 approximately one order of magnitude greater than those measured in other tissues (Figure
202 1).



203

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

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



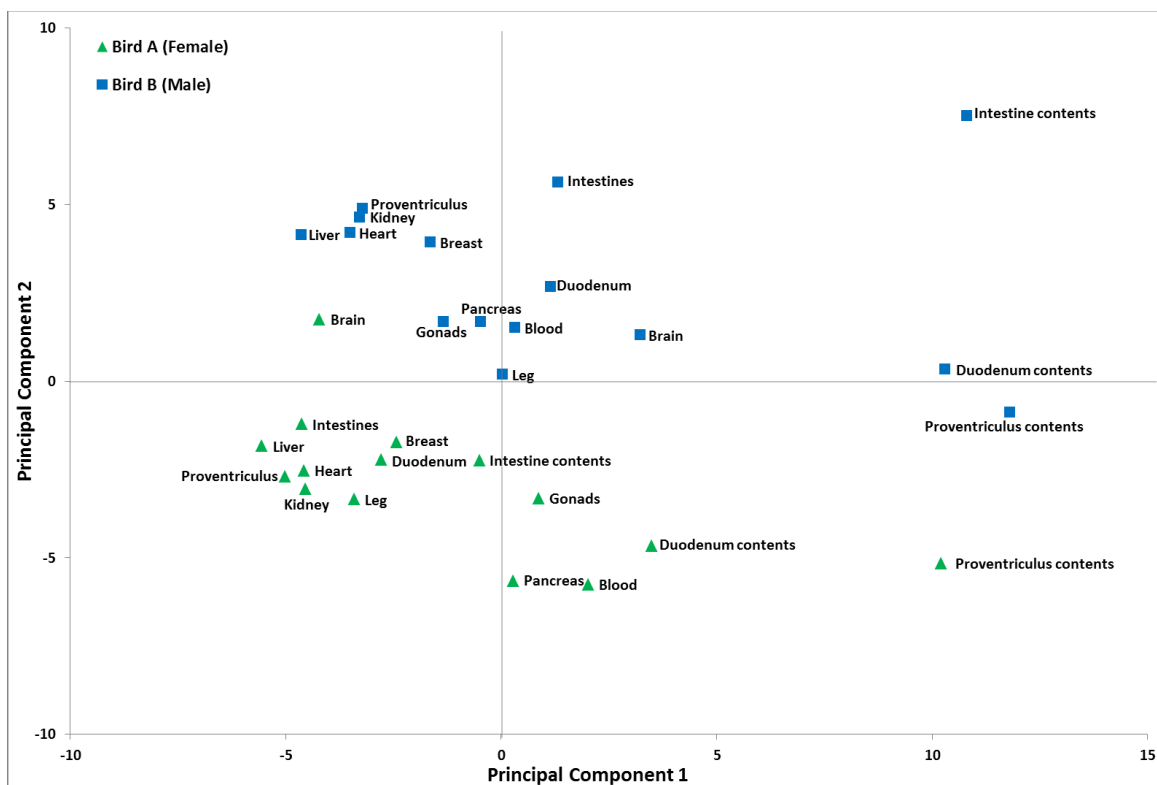
213

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

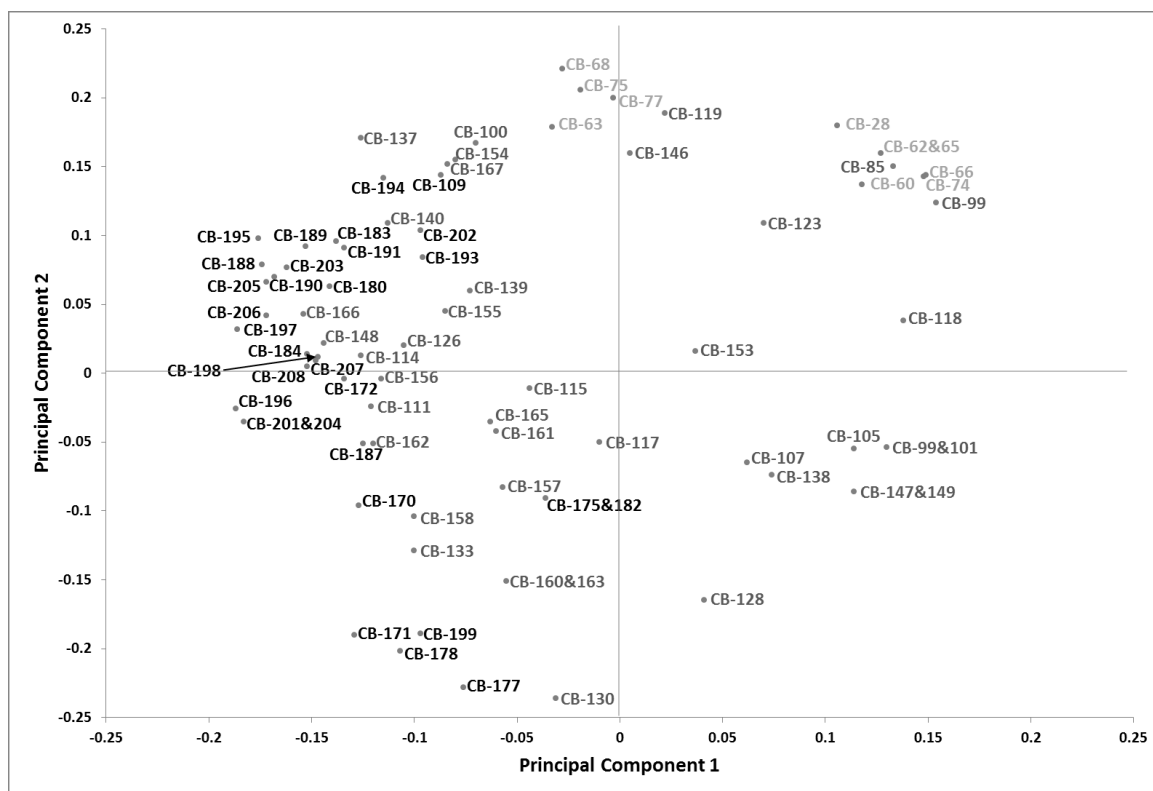
216 Despite both birds operating in the same foraging area it was still possible to identify subtle
 217 differences between samples from each bird using principal component analysis (Figure 3).
 218 Within Figure 3 it can be observed that organs from each bird can be differentiated based on
 219 the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

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

228 Figure 3 also highlights the variation in PCB signature in partially digested food and tissue.
229 The PCB signature derived from partially digested food items, in particular from the
230 proventriculus (first part of the birds stomach), differed from those measured in the
231 respective host bird (Supplementary Information 1) and strongly influenced principal
232 component 1 of the scores plot (Figure 3). Closer examination of the individual PCBs
233 revealed that this was primarily due to higher proportions of the less chlorinated PCBs, along
234 with CB-118, CB-99 & 101, CB-105 and CB-147 & 149, in the partially digested food (Figure
235 4; Supplementary Information 1). Uniquely, eight PCBs that were absent from the tissue
236 samples were recorded in all of the partially digested food samples (CB-44, CB-49, CB-64,
237 CB-71, CB-87, CB-88&95, and CB-179). The majority of these PCBs contained either -25 or
238 -236 substitution patterns. Many of the congeners present in high proportions in the
239 undigested food have also previously been reported in high proportions in members of the
240 *Gadidae* family (Megson et al., 2013) from the southwest coast of Great Britain, which
241 guillemots are known to feed on (Anderson et al., 2014). Since both samples from the
242 proventriculus contents grouped in a similar area in the scores plot (Figure 3), we suggest
243 that both birds had recently consumed a similar prey type.



244
245 Figure 3. Scores plot of PC1 and PC2 showing the difference in PCB signature between the
246 two birds, along with a different PCB signature in the partially digested food samples.



247

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

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

264 3.2 Chiral PCBs in different tissue types

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

272 2016). This study aims to help address this knowledge gap through the analysis of 12
273 different tissue samples and 3 partially digested food samples in two birds (Supplementary
274 Information 2).

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

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

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

305 **4 Conclusions**

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

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

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

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

323 **Acknowledgements**

324 The authors would like to thank Peter Venn at RSPCA West Hatch for supplying guillemot
325 carcasses and Alec Kettle, Alan Griffiths and Tony Hadley (LECO) for their help and support
326 with the research. Finally, David Megson would also like to thank Plymouth University for
327 funding the initial part of this project which formed part of his PhD research.

