Elucidating the Structural Properties that Influence the Persistence of PCBs in Humans using the National Health and Nutrition Examination Survey (NHANES) Dataset

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Abstract

In human exposure studies involving Polychlorinated Biphenyls (PCBs), it is useful to establish when an individual was potentially exposed. Age dating PCB exposure is complex but assessments can be made because different PCB congeners have different residence times in the human body. The less chlorinated congeners generally tend to have shorter residence times because they are biotransformed and eliminated faster than more chlorinated congeners. Therefore, the presence of high proportions of less chlorinated congeners is often indicative of recent exposure. The 2003-04 National Health and Nutrition Examination Survey (NHANES) dataset contains results for the concentration of 37 PCBs in a sub-sample of the US population. Multivariate statistical analysis of the NHANES data showed that less chlorinated congeners are not always biotransformed faster than higher chlorinated compounds. For example, PCB 28 (a tri-chlorobiphenyl) appears to be more resistant to biotransformation than PCB 101 and 110 (penta-chlorobiphenyls). Using statistical analysis of the NHANES data in conjunction with previously published studies on PCB persistence in humans, it was possible to identify the structural relationships that determine if a PCB is likely to be from a recent exposure (termed 'episodic') or from steady state exposure. Congeners with chlorine atoms in the 2,5- and 2,3,6- positions appear to be more susceptible to biotransformation whereas congeners with chlorine bonds in the 2,3,4- 2,4,5- 3,4,5- and 2,3,4,5- positions appear to be more persistent. This work shows that future investigations to date PCB exposure would benefit from the analysis of a wide range of congeners, including the selection of key congeners based not only on the degree of chlorination but also on the positions of the chlorine atoms on the biphenyl.

Key words

Polychlorinated biphenyls, PCBs, NHANES, age dating, biotransformation, residence time

1 Introduction

Polychlorinated biphenyls (PCBs), a group of 209 chlorinated organic compounds, were first synthesised for industrial purposes in 1929. They were widely used until the 1980s when their use was phased out due to environmental and human health risks (Erickson, 2001; Johnson et al., 2006). Concentrations of PCBs in the environment vary widely and depend on the solubility, volatility and lipophilicity (often indicated by the octanol-water partition coefficient (log Kow)) of the individual congener. PCBs concentrations in humans are dictated by the rates of intake, bioavailability, biotransformation and elimination. The average person in the United States of America will be exposed to background PCBs in low concentrations, estimated in the early 1990s at 0.53 µg/person/day, with food consumption accounting for 97% of this background (Duarte-Davidson and Jones, 1994). Because of their

persistence and toxicity, PCB concentrations in the US population are routinely monitored within The United States National Health and Nutrition Examination Survey (NHANES) (Centers for Disease Control and Prevention, 2011a; Patterson et al., 2008; Patterson et al., 2009b).

In environmental and human exposure studies, it is useful to establish when an individual was exposed to PCBs. 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. Predictions of the residence times of individual congeners based on the position of chlorine atoms on the biphenyl were pioneered by Brown (1994). Since then several studies have calculated different PCB residence times in humans (Ritter et al., 2011). Hansen (2001) separated PCBs into categories based on their perceived persistence in human samples. This resulted in the designation of two types of PCBs, steady state and episodic congeners. Hansen (2001) 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'. Throughout this paper reference is made to steady state and episodic congeners where steady state congeners are those that are persistent in the body and episodic congeners are susceptible to biotransformation and elimination and therefore are only identified in humans transiently.

Half-life calculations for several common PCB congeners showed a general trend of shorter half-lives for less chlorinated congeners (Seegal et al., 2011). There is a wide range of calculated PCB half-lives however and different studies have produced results which have varied by a factor of 50 for the same PCB. A recent review of the persistence of PCBs in humans calculated half-lives ranging from 2.6 years for PCB 52 up to 15.5 years for PCB 170 (Ritter et al., 2011).

PCBs are relatively insoluble and therefore require transformation into more polar compounds before they can be excreted (Sandau, 2001). Metabolic pathways include the

introduction of an epoxide, hydroxylation or conjugation to produce water soluble derivatives (Letcher et al., 1999). Generally PCBs with a higher degree of chlorination and co-planar PCBs with no *ortho* chlorines are most persistent in the body (Hansen, 1999; Herrick et al., 2011; James, 2001; Seegal et al., 2011). This is believed to be because these congeners are more resistant to the first metabolic breakdown step, which is hydroxylation involving the cytochrome P450 system (James, 2001).

There is some uncertainty surrounding the biotransformation and relative half-lives of PCBs in humans. The presence of high proportions of less chlorinated congeners are assumed to be indicative of recent exposure (Brown and Lawton, 2001; Duarte-Davidson and Jones, 1994; Herrick et al., 2011). However, this assumption has several limitations. If inhalation is the dominant rout of exposure then the individual is likely to be exposed to a higher proportion of the more volatile, less chlorinated congeners. If exposure is from a heavily weathered or more chlorinated Aroclor, the individual will be exposed to higher proportions of the more chlorinated compounds. Therefore, it is important to understand the source and contaminant pathway when attempting to age date exposure. The identification of both episodic and steady state congeners based on the degree of chlorination can be used to improve age dating techniques and provide a more consistent approach that is applicable to a wider range of potential PCB sources.

This paper presents a multivariate statistical analysis of the NHANES 2003-04 dataset to identify steady state and episodic congeners from background concentrations of PCBs present within the US population. These results were used in conjunction with other reviews on PCB metabolism (Brown and Lawton, 2001; Hansen, 2001) to identify how the structure of PCBs relates to rates of biotransformation in humans. Statistical analysis of the NHANES dataset for structure activity and rates of biotransformation has not previously been undertaken to the authors' knowledge.

