

2015-04-24

Can polychlorinated biphenyl (PCB) signatures and enantiomer fractions be used for source identification and to age date occupational exposure?

Megson, D

<http://hdl.handle.net/10026.1/4347>

10.1016/j.envint.2015.04.006

Environ Int

Elsevier BV

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation. Published in *Environment International*, 81, 56-63 (2015), doi; 10.10156/j.envint.2015.04.006.

Can polychlorinated biphenyl (PCB) signatures and enantiomer fractions be used for source identification and to age date occupational exposure?

*David Megson ^{*1}, Jean-François Focant ², Donald G. Patterson ³, Matthew Robson ^{4,5},
Maeve C. Lohan ¹, Paul J. Worsfold ¹, Sean Comber ¹, Robert Kalin ⁶, Eric Reiner ⁵, Gwen
O'Sullivan ⁷*

1 Biogeochemistry Research Centre, SoGEES, Plymouth University, Plymouth, Devon, PL4 8AA, UK

2 Department of Chemistry, University of Liège, Allée de la Chimie, Liège, Belgium

3 Exponent Inc, One Capital City Plaza, Suite 1620, 3350 Peachtree Road, Atlanta, GA 30326, USA

4 Department of Chemistry, Brock University, 500 Glenridge Ave, St. Catharines, ON. L2S 3A1, Canada,

5 Ontario Ministry of the Environment and Climate Change, 125 Resources Road, Toronto, Ontario, M9P 3V6, Canada

6 Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow G1 1XQ, UK

7 Department of Environmental Science, Mount Royal University, 4825 Mount Royal Gate SW, Calgary, Alberta, T3E 6K6, Canada

***Corresponding Author**
David Megson

Abstract

Detailed polychlorinated biphenyl (PCB) signatures and chiral Enantiomer Fractions (EFs) of CB-95, CB-136 and CB-149 were measured for 30 workers at a transformer dismantling plant. This was undertaken to identify sources of exposure and investigate changes to the PCB signature and EFs over different exposure periods. Approximately 1.5 g of serum was extracted and PCB signatures were created through analysis by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS) and EFs calculated following analysis by gas chromatography with high resolution mass spectrometry (GC-HRMS). A total of 84 PCBs were identified in the serum samples with concentrations of the 7 indicator PCBs ranging from 11 - 350 ng g⁻¹ of serum (1.2 - 39 µg g⁻¹ lipid). The PCB signatures were interpreted using principal component analysis (PCA) which was able to distinguish workers with background or recent minimal exposure from those with prolonged occupational exposure. Occupationally exposed individuals had a similar PCB profile to Aroclor A1260. However, individuals with prolonged exposure had depleted proportions of several PCB congeners that are susceptible to metabolism (CB-95, CB-101 and CB-151) and elevated proportions of PCBs that are resistant to metabolism (CB-74, CB-153, CB-138 and CB-180). The results also identified a third group of workers with elevated proportions of CB-28, CB-60, CB-66, CB-74, CB-105 and CB-118 who appeared to have been exposed to an additional source of PCBs. The results show near complete removal of the CB-95 E2 enantiomer in some participants, indicating that bioselective metabolism or preferential excretion of one enantiomer occurs in humans. By considering PCB concentrations along with detailed congener specific signatures it was possible to identify different sources of contamination and gain an insight into both the magnitude and duration of exposure.

Keywords

Polychlorinated biphenyls (PCBs); Human exposure, GCxGC-TOFMS, Chemical fingerprinting, PCB atropisomers, Chiral, Enantiomer Fractions

Highlights

- Eighty four different PCBs detected in human serum samples
- PCB signatures used to distinguish recent and prolonged exposure
- PCB signatures used to identify different sources of exposure
- Near complete removal of the CB-95 E2 enantiomer recorded in some humans

1 Introduction

Polychlorinated biphenyls (PCBs) are a group of 209 chlorinated organic compounds that were widely used throughout the 20th century. While PCBs have been largely phased out of commercial/industrial use, they remain an important legacy contaminant (O'Sullivan and Sandau, 2013). They are highly persistent and can still be found in closed systems in some countries, e.g. as dielectric fluids in electrical equipment and transformers. Many of these transformers containing PCBs are in the process of being replaced, and this process therefore presents the potential of PCB exposure for humans working in dismantling plants. In these instances it is important not only to determine the extent of the exposure, along with the potential risks to human health, but also to establish the source of the contamination and age date the exposure.

