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.

2 FACTORS AFFECTING THE PCB SIGNATURE IN HUMANS

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).

2.1 Intake of PCBs

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]

2.2 Uptake of PCBs

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 blood[16] 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 fish[18] 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]

2.3 Post uptake factors

Once PCBs are taken up by humans they do not remain in the body at constant levels. Individual PCB concentrations and the overall PCB signature are altered by biotransformation and elimination. PCBs are fairly insoluble and therefore need to be transformed into more soluble compounds before they can be excreted.[21] This usually involves the introduction of an epoxide, hydroxylation or conjugation to produce more water soluble derivatives.[22] The metabolic breakdown pathway is usually initiated through hydroxylation involving the cytochrome P450 isozymes (CYP1A and CYP2B). These are not only triggered by the presence of PCBs but can also be activated by PAHs from eating barbecued food, smoking, drinking coffee or by consuming cruciferous vegetables such as broccoli.[23,24] Not all PCBs are biotransformed in the same way or at the same rate. The structure of the PCB determines which enzyme preferentially transforms it. Co-planar PCBs with no or one *ortho* chlorines are preferred substrates for CYP1A, whereas CYP2B will transform most PCBs.[22] In general the more chlorinated congeners are more resistant to biotransformation than the less chlorinated congeners but the exact position of chlorine atoms on the biphenyl is also very important.[25,26] Based on their resistance in humans, PCBs can be classified into two groups; steady state PCBs and episodic PCBs. Hansen[27] classified steady state PCBs as ‘more persistent CBs that are commonly reported’, whereas episodic PCBs were classified as PCBs that are ‘generally present only transiently and may be detectable in a small fraction of a survey population’.

3 IDENTIFICATION OF EPISOIC AND STEADY STATE CONGENERS

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

3.1 Interrogation of the NHANES dataset

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

Once downloaded, the NHANES dataset was interrogated by splitting participants into 74 yearly age groups from 12 – 85 years old based on their age at the time of participation in the survey. A box and whisker plot of PCB concentrations in each age group was plotted for each congener to identify any difference in PCB concentration with age. Scatter plots of participant age versus $\log_{10}$ transformed PCB concentration were also produced for each congener. A linear regression was applied to each scatter plot and the significance of the resultant relationship between PCB concentration and age was calculated as a P-value. The results were used to identify two groups of PCBs; steady state congeners which showed a significant increase in concentration with age and episodic congeners which showed no significant increase in concentration with age (Figure 1).

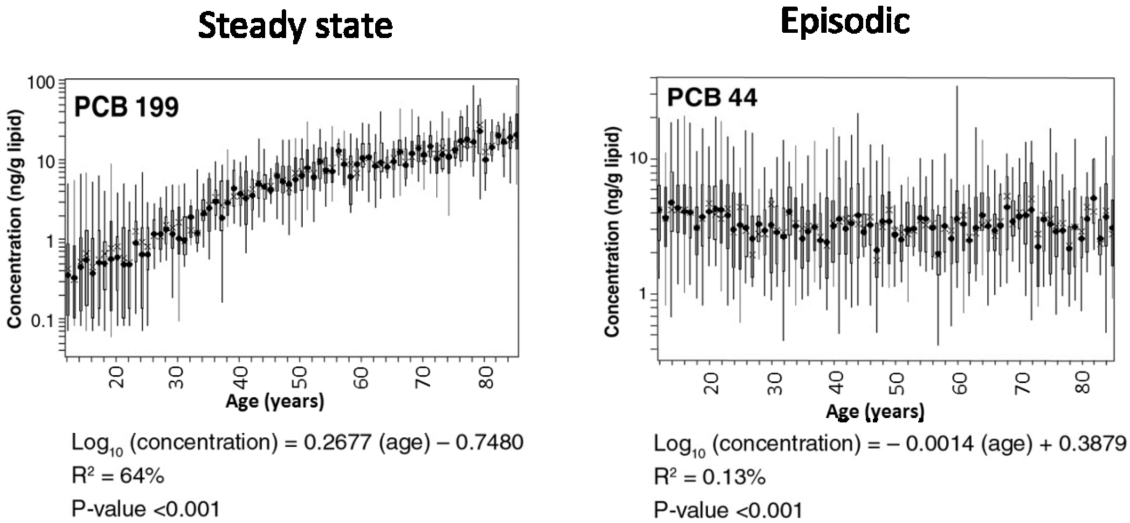

Figure 1 *Steady state and episodic congeners identified from regression analysis (Figure adapted from Science of the Total Environment, 461–462, Megson, D., O'Sullivan, G., Comber, S., Worsfold, P. J., Lohan, M. C., Edwards, M. R., Shields, W. J., Sandau, C. D. & Patterson Jr, D. G., Elucidating the 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)*