2 Methodology

2.1 NHANES Data

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. Data from the NHANES surveys are publically available from the CDC (Centers for Disease Control and Prevention, 2011b)

The NHANES survey uses a complex, stratified, multistage probability sampling strategy. This allows the study to be representative of the civilian, non-institutionalized U.S. population by age, gender, and race/ethnicity. In the 2003-04 survey PCB analysis was undertaken on serum obtained from approximately 2000 individuals, which corresponds to one third of the total number of participants in the survey who were aged 12 or above. Serum samples (1 – 1.5 mL) were spiked with $^{13}C_{12}$ labelled internal standards. PCBs were isolated using a C_{18} solid phase extraction (SPE) procedure followed by extraction through neutral silica and Florisil columns with hexane and 1:1 dichloromethane / hexane. Before quantification samples were reconstituted with 10 µL of ^{13}C labelled external standard. Samples were analysed using high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS). 1 µL of sample was injected into a Hewlett-Packard 6890 gas chromatograph operated in the splitless injection mode with a flow of 1 $mLmin^{-1}$ helium through a DB-5ms capillary column (30 m x 0.25 mm x 0.25 µm film thickness) where analytes are separated prior to entering a Thermo Finnigan MAT95 XP (5 kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley).Further information regarding the collection and analysis of serum samples together with data quality procedures are available from the CDC (Centers for Disease Control and Prevention, 2011b).

The 2003-04 dataset contains results from the most recent PCB analysis. Although these PCBs are mostly reported as individual congeners, they have the potential to co-elute with one or more other congeners. PCBs analysed by GC-MS are often a combination of several PCBs where only the predominant congener is named. HRMS operating with a resolving power of 10,000 is able to separate PCBs from co-contaminants with a similar mass but cannot separate PCB isomers. Therefore the analytical technique was not able to resolve PCB 138 from 158 or PCB 196 from 206 and these congeners were reported as PCB 138 & 158 and PCB196 & 206. Due to these co-elutions the NHANES data set contains results for a total of 37 PCBs presented as 35 variables. Results for PCB 81, 126 and 169 are also provided in the NHANES dataset. However, these were not included in this assessment as they made a negligible contribution to the total PCBs because of their very low parts per trillion (pg/g) concentrations compared to parts per billion (ng/g) for the other 37 PCBs (Patterson et al., 2009b).

The following data files were downloaded from the NHANES database, “L28DFP_C.xpt”, “L28NPB_C.xpt” and “DEMO_C.xpt”. These data files contain the results for dioxin like PCBs, non-dioxin like PCBs and the demographic variables for each participant, respectively. These datasets were transferred and combined to associate the demographics data with the analytical results. Only the lipid- adjusted PCB concentrations were used in the statistical analysis to allow comparison with other studies. The NHANES datasets reports concentrations below the limit of detection (LOD) as the LOD divided by the square root of two. This transformation was retained for the subsequent statistical analysis.

2.2 Statistical analysis

Participants were split into 74 yearly age groups from 12 – 85 years old based on their age at the time of participation in the survey. As the NHANES survey was a long term process, samples were not collected from all participants at the same time. Although the distribution of participants was not evenly spread across all of the age groups, there were sufficient participants in each group to provide a reliable assessment. There were approximately 30

participants in each age group above 20 years old and approximately 80 participants in each of the eight age groups from 12 and 19 years old. During the regression analysis a plot of the residuals against the fitted values showed no uneven distribution due to this bias.

A sensitivity assessment was undertaken to evaluate the effect of the LOD transformation of LOD divided by the square root of two compared with results below the LOD substituted as both zero and the LOD. Using different values for results below the LOD did not alter the conclusions drawn from the analyses reported. There is, however, less certainty in the trends found for PCBs containing a high proportion of results below the LOD.

A box and whisker plot of PCB concentrations in each age group was plotted for each congener. Scatter plots of participant age against $\log_{10}$ transformed PCB concentrations 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. Plots and significance testing were undertaken using Minitab v16.

Cluster analysis based on Euclidean distances was performed using the PCB congeners as the subject of analysis rather than the individual samples. This focused the analysis to associations among PCBs in sera rather than relationships between samples, i.e. people. Results of the cluster analysis were presented in a dendogram (tree diagram), based on the distance matrix between each PCB and every other PCB. PCBs with similar proportions in all serum samples will have small distances whereas PCBs with highly variable proportions across samples will have large distances. The dendrogram clustered PCBs based on how similar their proportions were across all serum samples.

Principal component analysis (PCA) was undertaken to evaluate if there was any relationship between age and PCB signature. 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). These results can be plotted as a loadings plot showing co-varying

contaminants, and a scores plot showing the similarity of samples based on their chemical composition.

For both the cluster analysis and 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 then secondly by subtracting the mean and dividing by the standard deviation. These transformations were undertaken to keep high concentration variables from dominating the analysis (Johnson et al., 2002). All multivariate analysis and plots were produced using PRIMER 6.

The NHANES PCB data was originally collated to undertake an assessment of the background concentrations of PCBs in the US population. The NHANES dataset has been used by others to identify differences in congener patterns within subgroups of the US population (Axelrad et al., 2009; Weintraub and Birnbaum, 2008). By using the data to investigate the relative rate of biotransformation of PCBs, the data has been used in a novel way for a purpose other than it was originally intended. This paper has used the NHANES sample measurements as a distinct study and the findings are not intended to be directly representative of the US population. For the data to be directly representative of the US population specific weighting factors would need to be applied to the individual data results. This would require further population statistics which is beyond the scope of this paper.

