

TECHNIQUES TO AGE DATE HUMAN EXPOSURE TO PCBs

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1 INTRODUCTION

PCBs were first produced in 1929 and were widely used throughout the 20th century for a variety of industrial uses such as dielectric fluids, hydraulic fluids and plasticisers. PCBs are a group of 209 'man made' compounds that were produced as blends, each containing a specific mixture of different PCB congeners. These blends were sold under trade names based on their chlorine content. Monsanto was the market leader in worldwide PCB production and produced approximately 1.2 million tonnes which accounted for more than half of the global total.¹ Monsanto produced nine main Aroclors, accounting for 99.97% of production,² each identified with a four digit code. For example, Aroclor 1260 contained an end product with 60% chlorine by weight. Similar naming systems and production methods were used by the other manufacturers, resulting in the proportions of congeners in Aroclor 1260, Clophen A60 and Kanechlor 600 being almost identical.³ PCB production in the US peaked in 1970.² However, production rates decreased steadily throughout the 70's as the health and environmental risks from PCBs began to be better understood. In the United States this resulted in passing of the Toxic Substances Control Act in 1976 and the phasing out of PCBs which began in 1979.⁴ PCBs have been phased out in open systems but they can still be found in closed systems, e.g. as dielectric fluids in electrical equipment. They are highly persistent and are still routinely found in environmental and human samples across the globe.

All humans have been exposed to background concentrations of PCBs; however there are also unfortunate events where individuals are exposed to elevated concentrations of PCBs. In litigation cases it is especially important to differentiate these specific exposure events from background exposure, as well as identifying the source of contamination and age dating the exposure period. Age dating PCB exposure is a complex task but assessments can be made because different PCB congeners have different residence times in the human body.

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2 FACTORS AFFECTING THE PCB SIGNATURE IN HUMANS

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2.1 Intake of PCBs

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2.2 Uptake of PCBs

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PCBs do not occur naturally in the environment but are present due to releases from human activity. A variety of post release processes, e.g. volatilization, dispersion and biodegradation, can all alter the PCB signature in the environment.³ However, further important changes to the PCB signature can also occur during and after an exposure event. These changes can occur during intake (how PCBs enter the body), uptake (how and where PCBs are absorbed once they enter the body), and post-uptake (factors such as biotransformation and elimination).

PCBs enter humans through three main pathways: ingestion, inhalation and dermal contact. The vast majority of people will only be exposed to background concentrations of PCBs, which will predominantly occur through the ingestion of contaminated foods.⁵ For the UK population it was calculated that food consumption was responsible for 97% of the total exposure to PCBs.⁶ As well as affecting the total intake of PCBs, a person's diet will also influence their specific congener profile because the PCB signature is different for different food groups.⁷ The PCB signature in plants tends to be more closely related to the atmospheric signature than the signature of the soil they were grown in.⁸ This means that vegetables and cereals contain higher proportions of the more volatile, less chlorinated congeners such as CB-28. Lipid rich food groups such as fish and red meat tend to contain higher proportions of the more chlorinated congeners along with higher total PCB concentrations.⁶ As a result, the major source of PCBs in western countries tends to be from fish and meat, whereas cereals are an important source of PCBs in countries such as India where less meat is consumed.⁹ Dermal intake and inhalation are normally considered to be only minor pathways for PCB exposure. However, PCBs do have a relatively high dermal absorption factor (0.14)¹⁰ and there have been some instances where inhalation has proved to be an important pathway.^{11,12} In instances where exposure was through dermal and inhalation pathways, individuals have contained high proportions of the less chlorinated congeners.^{11,13}