Principal component analysis (PCA) was undertaken to further evaluate if there was any relationship between age and PCB signature. Prior to undertaking PCA, PCB concentrations were standardised by dividing the concentration of each PCB by the sum of the concentrations of the 37 PCBs reported in the sample. The data were then normalised in two steps, initially taking the square root of the proportion and then subtracting the mean and dividing by the standard deviation. These transformations were undertaken to prevent high concentration variables from dominating the analysis.[31] The scores plot showed a clear gradient in the age of participant along the first principal component axis (Figure 2).

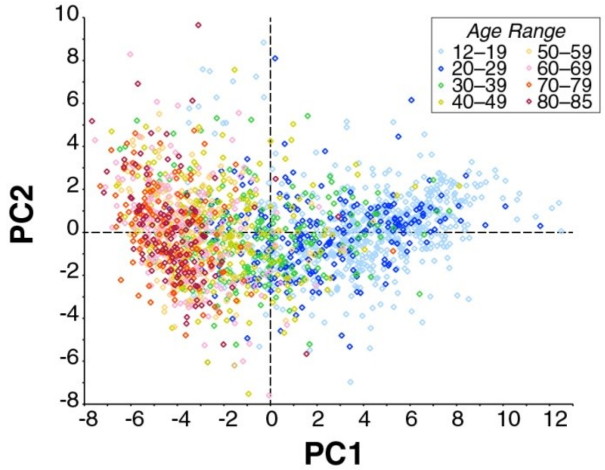

Figure 2 *Scores plot showing gradient in participant age along PC1 (Reprinted from Science of the Total Environment, 461–462, Megson, D., O'Sullivan, G., Comber, S., Worsfold, P. J., Lohan, M. C., Edwards, M. R., Shields, W. J., Sandau, C. D. & Patterson Jr, D. G., Elucidating the structural properties that influence the persistence of PCBs in humans using the National Health and Nutrition Examination Survey (NHANES) dataset. Copyright (2013), with permission from Elsevier)*

The loadings plot was used to identify three groups of congeners (Figure 3). Group 1 (with positive PC1 values) contained PCBs with predominantly 25- and 236- substitution which were present in higher proportions in the younger participants. Group 2 (with negative PC1 values) contained PCBs with predominantly 2345- and 23456- substitution which were present in higher proportions in the older participants. A third group was also separated (with negative PC2 values) containing PCBs with predominantly 245- substitution. The PCBs in Group 2 are indicative of steady state congeners that have accumulated in higher proportions over time as they are more resistant to biotransformation and elimination. The PCBs in Group 1 are indicative of episodic PCBs as the younger individuals have not been alive as long at the older participants and therefore the fractionation of episodic and steady state congeners is less pronounced. Group 3 was poorly correlated with age but contained PCBs that have previously been recorded in depleted proportions due to biotransformation.[32]

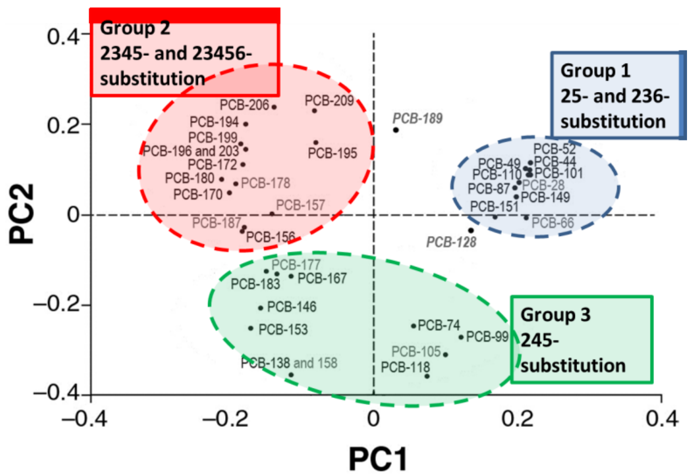

Figure 3 *Loadings plot showing groups of PCBs based on chlorine position (Figure adapted from Science of the Total Environment, 461–462, Megson, D.,*