328

329 **References**

330

- 331 Acampora, H., White, P., Lyashevskaya, O. and O'Connor, I. (2017) 'Presence of persistent
332 organic pollutants in a breeding common tern (*Sterna hirundo*) population in Ireland',
333 *Environmental Science and Pollution Research*, 24(14), pp. 13025-13035.
- 334 Anderson, H. B., Evans, P. G. H., Potts, J. M., Harris, M. P. and Wanless, S. (2014) 'The diet
335 of Common Guillemot *Uria aalge* chicks provides evidence of changing prey
336 communities in the North Sea', *Ibis*, 156(1), pp. 23-34.
- 337 Boumphrey, R. S., Harrad, S. J., Jones, K. C. and Osborn, D. (1993) 'Polychlorinated
338 biphenyl congener patterns in tissues from a selection of British birds', *Archives of
339 Environmental Contamination and Toxicology*, 25(3), pp. 346-352.
- 340 Bradstreet, M. S. W. and Brown, R. G. B. (1985) 'Feeding ecology of the Atlantic Alcidae', in
341 Nettleship, D.N. & Birkhead, T.R. (eds.) *The Atlantic Alcidae*. London, UK: Academic
342 Press, pp. 263-318.
- 343 Brown, T. A., Belt, S. T., Ferguson, S. H., Yurkowski, D. J., Davison, N. J., Barnett, J. E. F.
344 and Jepson, P. D. (2013) 'Identification of the sea ice diatom biomarker IP25 and
345 related lipids in marine mammals: A potential method for investigating regional
346 variations in dietary sources within higher trophic level marine systems', *Journal of
347 Experimental Marine Biology and Ecology*, 441, pp. 99-104.
- 348 Buckman, A. H., Wong, C. S., Chow, E. A., Brown, S. B., Solomon, K. R. and Fisk, A. T.
349 (2006) 'Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of
350 hydroxylated PCBs in fish', *Aquatic Toxicology*, 78(2), pp. 176-185.
- 351 Chu, S. G., Covaci, A. and Schepens, P. (2003) 'Levels and chiral signatures of persistent
352 organochlorine pollutants in human tissues from Belgium', *Environmental Research*,
353 93(2), pp. 167-176.
- 354 Fernie, K. J., King, R. B., Drouillard, K. G. and Stanford, K. M. (2008) 'Temporal and spatial
355 patterns of contaminants in Lake Erie watersnakes (*Nerodia sipedon insularum*)
356 before and after the round goby (*Apollonia melanostomus*) invasion', *Science of the
357 Total Environment*, 406(1-2), pp. 344-351.
- 358 Frame, G. M., Cochran, J. W. and Bowadt, S. S. (1996) 'Complete PCB congener
359 distributions for 17 aroclor mixtures determined by 3 HRGC systems optimized for

360 comprehensive, quantitative, congener-specific analysis', *Hrc-Journal of High*
361 *Resolution Chromatography*, 19(12), pp. 657-668.

362 Hansen, L. G. (1999) *The ortho side of PCBs*. Kluwer Academic Publishers.

363 Harner, T., Wiberg, K. and Norstrom, R. (2000) 'Enantiomer fractions are preferred to
364 enantiomer ratios for describing chiral signatures in environmental analysis',
365 *Environmental Science & Technology*, 34(1), pp. 218-220.

366 Harrad, S., Ren, J. and Hazrati, S. (2006) 'Chiral signatures of PCB#s 95 and 149 in indoor
367 air, grass, duplicate diets and human feces', *Chemosphere*, 63, pp. 1368-1376.

368 Harris, M. P. and Wanless, S. (2004) 'Common Guillemot (*Uria aalge*)', in Mitchell, P.I.,
369 Stephen, F.N., Ratcliffe, N. & Dunn, T.E. (eds.) *Seabird Populations of Britain and*
370 *Ireland*: T. & A.D. Poyser, London, UK, pp. 350-363.

371 Helsel, D. R. (2006) 'Fabricating data: How substituting values for nondetects can ruin
372 results, and what can be done about it', *Chemosphere*, 65(11), pp. 2434-2439.

373 Hu, D., Martinez, A. and Hornbuckle, K. C. (2011) 'Sedimentary Records of Non-Aroclor and
374 Aroclor PCB mixtures in the Great Lakes', *J Great Lakes Res*, 37(2), pp. 359-364.