3 Results

Four different techniques were used to identify episodic and steady state congeners in serum based on the NHANES data. These techniques were regression analysis, PCA, cluster analysis and signature plots.

3.1 Regression analysis

Box and whisker plots of lipid adjusted PCB concentrations in each age group were plotted for each congener using Minitab v16. The results for a selection of 4 PCBs (PCB 44, 99, 187 and 199) are displayed as Figure 1. These congeners were selected as they represent the range in fitted gradients. Scatter plots of the data were also produced and fitted with a linier trend line, P values were calculated to test if the gradient of the line was significantly greater than 0.

32 out of 37 PCBs displayed an increase in PCB concentration with age, with the exception of PCB 87, PCB128 and PCB 149 the increase could be considered significant at the 95% confidence level. 5 out of 37 PCBs, (PCB 44, 49, 52, 101 and 110) displayed a decrease in concentration with age that was significant at the 95% confidence level. PCB 44, 49, 52, 87, 101, 110 and 149 did not display a significant increase in concentration with age are therefore considered to be susceptible to biotransformation and elimination and are therefore cleared from the body relatively quickly. PCB 128 also did not show a significant increase in concentration with age; however this was believed to be because 75% of the samples had concentrations below the limits of detection.

<Figure 1>

3.2 Principal Component Analysis (PCA)

PCA was undertaken to identify any differences in the PCB signature of participants of different ages. If the source of contamination was the dominant factor controlling the PCB signature there should be no association with age. However, if biotransformation and elimination had the greatest influence on the PCB signature, then the older participants would show a chemically different signature to younger participants and contain higher proportions of the more persistent congeners. This appears to be the case for the NHANES data; principal component 1 (PC1) is closely correlated with participant age. PC1 accounted for 50% of the variance in the model (with PC2 accounting for 11%) and although there is a

good correlation with age there is a high degree of variance in the data which could not be accounted for by age alone. This is likely to be attributed to other factors such as different individual's diets and rates of metabolism. The scores plot (Figure 2) shows a gradient with age along the PC1 axis. The scores plot can be compared with the loadings plot (Figure 3) to identify patterns in the congener profiles of the individuals. For example, participants who are positioned in the top right quadrant of the scores plot (with positive scores on PC1 and PC2) will have signatures containing elevated proportions of the PCBs located in the top right quadrant of the loadings plot (with positive eigenvector values on PC1 and PC2).

The loadings plot (Figure 3) shows a tight grouping of PCBs with eigenvector values for PC1 >0 and for PC2 close to or >0 (Group 1), including PCBs 28, 44, 49, 52, 66 87, 101, 110, 149 and 151. This group was predominantly comprised of congeners with 2,5 and 2,3,6 substitution which are present on the right side of the plot, indicating enrichment of these congeners in the younger participants. A second group of congeners were identified with eigenvector values for of PC1 of <0 and for PC2 close to or >0 (Group 2). This group was predominantly comprised of congeners with 2,3,4,5 and 2,3,4,5,6 substitution which are present on the left side of the plot, indicating enrichment of these congeners in the older participants. The third group of congeners were those with the most negative eigenvector values for PC2 and were not well correlated with age. This group was predominantly comprised of congeners with 2,4,5 substitution.

<Figure 2>

<Figure 3>

3.3 Cluster Analysis

Results of a cluster analysis based on Euclidean distances between all PCBs were plotted as a dendrogram (Figure 4). The dendrogram separates the PCBs into similar groupings as was observed in the PCA. The first degree of separation at a distance of 73, produces a grouping which contains all PCBs with a value for PC1 of <1. The remaining group contains

the tight subgroup of 7 PCBs with a distance of less than 27, which comprises only congeners with 25 and 236 substitution (PCB 149, 87, 101, 110, 49, 44 and 52).

<Figure 4>

3.4 Signature plots

To further highlight the differences in PCB signature, box plots of the proportions of each congener from the 12 year old participants and 85 year old participants were compared. The proportions of each congener were calculated for the two age groups to show the relative enrichment in each group. The results are presented as Figure 5 and show relative enrichment of several congeners in the 12 year olds sera, particularly PCBs 44, 49, 52, 87, 101, 110, and PCB 149. Relative enrichment of PCB 128 and PCB 189 was also recorded in the 12 year olds sera; however these PCBs were not detected in around 75% of participants so the results from these congeners should be treated with caution.

<Figure 5>

3.5 Summary of results

The four statistical techniques show similar conclusions for most congeners. Table 1 presents the results of the regression analysis and loadings for PC1. This data was used along with the cluster analysis and signature plots to identify episodic and steady state congeners. Congeners exhibiting a negative or non-significant gradient, a value for PC1 and PC2 of >0 and clustering on the loadings plot and dendrogram were identified as episodic. These were PCBs 44, 49, 52, 87, 101, 110, and 149. Congeners that showed conflicting results across the four techniques (PCB 66 and 151) and those that had a high percentage of results below the LOD (PCB 128 and PCB 189) were marked as ‘unsure’ in Table 1. The remaining congeners were considered steady state as they exhibited a significant increase in concentration with participant age. Table 1 includes the structural formula for each PCB, and is ordered based on the relative increase/decrease in PCB concentration with age.

Congeners showing the greatest relative increase with age are at the bottom of the table with the episodic congeners at the top.