Once PCBs have entered the body the rate of uptake is relatively high, a study undertaken in 1972 showed that 88% of the sum of various PCB congeners was absorbed in preadolescent girls.¹⁴ PCBs are highly lipophilic, with log K_{ow} values ranging from 4.3 (for PCB 2) up to 8.26 (for PCB 209),¹⁵ which helps to explain why they accumulate in the body. As PCBs are hydrophobic, individuals with higher body lipid contents tend to have higher concentrations of PCBs. Therefore, when comparing PCB concentrations between individuals, results are often lipid normalised and recorded in $ng\ g^{-1}$ lipid. Due to obvious ethical reasons PCB fractionation in different human organs has not been widely studied. However, fractionation has been observed in humans for polybrominated diphenyl ethers, with enrichment of the less brominated congeners in the umbilical cord blood¹⁶ and breast milk.¹⁷ The majority of fractionation studies have been undertaken on animals where fractionation driven by the log K_{ow} values has resulted in accumulation of less chlorinated PCBs (along with dioxins and furans) in the eggs of fish¹⁸ and birds.¹⁹ Fractionation may not occur across all tissue types however; e.g. no significant difference was observed in the PCB signature in liver and muscle tissue from 7 different predatory bird species in Belgium.²⁰

101 **2.3 Post uptake factors**

102 Once PCBs are taken up by humans they do not remain in the body at constant levels.
103 Individual PCB concentrations and the overall PCB signature are altered by
104 biotransformation and elimination. PCBs are fairly insoluble and therefore need to be
105 transformed into more soluble compounds before they can be excreted.²¹ This usually
106 involves the introduction of an epoxide, hydroxylation or conjugation to produce more
107 water soluble derivatives.²² The metabolic breakdown pathway is usually initiated through
108 hydroxylation involving the cytochrome P450 isozymes (CYP1A and CYP2B). These are
109 not only triggered by the presence of PCBs but can also be activated by PAHs from eating
110 barbecued food, smoking, drinking coffee or by consuming cruciferous vegetables such as
111 broccoli.^{23,24} Not all PCBs are biotransformed in the same way or at the same rate. The
112 structure of the PCB determines which enzyme preferentially transforms it. Co-planar
113 PCBs with no or one *ortho* chlorines are preferred substrates for CYP1A, whereas CYP2B
114 will transform most PCBs.²² In general the more chlorinated congeners are more resistant
115 to biotransformation than the less chlorinated congeners but the exact position of chlorine
116 atoms on the biphenyl is also very important.^{25,26} Based on their resistance in humans,
117 PCBs can be classified into two groups; steady state PCBs and episodic PCBs. Hansen²⁷
118 classified steady state PCBs as ‘more persistent CBs that are commonly reported’, whereas
119 episodic PCBs were classified as PCBs that are ‘generally present only transiently and may
120 be detectable in a small fraction of a survey population’.

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123 **3 IDENTIFICATION OF EPISODIC AND STEADY STATE CONGENERS**

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125 As stated in section 2, there are many factors that can affect the PCB signature in humans.
126 One of the most important factors is the resistance of a PCB to biotransformation and
127 elimination.²⁶ The difference in resistance of certain PCBs can result a noticeable shift in
128 the PCB signature over time. Recently exposed individuals contain higher proportions of
129 episodic congeners and historically exposed individuals contain higher proportions of
130 steady state congeners. Therefore, when age dating human exposure, it is important to
131 correctly identify which congeners are steady state and which are episodic. The following
132 section outlines how Megson *et al.*²⁶ used the National Health and Nutrition Examination
133 Survey (NHANES) to identify steady state and episodic PCBs.

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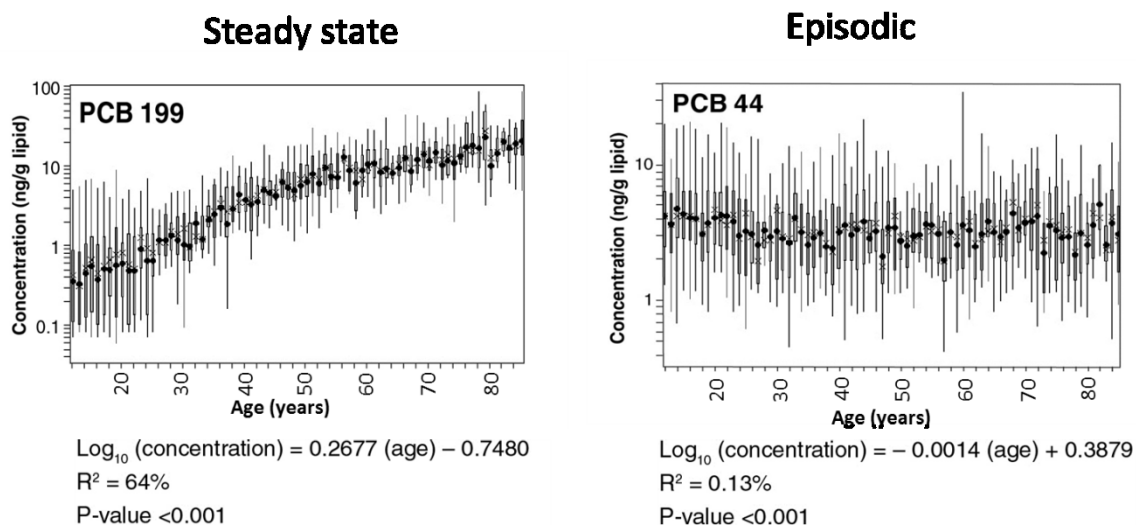
135 **3.1 Interrogation of the NHANES dataset**