As PCBs were produced as commercial mixtures, such as Aroclors, each blend has a specific congener profile (signature) based on the relative proportions of each PCB in the total mixture. This signature can be used to easily distinguish commercial mixtures, however environmental investigations involving humans are more complex as there are often multiple potential sources of PCB exposure. The signature can be altered by changes such as volatilization, dissolution and biodegradation (Jaspers et al., 2013; Johnson et al., 2006). The signature in humans can also vary depending on different exposure pathways e.g. oral, inhalation or dermal, and can be altered through post uptake processes such as biotransformation and elimination (Jaspers et al., 2013; Megson et al., 2013a). If a dominant source of exposure can be identified then alterations to this signature from post uptake processes such as biotransformation and elimination may provide useful information to distinguish between recent and prolonged exposure. The sera of an individual who has been historically exposed may contain higher proportions of the PCBs that are more resistant to

biotransformation and elimination. Due to all of the subtle changes that can occur to the PCB profile, it is imperative that signatures are created using detailed congener specific datasets when attempting to identify the source of exposure. Analysis using comprehensive two dimensional chromatography has proven to be an excellent technique for this purpose as it is able to separate over 190 individual PCB congeners (Focant et al., 2004; Korytar et al., 2006; Harju et al., 2003; Zapadlo et al., 2011; Megson et al., 2013b).

Of the 209 PCBs there are 19 which are predicted to exist as stable atropisomers (Oki, 1983). They have a high degree of *ortho* chlorine substitution which inhibits rotation, and asymmetrical *meta* and *para* substitution on each biphenyl, resulting in two optical isomers. In commercial mixtures both enantiomers are produced in equal proportions; however in animals, metabolic processes such as enzyme mediated oxidation have been proven to preferentially target one stereoisomer, resulting in atropisomeric enrichment (Harrad et al. 2006; Wong et al., 2002; Wu et al., 2014). Therefore, the sera of an individual who has recently been exposed to a commercial PCB mixture may be expected to contain near equal proportions of each stereoisomer whereas the sera of a historically exposed individual may show a greater degree of fractionation. However, this signal is likely to be complicated by interferences such as other background sources of PCBs.

The goal of this study was to determine if PCB signatures and enantiomer fractions could be used to identify the source of contamination and distinguish between recent and prolonged exposure periods for 30 workers at a transformer dismantling plant.

2 Experimental

2.1 Sample collection

Samples of whole blood were collected from 30 people working at a transformer dismantling plant in Europe. Samples were obtained from workers performing a range of different roles at the plant, including workers on the dismantling floor who were likely to have had direct contact with PCBs and those who were not expected to have had any direct contact with PCBs such as administrative staff and a security guard. Samples were obtained from employees who had been working at the plant from 3 - 21 years. However, information on occupation and length of time at the plant was not available for three participants (id no. S028, S029 and S030). A 10 mL sample of whole blood was obtained from each worker, the blood was collected in vacutainers, then centrifuged and the serum collected and stored at -20 °C.

Total lipid concentrations were determined by enzymatic analysis which was performed by a sub-contractor clinical laboratory on a dedicated 2 mL serum sub-sample. Four types of lipids were targeted and measured; triglycerides, total cholesterol, non-esterified (free) cholesterol, and phospholipids. Sample sizes were as follows: triglycerides (2 µL), total cholesterol (2 µL), non-esterified (free) cholesterol (50 µL), and phospholipids (20 µL). Total lipid concentrations were estimated using the summation method of Akins et al. (1989). The total lipid content was expressed in g L⁻¹. For the inter-conversion of volumetric and gravimetric data, a value of 1.026 g mL⁻¹ for serum specific gravity was used.