<Table 1>

4 Discussion

The biotransformation and elimination of PCBs is a highly complex process that is not fully understood. Rates of biotransformation of both total PCBs and individual congeners vary greatly between individuals. Differences have been observed between individuals of different ethnic groups, age groups, body weights and even those with different diets and social habits such as smoking and coffee consumption (Axelrad et al., 2009; International Programme on Chemical Safety (IPCS), 1993; Jain and Wang, 2010; Jain and Wang, 2011; Nichols et al., 2007; Weintraub and Birnbaum, 2008).

PCBs need to be biotransformed into more polar compounds before they can be eliminated from the body (Sandau, 2001). Metabolic pathways to produce water soluble derivatives include hydroxylation or the formation of an epoxide followed by Phase II enzymatic conjugation (Sandau, 2001). 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 less than one *ortho* chlorine are preferred substrates for cytochrome P450 1A isozymes (CYP1A) , whereas cytochrome P450 2B isozymes (CYP2B) transform most of the PCBs (Letcher et al., 1999). These enzymes are not only induced by the presence of PCBs, environmental and dietary conditions are also important, and CYP1A can be induced by exposure to PAHs and CYP2B by eating cruciferous vegetables (James et al., 2008).

The metabolic processes that control PCB biotransformation are highly variable. Different processes have been reported in different animal species and also within different human sub-groups (Brown, 1994). Some metabolic processes also appear to be dependent on the

source of contamination. Brown and Lawton (2001) reported the findings of two types of degradation patterns that had been recorded in humans, Pattern A and Pattern B (called Pattern C when involved with more heavily chlorinated Kanechlors) (Masuda et al., 1974). Biotransformation through the CYP1A-like pathway produces a signature with depleted proportions of PCBs 28, 74, 105, 118 and 167 (Pattern A), whereas biotransformation through the CYP2B-like pathway produces a signature with depleted proportions of PCBs 99, 138, 153 and 163 (Pattern B).

The results of this study were assessed to see if these different metabolic pathways had a significant impact on the signature of the participants in the NHANES data. PC1 showed a good correlation with the age of the participant (Figure 2), which was used to help separate PCBs into groups of episodic and steady state congeners. However, principal component analysis also identified a third group of PCBs. These were identified as Group 3 on the loadings plot (Figure 3) and were poorly correlated with age. This group included PCB 74, 99, 105, 118, 138, 146, 153, 167, 177 and 183. With the exception of PCB 28 (and PCB 163 which was not included in the NHANES dataset) this group contains all the congeners that were identified by Masuda et al. (1974) in depleted proportions due to biotransformation through CYP1A and CYP2B pathways. This indicates that PC2 may be linked to the different metabolic rates of different participants.

The results indicated the enrichment of several congeners in the 12 year olds sera, particularly PCBs 44, 49, 52, 87, 101, 110, and PCB 149. Similar trends were detected in other school aged children by Patterson et al. (2009a). However, several of these congeners (44, 49, 52, 87, 149) have been reported to be associated with inhalation exposure by DeCaprio (2005) and Herrick (2011).

The classification of episodic and steady state congeners shown in Table 1 is loosely related to the degree of chlorination. Congeners with a higher degree of chlorination are generally more resistant to biotransformation and elimination from the body and therefore have longer

relative residence times (Hansen, 1999; Herrick et al., 2011; James, 2001; Seegal et al., 2011). However, there are some exceptions to this trend. For example PCB 28 which has 3 chlorine atoms, appears to be more resistant to biotransformation and elimination than PCB 149 which has 6 chlorine atoms and PCB 110 and 101 which both have 5 chlorine atoms. Although PCB 28 was located within the group of episodic congeners in the PCA and cluster analysis, the regression analysis indicated a significant increase in the concentration of PCB 28 with age. It has also previously been classed as a steady state congener when compared with other less chlorinated congeners (Brown and Lawton, 2001; Hansen, 2001).

To understand why PCBs have different residence times in the body the results from this study and two previous studies (Brown and Lawton, 2001; Hansen, 2001) were plotted on a chlorine bond matrix to see how the exact position of chlorine atoms affected the apparent rate of biotransformation. The results are presented as two chlorine bond matrices in Figure 6a and 6b. Figure 6a shows congeners that were identified as episodic from this study and by Hansen (2001). Figure 6b shows congeners that were identified as steady state by this study and by Hansen (2001), plus congeners identified as resistant to metabolism by Brown and Lawton (2001).

<Figure 6a,6b>

The results in Figure 6a and 6b show some clear trends in the apparent rate of biotransformation and elimination of PCBs. Congeners with chlorine bonding in the 2,5 and 2,3,6 positions (and 2- in di- and tri-chlorinated congeners) are often rapidly biotransformed and so are likely to be classed as episodic (Hansen, 2001). PCBs with chlorine bonding in the 2,3,4, 2,4,5, 3,4,5, and 2,3,4,5 positions are often more resistant to biotransformation and therefore are likely to be classed as steady state congeners.

The 3,5 and 2,4,6 chlorine bonds were identified by Frame (2001) as highly un-favoured positions during PCB production. They are present in Aroclors in relatively low concentrations (Frame et al., (1996) and thus are difficult to detect in environmental samples

and human serum by routine analysis. This may explain why no PCBs with either 3,5 or 2,4,6 chlorine bonds were identified as either episodic or steady state.

5 Conclusions

The results reported here provide supporting evidence that different PCBs have different residence times in the body. These differences have been previously explained by the degree of chlorination or the number of *ortho* chlorines on the biphenyl structure (Herrick et al., 2011; James, 2001; Seegal et al., 2011). Statistical analysis of the NHANES data was used in conjunction with previously published studies (Brown and Lawton, 2001; Hansen, 2001) to identify structural relationships that determine if a PCB is likely to be episodic or steady state. It is recommended that future investigations estimating PCB exposure focus on using relative proportions of key congeners identified in this study, rather than simply selecting congeners based on the degree of chlorination.