136 NHANES is a continuous survey that was designed to monitor the health of the US
137 population through interviews, physical examination and laboratory analysis. This includes
138 the routine determination of a range of contaminants, including a total of 37 PCBs in
139 serum. The most recent survey to include PCB analysis was undertaken in 2003-04 using
140 serum obtained from approximately 2000 individuals. Samples were analysed using high
141 resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS) and
142 quantified by isotope dilution. Data from the NHANES surveys are publically available
143 from the Centers for Disease Control and Prevention (CDC)^{28,29} along with information
144 regarding the collection and analysis of the serum samples and data quality procedures.³⁰

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146 Once downloaded, the NHANES dataset was interrogated by splitting participants into 74
147 yearly age groups from 12 – 85 years old based on their age at the time of participation in
148 the survey. A box and whisker plot of PCB concentrations in each age group was plotted
149 for each congener to identify any difference in PCB concentration with age. Scatter plots
150 of participant age versus log₁₀ transformed PCB concentration were also produced for each

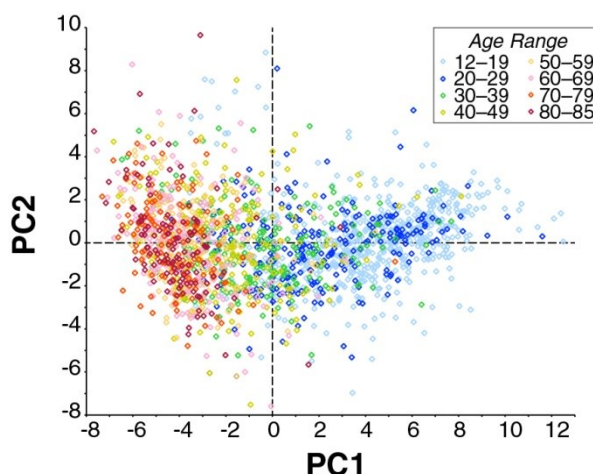
151 congener. A linear regression was applied to each scatter plot and the significance of the
152 resultant relationship between PCB concentration and age was calculated as a P-value. The
153 results were used to identify two groups of PCBs; steady state congeners which showed a
154 significant increase in concentration with age and episodic congeners which showed no
155 significant increase in concentration with age (Figure 1).
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159 **Figure 1** *Steady state and episodic congeners identified from regression analysis*
160 *(Figure adapted from Science of the Total Environment, 461–462, Megson, D.,*
161 *O’Sullivan, G., Comber, S., Worsfold, P. J., Lohan, M. C., Edwards, M. R.,*
162 *Shields, W. J., Sandau, C. D. & Patterson Jr, D. G., Elucidating the structural*
163 *properties that influence the persistence of PCBs in humans using the National*
164 *Health and Nutrition Examination Survey (NHANES) dataset. Copyright*
165 *(2013), with permission from Elsevier)*
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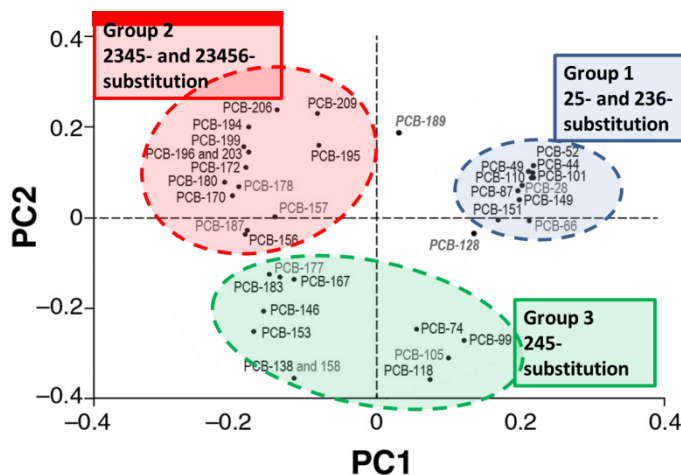
167 Principal component analysis (PCA) was undertaken to further evaluate if there was any
168 relationship between age and PCB signature. Prior to undertaking PCA, PCB
169 concentrations were standardised by dividing the concentration of each PCB by the sum of
170 the concentrations of the 37 PCBs reported in the sample. The data were then normalised
171 in two steps, initially taking the square root of the proportion and then subtracting the mean
172 and dividing by the standard deviation. These transformations were undertaken to prevent
173 high concentration variables from dominating the analysis.³¹ The scores plot showed a
174 clear gradient in the age of participant along the first principal component axis (Figure 2).
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Figure 2 Scores plot showing gradient in participant age along PC1 (Reprinted from *Science of the Total Environment*, 461–462, Megson, D., O’Sullivan, G., Comber, S., Worsfold, P. J., Lohan, M. C., Edwards, M. R., Shields, W. J., Sandau, C. D. & Patterson Jr, D. G., *Elucidating the structural properties that influence the persistence of PCBs in humans using the National Health and Nutrition Examination Survey (NHANES) dataset*. Copyright (2013), with permission from Elsevier)