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)

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]

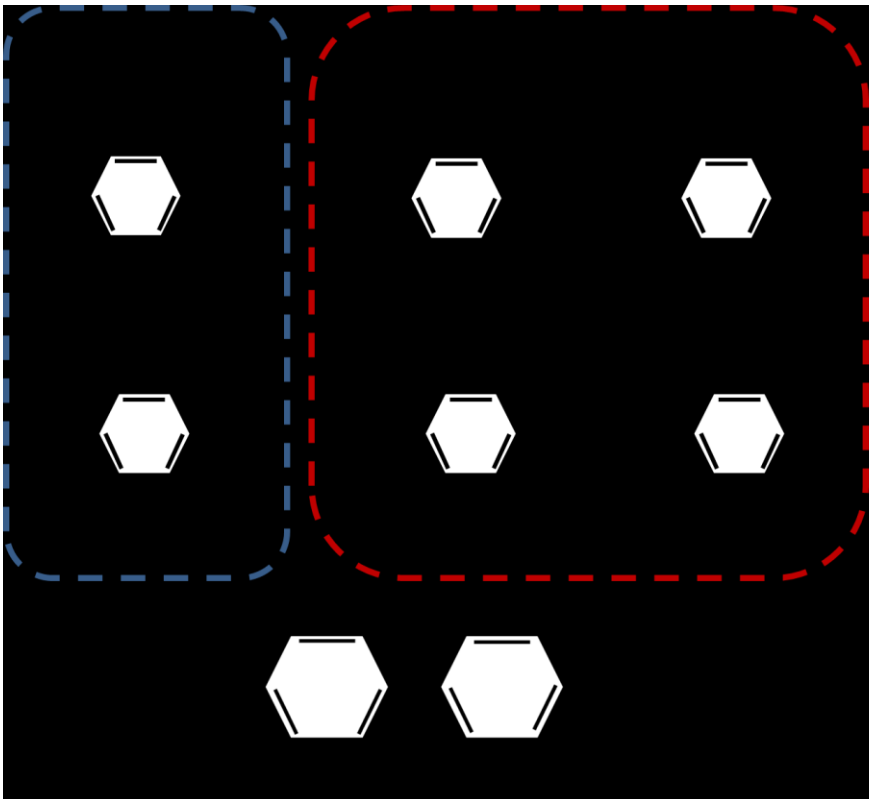

Figure 4 *Structure of some episodic and steady state PCBs*

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

4.1 Identification of episodic and steady state PCBs

Nine PCB congener standard calibration mixtures (CS1 to CS9; AccuStandard) containing 10 µg mL^{-1} of each PCB in 1 mL of isooctane were combined to produce a solution containing all 209 congeners. This was analysed 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) 1D column and a Rxi-17 (1.5 m x 0.1 mm x 0.1 µm) 2D column (further settings are reported in [39]). Two hundred of the 209 congeners were separated, with the remaining 9 co-eluting congeners comprising; three doublets (CB65 + CB62, CB160 + CB163 and CB201 + CB204) and one triplet (CB20 + CB21 + CB33). Of these 9 congeners only 4 (CB20, CB33, CB163, CB201) were present in detectable concentrations in the 5 most common commercial Aroclor mixtures, and only 1 (CB21) has a chlorine substitution pattern indicative of a steady state or episodic congener. This method therefore provides excellent separation of episodic and steady state congeners. A 2D contour plot produced from the chromatogram of the 209 PCB mixture is shown in Figure 5.