In any assessment pertaining to age dating PCB exposure, the source of PCBs and relevant pathway of exposure should be identified. For example, recent studies undertaken by Herrick et al. (2011) and DeCaprio et al. (2005) have used several of the less chlorinated congeners to identify PCB exposure via inhalation. These types of findings can be used in conjunction with this study to enable assessors to identify relevant PCBs from the degree of chlorination that will be most applicable to their investigation.

Conflicts of interest

The authors declare no conflict of interest.

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Table 1. Summary of regression data and PCA for the 37 NHANES PCBs

Congener	Structural formula	Number of chlorines	Mean concentration (ng/g lipid)	% of results below LOD	Increasing or decreasing concentration with age	P value	Eigenvector for PC1	Episodic (e), Steady State (s), unsure (-)
PCB 44	23-2'5'	4	2.7	0.2	Decreasing	0.000	0.220	e
PCB 49	24-2'5'	4	1.7	0.6	Decreasing	0.000	0.214	e
PCB 110	236-3'4'	5	1.8	2	Decreasing	0.001	0.218	e
PCB 101	245-2'5'	5	2.4	3	Decreasing	0.000	0.221	e
PCB 52	25-2'5'	4	3.6	0	Decreasing	0.000	0.221	e
PCB 149	236-2'4'5'	6	0.84	4	Increasing	0.428	0.201	e
PCB 87	234-2'5'	5	1.1	17	Increasing	0.383	0.199	e
PCB 28	24-4'	3	6	0	Increasing	0.000	0.205	e/s*
PCB 128	234-2'3'4'	6	0.019	75	Increasing	0.929	0.137	-
PCB 151	2356-2'5'	6	0.41	21	Increasing	0.000	0.171	-
PCB 66	24-3'4'	4	1.8	1	Increasing	0.000	0.215	-
PCB 189	2345-3'4'5'	7	0.34	76	Increasing	0.000	0.031	-
PCB 99	245-2'4'	5	6.2	0.1	Increasing	0.000	0.123	s
PCB 105	234-3'4'	5	2.1	3	Increasing	0.000	0.101	s
PCB 118	245-3'4'	5	10	0	Increasing	0.000	0.075	s
PCB 74	245-4'	4	7.4	0	Increasing	0.000	0.056	s
PCB 138 & 158	234-2'4'5' 2346-3'4'	6	24	0	Increasing	0.000	-0.117	s
PCB 195	23456-2'3'4'	8	1.3	46	Increasing	0.000	-0.082	s
PCB 183	2346-2'4'5'	7	2.5	11	Increasing	0.000	-0.137	s
PCB 153	245-2'4'5'	6	32	0	Increasing	0.000	-0.174	s
PCB 146	235-2'4'5'	6	3.9	2	Increasing	0.000	-0.16	s
PCB 177	2356-2'3'4'	7	2.3	20	Increasing	0.000	-0.152	s
PCB 187	2356-2'4'5'	7	7.7	2	Increasing	0.000	-0.183	s
PCB 170	2345-2'3'4'	7	9.1	3	Increasing	0.000	-0.204	s
PCB 180	2345-2'4'5'	7	26	8	Increasing	0.000	-0.215	s
PCB 172	2345-2'3'5'	7	1.3	35	Increasing	0.000	-0.185	s
PCB 196 & 203	2345-2'3'4'6' 23456-2'4'5'	8	4.8	13	Increasing	0.000	-0.181	s
PCB 157	234-3'4'5'	6	1.2	37	Increasing	0.000	-0.144	s
PCB 156	2345-3'4'	6	4.9	17	Increasing	0.000	-0.186	s
PCB 178	2356-2'3'5'	7	1.8	24	Increasing	0.000	-0.195	s
PCB 206	23456-2'3'4'5'	9	4.2	7	Increasing	0.000	-0.141	s
PCB 167	245-3'4'5'	6	1.2	42	Increasing	0.000	-0.117	s
PCB 194	2345-2'3'4'5'	8	5.8	22	Increasing	0.000	-0.181	s
PCB 209	23456-2'3'4'5'	10	3.3	7	Increasing	0.000	-0.084	s
PCB 199	2345-2'3'5'6'	8	6.2	14	Increasing	0.000	-0.188	s

* PCB 28 is grouped with episodic congeners when compared with the total PCB mixture as a whole. However, in comparison with other tri-chlorinated biphenyls this congener would be considered a steady state congener