2.2 Sample preparation

All reagents required for extraction and clean-up were sourced specifically for dioxin, furan and PCB analysis or of the closest grade available of similar quality. Approximately 1.5 g of serum was accurately weighed (to 4 decimal places) and transferred to a vial and 5 µL of ¹³C₁₂ labelled CB-60, CB-127 and CB-159 at a concentration of 100 pg µL⁻¹ (CIL-EC-5370

EN-1948-4 PCB sampling standard) was added to determine recovery. A volume of formic acid equal to the mass of the sample was added to the serum followed by the same volume of high purity water. During each addition the solution was vortexed and allowed to degas for several minutes. The whole extract was then applied to a C₁₈ SPE cartridge (1 g / 6 mL) and the PCBs were eluted with hexane. The eluent was treated using EPA method 3665A sulphuric acid / permanganate clean-up followed by EPA method 3620 Florisil clean-up. Extracts were reduced to approximately 50 µL by nitrogen evaporation and 100 µL of ¹³C₁₂ labelled PCBs (CIL-EC-5367 CDC PCB Spiking Standard), each at a concentration of 7.5 pg µL⁻¹, were added. Extracts were left overnight to evaporate to incipient dryness (the spiking standard contained a dodecane keeper). Samples were reconstituted with 10 µL of hexane prior to analysis.

2.3 Congener specific analysis by GCxGC-TOFMS

Analysis was conducted based on the method described by Megson et al. (2013b) which is summarised below. Samples were 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 fitted with a Rtx-PCB (60 m x 0.18 mm x 0.18 µm) ¹D column and a Rxi-17 Sil MS (1.5 m x 0.18 mm x 0.18 µm) ²D column. One µL of sample was injected in splitless mode.

Procedural blanks were prepared for each batch of 8 samples. Contamination with CB-11 was identified in the blanks and so this congener was excluded from the results. All samples were spiked with two sets of ¹³C₁₂ labelled internal standards which were used to quantify PCB concentrations and calculate recovery. Quantification was undertaken through isotope dilution; calibration data was produced for 41 of the most commonly encountered congeners

(CIL-EC-4133 DSJ PCB Mixture). Quantification of other congeners present in the samples was undertaken using the calibration data from the closest eluting calibrated congener with the same level of chlorination. Recovery for all samples was within the accepted range specified by EPA method 1668C (10 % to 145 %), the mean recovery was 55 % (± 16 %; 1σ). Concentrations were recovery corrected and lipid normalised and reported as ng g^{-1} lipid weight. The instrument limit of detection (LOD) was estimated empirically using the calibration standard mixtures, LODs for CB-18 and CB-206 were calculated at a concentration of at 1 and $50 \text{ pg } \mu\text{L}^{-1}$ respectively. Accuracy and precision were measured for the sum of the European Union 7 indicator congeners (EC7; CB-28, CB-52, CB-101, CB-118, CB-138, CB-153, CB-180) by analysing a $10 \text{ ng } \mu\text{L}^{-1}$ Aroclor 1248 standard three times. The accuracy of the sum of the EC7 congeners for the three samples was 105% ($\pm 0.9\%$; 1σ).

2.4 Chiral analysis by GC-HRMS

The Enantiomeric Fractions (EFs) of CBs 95, 136 and 149 were analysed according to the method of Robson and Harrad (2004), using an Agilent 7890 Gas Chromatograph coupled to a Micromass Auto Spec Premier High Resolution MS tuned to greater than 10000 mass resolution. The two most abundant isotopes of each enantiomer were recorded in Single Ion Recording Mode (SIR). This was 325.88040 and 327.87750 for CB 95 and 359.84150 and 361.83850 for CBs 136 and 149. These PCBs were chosen because they are; (a) able to be baseline separated on the Chirasil Dex column, (b) free from any co-eluting congeners that may bias the results and (c) normally present in the environment in high enough concentrations to be accurately measured.