185 The loadings plot was used to identify three groups of congeners (Figure 3). Group 1 (with
186 positive PC1 values) contained PCBs with predominantly 25- and 236- substitution which
187 were present in higher proportions in the younger participants. Group 2 (with negative PC1
188 values) contained PCBs with predominantly 2345- and 23456- substitution which were
189 present in higher proportions in the older participants. A third group was also separated
190 (with negative PC2 values) containing PCBs with predominantly 245- substitution. The
191 PCBs in Group 2 are indicative of steady state congeners that have accumulated in higher
192 proportions over time as they are more resistant to biotransformation and elimination. The
193 PCBs in Group 1 are indicative of episodic PCBs as the younger individuals have not been
194 alive as long as the older participants and therefore the fractionation of episodic and steady
195 state congeners is less pronounced. Group 3 was poorly correlated with age but contained
196 PCBs that have previously been recorded in depleted proportions due to
197 biotransformation.³²
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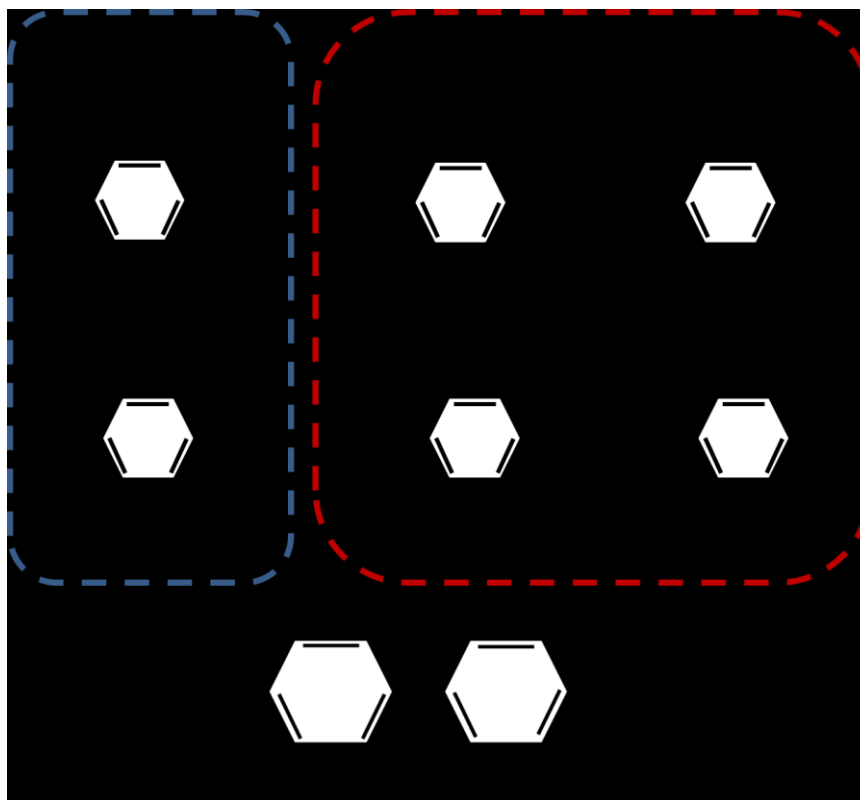
Figure 3 Loadings plot showing groups of PCBs based on chlorine position (Figure adapted from *Science of the Total Environment*, 461–462, Megson, D.,