375 Hu, D. F., Martinez, A. and Hornbuckle, K. C. (2008) 'Discovery of Non-Aroclor PCB (3,3 '-
376 Dichlorobiphenyl) in Chicago Air', *Environmental Science & Technology*, 42(21), pp.
377 7873-7877.

378 Jaspers, V. L. B., Megson, D. and O'Sullivan, G. (2013) 'Chapter 7, POPs in the Terrestrial
379 Environment', in O'Sullivan, G. & Sandau, C.D. (eds.) *Environmental Forensics for*
380 *Persistent Organic Pollutants*: Elsevier.

381 Jepson, P. D., Deaville, R., Barber, J. L., Aguilar, A., Borrell, A., Murphy, S., Barry, J.,
382 Brownlow, A., Barnett, J., Berrow, S., Cunningham, A. A., Davison, N. J., ten
383 Doeschate, M., Esteban, R., Ferreira, M., Foote, A. D., Genov, T., Gimenez, J.,
384 Loveridge, J., Llavona, A., Martin, V., Maxwell, D. L., Papachlimitzou, A., Penrose, R.,
385 Perkins, M. W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez,
386 A. and Law, R. J. (2016) 'PCB pollution continues to impact populations of orcas and
387 other dolphins in European waters', *Scientific Reports*, 6.

388 Johnson, G. W., Ehrlich, R., Full, W. and Ramos, S. (2007) 'Chapter 7: Principal
389 components analysis and receptor models in environmental forensics', in Morrison, R.
390 & Murphy, B.L. (eds.) *An Introduction to Environmental Forensics. 2nd Edition*.
391 Amsterdam: Elsevier, pp. 207-272.

392 Kania-Korwel, I., El-Komy, M., Veng-Pedersen, P. and Lehmler, H. J. (2010) 'Clearance of
393 Polychlorinated Biphenyl Atropisomers is Enantioselective in Female C57Bl/6 Mice',
394 *Environmental Science & Technology*, 44(8), pp. 2828-2835.

395 Kania-Korwel, I., Hornbuckle, K. C., Robertson, L. W. and Lehmler, H. J. (2008a) 'Dose-
396 dependent enantiomeric enrichment of 2,2 ',3,3 ',6,6 '-hexachlorobiphenyl in female
397 mice', *Environmental Toxicology and Chemistry*, 27(2), pp. 299-305.

398 Kania-Korwel, I. and Lehmler, H. J. (2016) 'Chiral polychlorinated biphenyls: absorption,
399 metabolism and excretion-a review', *Environmental Science and Pollution Research*,
400 23(3), pp. 2042-2057.

401 Kania-Korwel, I., Shaikh, N. S., Hornbuckle, K. C., Robertson, L. W. and Lehmler, H. J.
402 (2007) 'Enantioselective disposition of PCB 136 (2,2 ',3,3 ',6,6 '-hexachlorobiphenyl)
403 in C57BL/6 mice after oral and intraperitoneal administration', *Chirality*, 19(1), pp. 56-
404 66.

405 Kania-Korwel, I., Xie, W., Hornbuckle, K. C., Robertson, L. W. and Lehmler, H. J. (2008b)
406 'Enantiomeric enrichment of 2,2 ',3,3 ',6,6 '-hexachlorobiphenyl (PCB 136) in mice
407 after induction of CYP enzymes', *Archives of Environmental Contamination and*
408 *Toxicology*, 55(3), pp. 510-517.

409 Karjalainen, A., Paakkonen, J. P. J. and Karjalainen, J. (2006) 'Tissue-specific and whole-
410 fish accumulation of polychlorinated biphenyls by juvenile Baltic salmon (*Salmo salar*
411 L.) after oral gavage exposure', *Boreal Environment Research*, 11(6), pp. 421-430.

412 King, T. L., Yeats, P., Hellou, J. and Niven, S. (2002) 'Tracing the source of 3,3 '-
413 dichlorobiphenyl found in samples collected in and around Halifax Harbour', *Marine*
414 *Pollution Bulletin*, 44(7), pp. 590-596.

415 Kodavanti, P. R., Ward, T. R., Derr-Yellin, E. C., Mundy, W. R., Casey, A. C., Bush, B. and
416 Tilson, H. A. (1998) 'Congener-specific distribution of polychlorinated biphenyls in
417 brain regions, blood, liver, and fat of adult rats following repeated exposure to Aroclor
418 1254', *Toxicology and Applied Pharmacology*, 153, pp. 199-210.