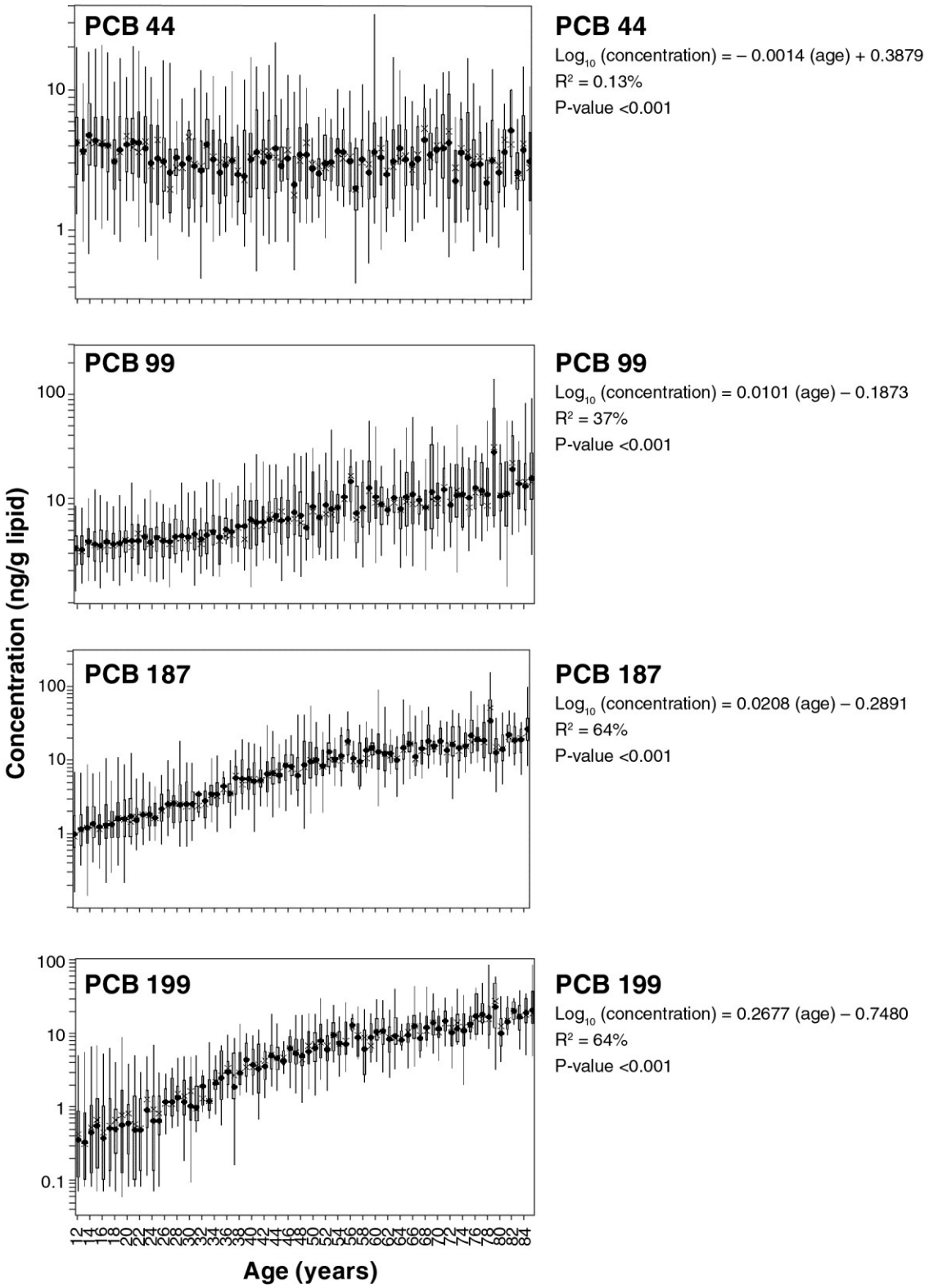

Figure 1) Plots of PCB44, PCB99, PCB187 and PCB199 lipid adjusted concentrations (ng/g lipid) against participant age. Results were obtained from the 2003-04 NHANEs study. Plots were produced using Minitab V16 and display the inter quartile range, the mean (x) and the median (•) along with the results of the regression analysis

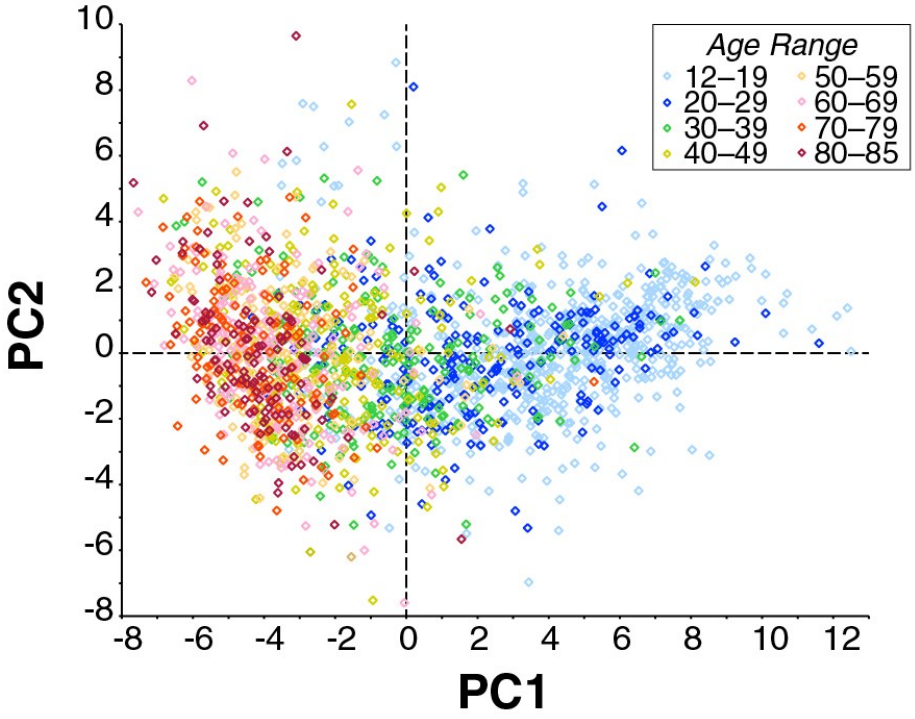

Figure 2) PCA scores plot of PC1(accounting for 50% of the variance) and PC2 (accounting for 11% of the variance) showing the strong relationship between age and PC1