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O'Sullivan, G., Comber, S., Worsfold, P. J., Lohan, M. C., Edwards, M. R., Shields, W. J., Sandau, C. D. & Patterson Jr, D. G., Elucidating the structural properties that influence the persistence of PCBs in humans using the National Health and Nutrition Examination Survey (NHANES) dataset. Copyright (2013), with permission from Elsevier)

3.2 Structure of episodic and steady state congeners

The results of the regression analysis and PCA were considered along with the cluster analysis presented in Megson *et al.*²⁶. These results were compared with previous studies on PCB metabolism^{25,27,33} to establish how the structure of PCBs affects their resistance to biotransformation and elimination in humans. The results showed that PCBs with chlorine substitution in the 25- and 236- positions appeared to be particularly susceptible to biotransformation and elimination whereas PCBs with 234-, 245-, 345- and 2345- substitution appeared to be more resistant. PCBs with no *para* chlorine and adjacent *meta* chlorine appeared to be the most susceptible (Figure 4). This is believed to be because these are more easily hydrolysed, which is the first metabolic step in the cytochrome P450 breakdown pathway.¹³



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Figure 4 Structure of some episodic and steady state PCBs

4 PCB ANALYSIS BY GCxGC-TOFMS

PCBs were first discovered in environmental samples in 1966.³⁴ However, due to their structural similarity, the separation of all 209 PCBs still presents a significant analytical challenge. When age dating exposure it is essential to be able to resolve episodic and steady state PCBs. The first fully comprehensive PCB analysis was undertaken by Frame

231 in 1997; retention times for all 209 PCBs were documented using multiple analysis on
232 several different GC columns.³⁵ However, to this day, all 209 PCBs have not been
233 separated in one analytical run. Several authors have reported the separation of over 190
234 PCBs using GCxGC-TOFMS.³⁶⁻³⁹ The following section outlines the separation of 200
235 congeners by Megson *et al.*³⁹ and gives examples of how the method can be applied to the
236 determination of PCBs in biological samples, thus demonstrating its potential for age
237 dating human exposure to PCBs.