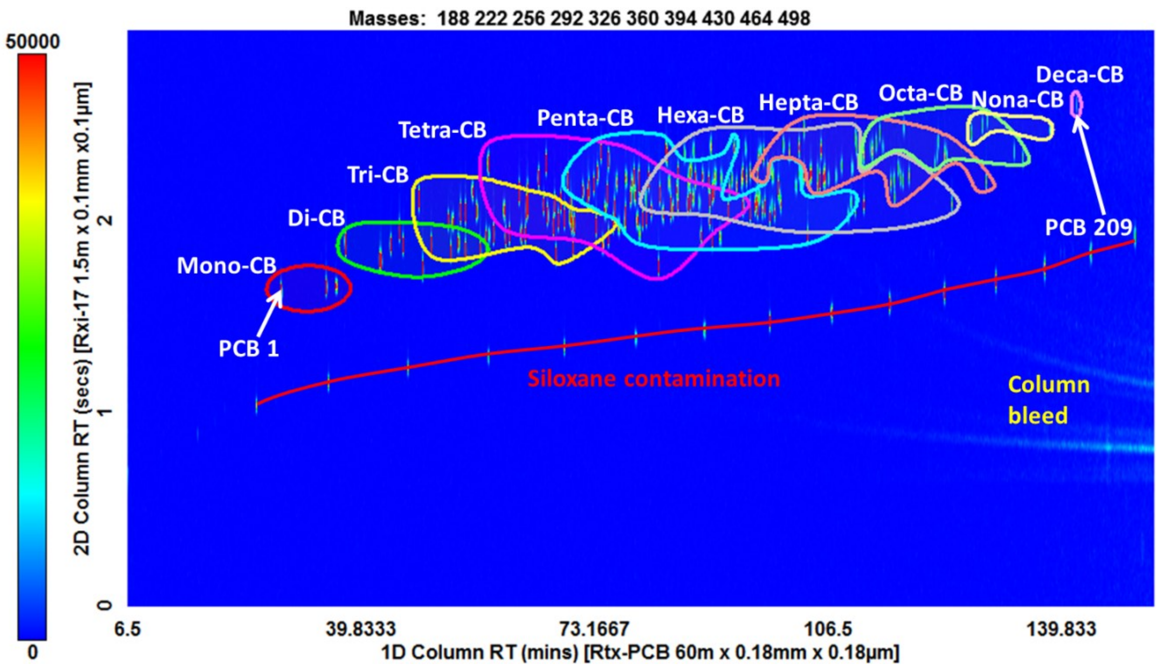

Figure 5 *Contour plot produced from a mixture containing all 209 PCBs using GCxGC-TOFMS*

4.2 Separation of PCBs in biological samples

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.

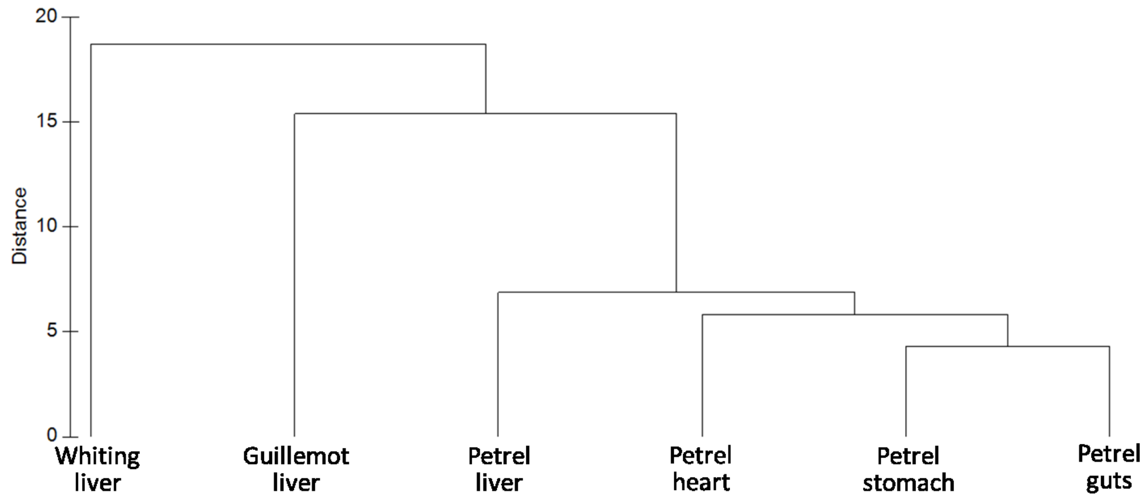

Figure 6 *Cluster plot of Euclidian distance of the PCB signature in four petrel organs, guillemot liver and whiting liver*

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

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

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