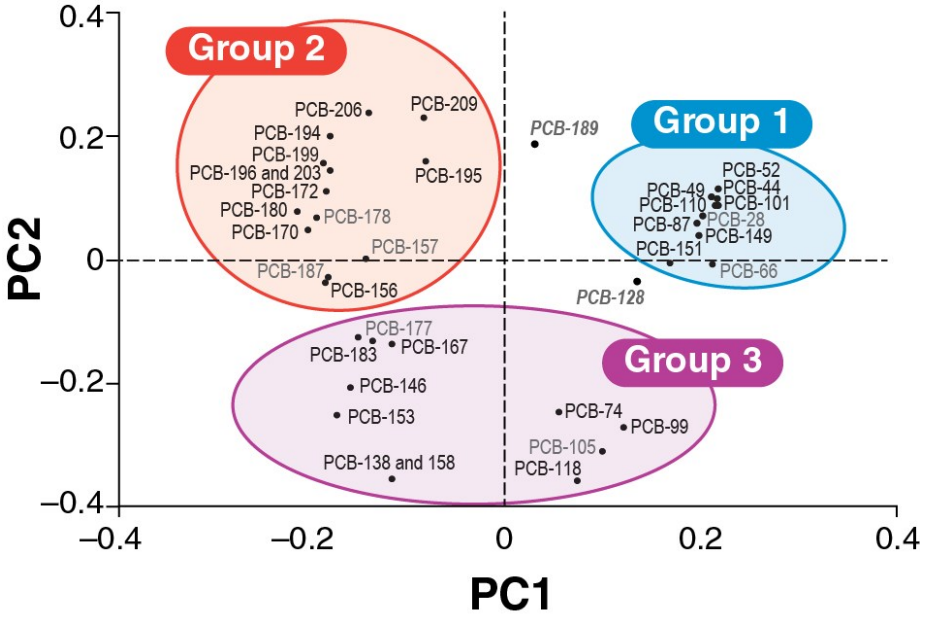

Figure 3) PCA loadings plot showing the grouping of congeners based on chlorine positions. Group 1 mainly contained PCBs with 25- and 236- chlorine substitution (listed in black), Group 2 mainly contained PCBs with 2345- and 23456- chlorine substitution (listed in black) and Group 3 mainly contained PCBs with 245- chlorine substitution (listed in black). PCBs in italics had over 75% of results below the analytical limits of detection