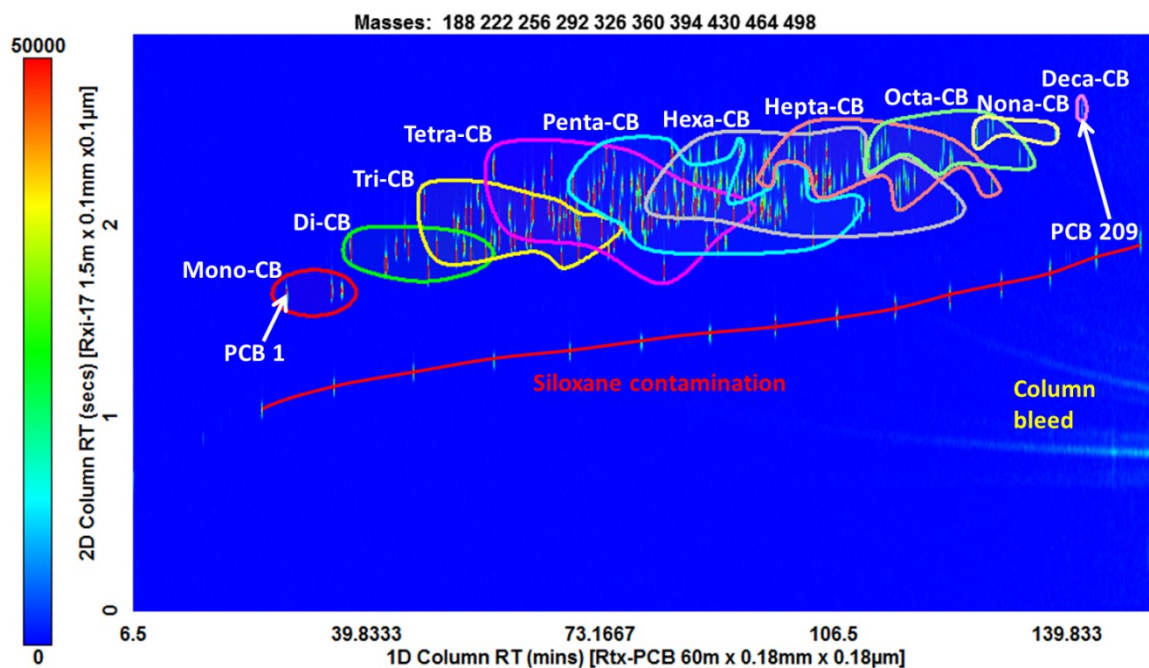
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239 4.1 Identification of episodic and steady state PCBs

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241 Nine PCB congener standard calibration mixtures (CS1 to CS9; AccuStandard) containing
242 $10 \mu\text{g mL}^{-1}$ of each PCB in 1 mL of iso-octane were combined to produce a solution
243 containing all 209 congeners. This was analysed using a time-of-flight mass spectrometer,
244 (LECO, St. Joseph, MI Pegasus 4D) coupled to a two dimensional gas chromatograph
245 (Agilent Technologies 7890A) equipped with a thermal modulator (LECO, St. Joseph,
246 MI). The gas chromatograph was installed with a Rtx-PCB (60 m x 0.18 mm x 0.18 μm)
247 1D column and a Rxi-17 (1.5 m x 0.1 mm x 0.1 μm) 2D column (further settings are
248 reported in ³⁹). Two hundred of the 209 congeners were separated, with the remaining 9
249 co-eluting congeners comprising; three doublets (CB65 + CB62, CB160 + CB163 and
250 CB201 + CB204) and one triplet (CB20 + CB21 + CB33). Of these 9 congeners only 4
251 (CB20, CB33, CB163, CB201) were present in detectable concentrations in the 5 most
252 common commercial Aroclor mixtures, and only 1 (CB21) has a chlorine substitution
253 pattern indicative of a steady state or episodic congener. This method therefore provides
254 excellent separation of episodic and steady state congeners. A 2D contour plot produced
255 from the chromatogram of the 209 PCB mixture is shown in Figure 5.

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259 **Figure 5** Contour plot produced from a mixture containing all 209 PCBs using
260 GCxGC-TOFMS