The chromatographic performance of the method was assessed prior to each run of 8 samples by analysing a 1:1:1 mixture of Aroclors 1248, 1252 and 1260.

Enatiomeric Fractions were calculated as per Harner et al. (2000). Whereby

EF=E1/(E1+E2)

where E1 equals the first eluting or the (+) enantiomer and E2 the second eluting enantiomer.

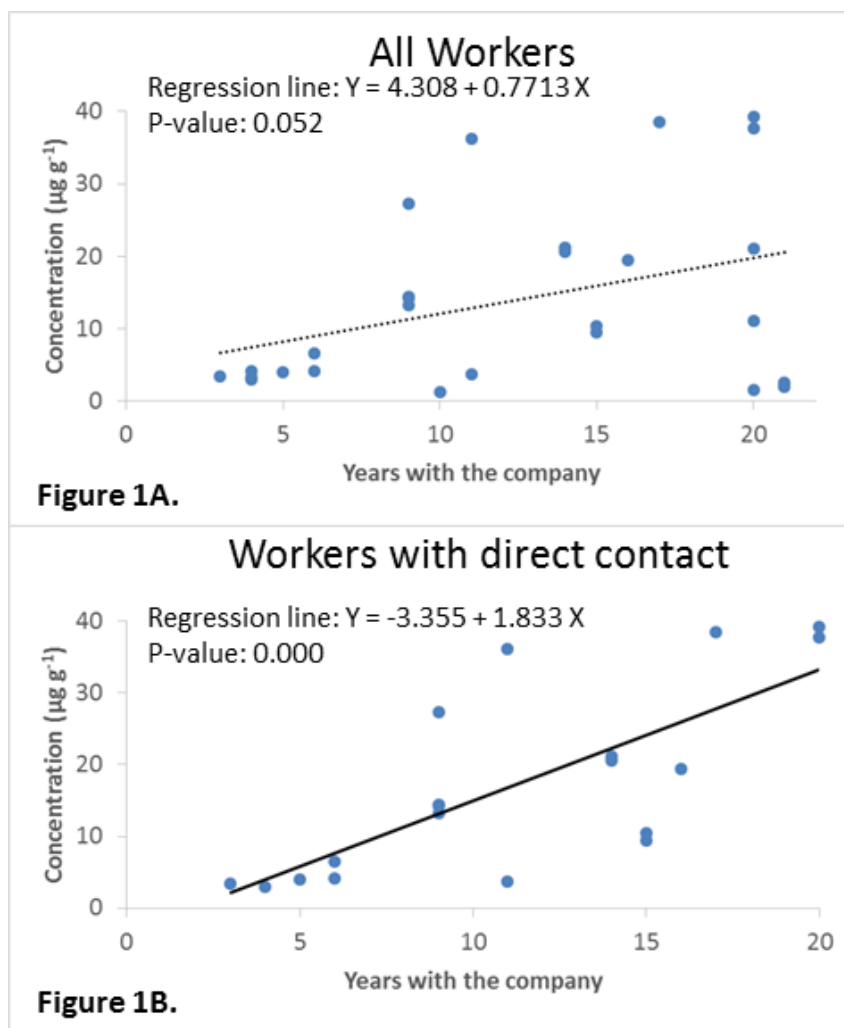
Samples were only accepted for quantitation if the enantiomeric fractions of the three atropisomers studied were 0.50 (± 0.01) in the Aroclor mixture, the least abundant enantiomer of the pair had a signal to noise (S:N) ratio greater than 10:1, the isotope ratios were within 20% of their theoretical values and the analytical recovery of the sample was greater than 30%. The instrument LOD was estimated by analysing a standard mixture of CB-95 and CB-149, LODs were calculated at a concentration of 2.5 pg μL^{-1} per enantiomer. Procedural blanks were prepared for each batch of 8 samples; no chiral PCBs were detected in the blanks above the limits of detection.