419 Maervoet, J., Beck, V., Roelens, S. A., Covaci, A., Voorspoels, S., Geuns, J. M. C., Darras,
420 V. M. and Schepens, P. (2005) 'Uptake and tissue-specific distribution of selected
421 polychlorinated biphenyls in developing chicken embryos', *Environmental Toxicology
422 and Chemistry*, 24(3), pp. 597-602.

423 Megson, D., Brown, T. A., Johnson, G. W., O'Sullivan, G., Bicknell, A. W. J., Votier, S. C.,
424 Lohan, M. C., Comber, S., Kalin, R. B. and Worsfold, P. J. (2014) 'Identifying the
425 provenance of Leach's storm petrels in the North Atlantic using polychlorinated
426 biphenyl signatures derived from comprehensive two-dimensional gas
427 chromatography with time-of-flight mass spectrometry', *Chemosphere*, 114, pp. 195-
428 202.

429 Megson, D., Focant, J. F., Patterson, D. G., Robson, M., Lohan, M. C., Worsfold, P. J.,
430 Comber, S., Kalin, R., Reiner, E. and O'Sullivan, G. (2015) 'Can polychlorinated
431 biphenyl (PCB) signatures and enantiomer fractions be used for source identification
432 and to age date occupational exposure?', *Environment International*, 81, pp. 56-63.

433 Megson, D., Kalin, R. B., Worsfold, P., Gauchotte-Lindsay, C., Patterson Jr, D. G., Lohan, M.
434 C., Comber, S., Brown, T. A. and O'Sullivan, G. (2013) 'Fingerprinting
435 polychlorinated biphenyls in environmental samples using comprehensive two-
436 dimensional gas chromatography with time-of-flight mass', *Journal of
437 Chromatography A*, 1318, pp. 276-283.

438 Megson, D., Robson, M., Jobst, K. J., Helm, P. A. and Reiner, E. J. (2016) 'Determination of
439 Halogenated Flame Retardants Using Gas Chromatography with Atmospheric
440 Pressure Chemical Ionization (APCI) and a High-Resolution Quadrupole Time-of-
441 Flight Mass Spectrometer (HRqTOFMS)', *Analytical Chemistry*, 88(23), pp. 11406-
442 11411.

443 Muir, D. C. G., Norstrom, R. J. and Simon, M. (1988) 'Organochlorine contaminants in arctic
444 marine food-chains - accumulation of specific polychlorinated-biphenyls and
445 chlordane-related compounds', *Environmental Science & Technology*, 22(9), pp.
446 1071-1079.

447 Oki, M. (1983) 'Recent Advances in Atropisomerism', *Topics in Stereochemistry*, 14, pp. 1-
448 81.

449 Robson, M. and Harrad, S. (2004) 'Chiral PCB signatures in air and soil: Implications for
450 atmospheric source apportionment', *Environmental Science & Technology*, 38(6), pp.
451 1662-1666.

452 Rodenburg, L. A., Guo, J., Du, S. and Cavallo, G. J. (2010) 'Evidence for unique and
453 ubiquitous environmental sources of 3,3'-dichlorobiphenyl (PCB 11)', *Environ Sci
454 Technol*, 44(8), pp. 2816-21.

455 Warner, N. A., Martin, J. W. and Wong, C. S. (2009) 'Chiral Polychlorinated Biphenyls Are
456 Biotransformed Enantioselectively by Mammalian Cytochrome P-450 Isozymes to
457 Form Hydroxylated Metabolites', *Environmental Science & Technology*, 43(1), pp.
458 114-121.

459 Wong, C. S., Lau, F., Clark, M., Mabury, S. A. and Muir, D. C. G. (2002) 'Rainbow trout
460 (*Oncorhynchus mykiss*) can eliminate chiral organochlorine compounds
461 enantioselectively', *Environmental Science & Technology*, 36(6), pp. 1257-1262.

462 Wu, X. A., Kammerer, A. and Lehmler, H. J. (2014) 'Microsomal Oxidation of 2,2',3,3',6,6'-
463 Hexachlorobiphenyl (PCB 136) Results in Species-Dependent Chiral Signatures of
464 the Hydroxylated Metabolites', *Environmental Science & Technology*, 48(4), pp.
465 2436-2444.

466