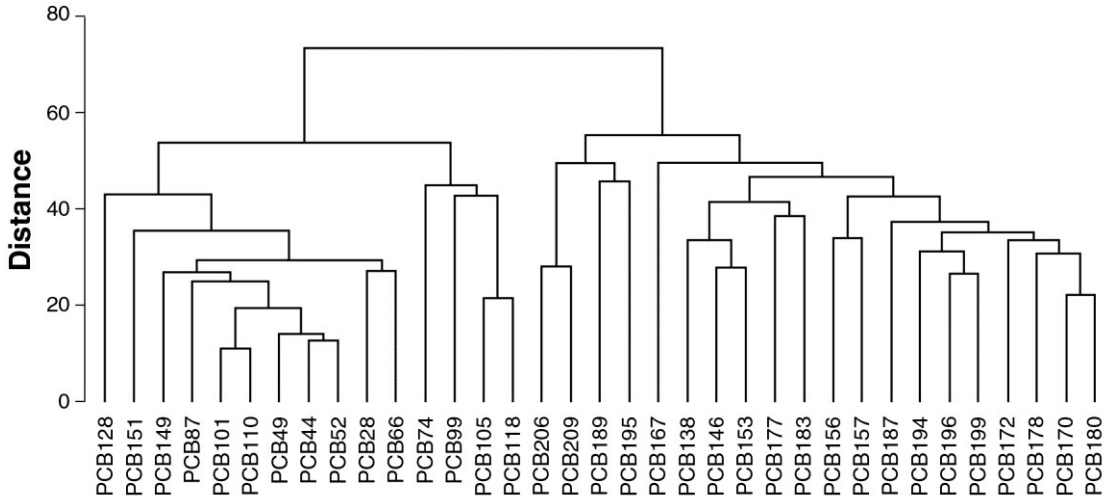

Figure 4) Dendrogram of Euclidean distance analysis

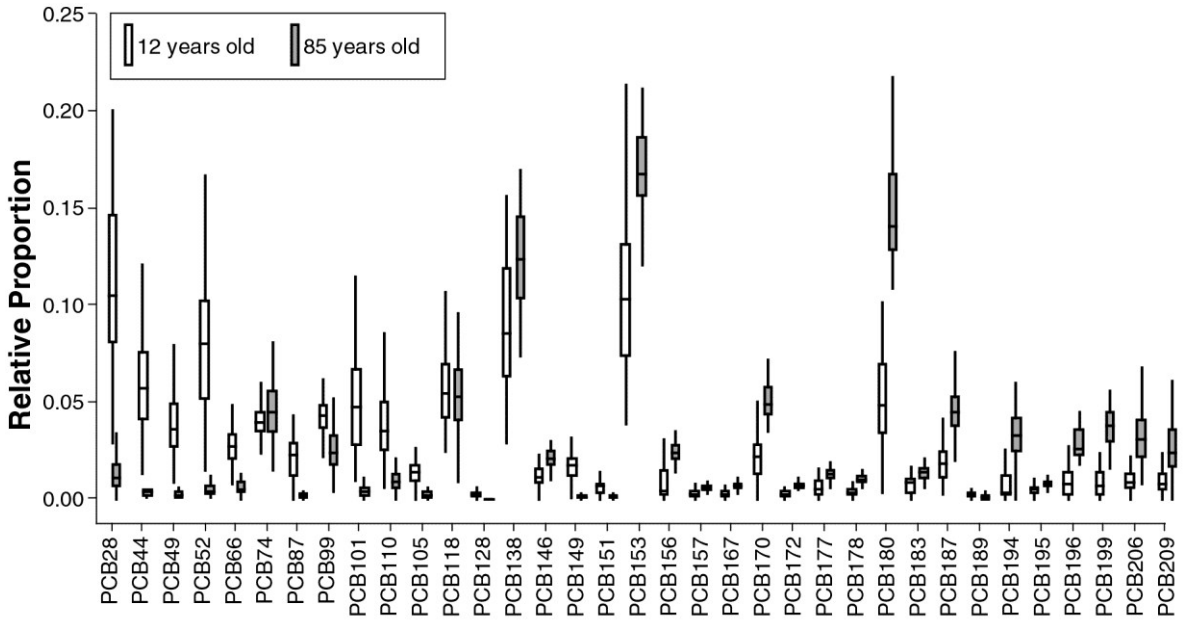

Figure 5) Signature plot of the average proportion of each congener analysed in the NHANEs survey for the 12 and 85 year old participants. Plots display the inter quartile range and the mean

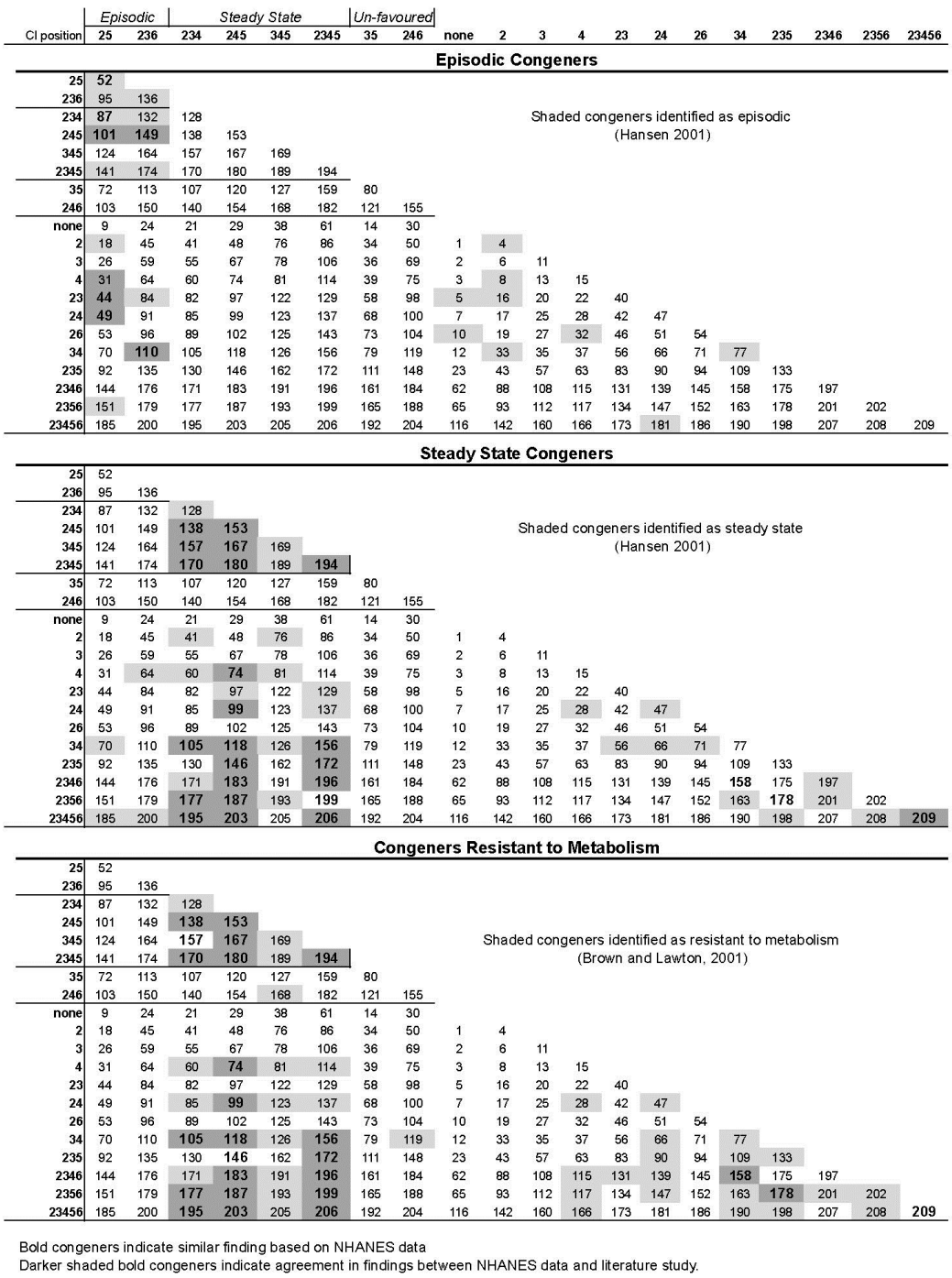

Figure 6) Chlorine bond matrix showing PCBs that were identified as episodic and steady state from a review of the NHANES data, Hansen (2001) and Brown and Lawton (2001). The top horizontal row shows chlorine position on the first phenyl ring, and the left vertical column shows the chlorine position on the second phenyl ring

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