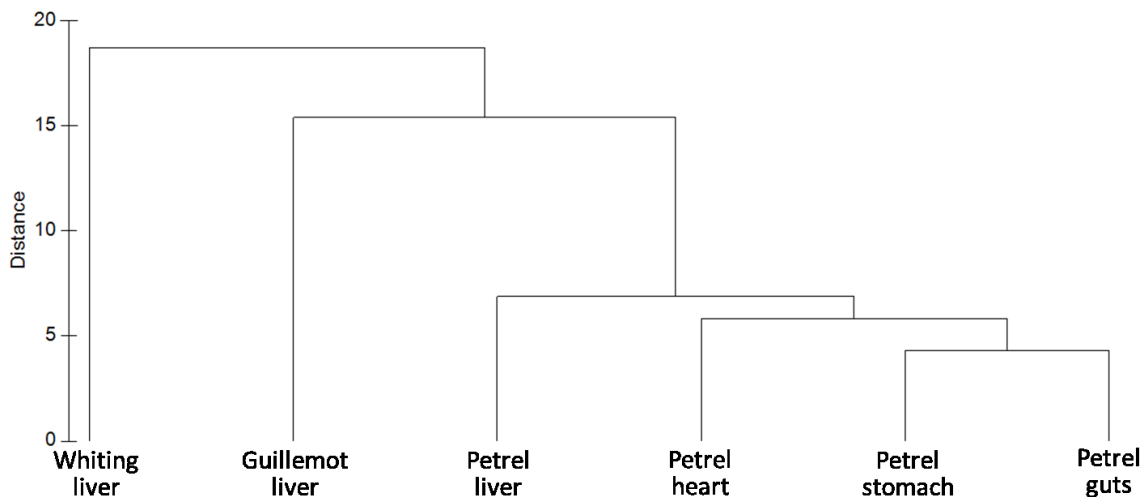
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263 4.2 Separation of PCBs in biological samples

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The application of the method to biological matrices was demonstrated through analysis of several marine animal tissues including; a whiting (*Merlangius merlangus*) liver, a guillemot (*Uria aalge*) liver and a liver, stomach, gut and heart obtained from the same Leach's storm petrel (*Oceanodroma leucorhoa*). The largest number of PCBs identified in these samples was 137 in the whiting liver, with a further 18 tentatively identified with a signal-to-noise ratio <10. The method also identified 120 PCBs in the guillemot liver with a further 11 tentatively identified with a signal-to-noise ratio <10. In the petrel samples 77 PCBs were identified in the heart, 83 in the stomach, 95 in the liver and 100 in the gut. Results from all samples were compared using Euclidian distance analysis to determine if the PCB signature was similar for the different petrel organs and/or if the signature in the liver was similar across the three species. Prior to statistical analysis all PCBs detected in less than 4 of the 6 samples were removed from the data set. This was undertaken to reduce the effect of using a LOD substitution value for instances where around 30% of the data was below the detection limit.⁴⁰ The remaining 93 PCBs with values < LOD were reported at 0.5 LOD. All PCB results were standardised by converting each PCB to proportions of the total PCB count in that sample. The data were normalised by taking the square root of the proportion then subtracting the mean and dividing by the standard deviation. Euclidian distance was calculated for the samples and the results presented as a cluster diagram (Figure 6). The cluster diagram shows the high similarity in the PCB signature between the samples obtained from the petrel, indicating that the signature was well retained throughout the animal's organs. However, there were distinct differences in the signature from the stomach sample which showed elevated proportions of PCB 138 and PCB 180. This may be associated with the PCB signature in undigested food within the stomach.



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Figure 6 Cluster plot of Euclidian distance of the PCB signature in four petrel organs, guillemot liver and whiting liver

5 CONCLUSIONS

Age dating PCB exposure is a complex task; however assessments can be made because different PCB congeners have different residence times in the human body. Episodic PCBs with chlorine substitution in the 25- and 236- positions appeared to be particularly

301 susceptible to biotransformation and elimination whereas steady state PCBs with 234-,
302 245-, 345- and 2345- substitution appeared to be more resistant. When age dating exposure
303 it is essential to be able to resolve these episodic and steady state PCBs. Analysis using
304 single column chromatography will often result in the co-elution of several key congeners.
305 However, analysis by comprehensive two-dimensional gas chromatography was able to
306 resolve 200 of the 209 congeners, a significant improvement on one dimensional gas
307 chromatography. The method was tested on biological matrices by conducting a
308 fingerprinting exercise which distinguished different Lech's storm petrel organs from
309 whiting and guillemot samples. The results showed that although different organs from the
310 same petrel contained different total PCB concentrations the signature was well retained
311 throughout each of the analysed organs. However, there were still subtle differences
312 between the PCB signature of the different storm petrel organs.

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314 Research is currently on-going to apply the statistical and analytical methods discussed in
315 this paper to age date human exposure to PCBs.

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Acknowledgements

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