3 Results and discussion

3.1 Concentrations of PCBs in workers at a transformer dismantling plant

A total of 84 different PCB congeners were identified in the serum of the 30 workers at the transformer dismantling plant. Concentrations of the 7 indicator PCBs ranged from 11 - 350 ng g^{-1} of serum (1.2 - 39 $\mu\text{g g}^{-1}$ lipid). Background concentrations of these congeners in humans are in the range of 0.1 - 10 ng g^{-1} serum (0.01 - 1 $\mu\text{g g}^{-1}$ lipid) (Longnecker, 2001). This shows that some workers had close to background exposure whereas others had elevated PCB concentrations indicating that they may have been exposed to PCBs through their occupation. The PCB concentrations in the different workers are summarised in Table 1. The results show that the workers with job roles involving direct contact with PCBs generally had higher PCB concentrations than those with no direct contact. The mean concentration of the EC7 PCBs in workers with direct contact was 17.2 $\mu\text{g g}^{-1}$ lipid (± 13.0 ; 1σ) which was significantly greater (P-value 0.004) than workers with no direct contact (5.83 $\mu\text{g g}^{-1}$ lipid

140 (± 6.90 ; 1σ). However, this was not true for all workers, as a concentration of $21.0 \mu\text{g g}^{-1}$
141 lipid was recorded in a chief of operations (chief) who was believed to have had no direct
142 contact with PCBs. PCB concentrations generally increased with the number of years the
143 employee had worked with the company (Figure 1A), although this increase was not
144 statistically significant (P-value of 0.052). However, when PCB concentrations of the 18
145 workers who were working at the dismantling plant with direct contact dismantling
146 transformers were considered, this increase was statistically significant (P-value of 0.000)
147 (Figure 1B). Although the PCB concentration is well correlated with the number of years an
148 individual has worked at the company it should not be used in isolation as proof that
149 occupational exposure has occurred. Higher PCB concentrations have been reported in older
150 individuals as a result of accumulation of the more persistent congeners and exposure to
151 higher historical background concentrations (Megson et al., 2013a; Quinn and Wania 2012).
152 There are also several physiological characteristics, such as body fat, serum albumin and age,
153 that can influence the uptake and retention of PCBs along with social preferences such as diet
154 and smoking (Axelrad et al., 2009; Brown and Lawton, 2001; Jain and Wang, 2011;
155 Weintraub and Birnbaum, 2008). This highlights the importance of looking at the specific
156 PCB signature of each individual to determine their exposure source rather than relying
157 solely on a total PCB concentration.



158

159 **Figure 1. Relationship between PCB concentration and number of years a worker had**
 160 **been with the company. Results are presented for all workers (Figure 1A) and only**
 161 **those with direct contact dismantling transformers (Figure 1B).**

162 **Table 1. PCBs concentrations and enantiomer fractions recorded in workers. DPW =**
163 **dismantling plant worker and DPC = dismantling plant chief, Concentrations are**
164 **presented to 3 significant figures and EFs to 2 decimal places. <LOD = below the limit**
165 **of detection (i.e. S:N ratio <10)**

	Sample ID	Job role	Years with the company	Concentration in serum (ng g ⁻¹)	Lipid corrected concentration in serum (µg g ⁻¹ lipid)		Enantiomer fractions					
					Per sample	Mean [±1 σ]	CB-95	Mean [±1 σ]	CB-136	Mean [±1 σ]	CB-149	Mean [±1 σ]
No direct contact	S001	DPC	21	18.4	2.49	5.83 [± 6.90]	0.51	0.63 [± 0.14]	<LOD	0.44 [± 0.00]	0.47	0.37 [± 0.08]
	S002	Administrative	20	13.7	1.49		0.74		<LOD		<LOD	
	S003	Workshop DPC	10	11.2	1.23		0.41		<LOD		0.41	
	S004	Cleaner	4	29.4	4.08		0.55		<LOD		0.26	
	S005	DPC	20	166	21		0.59		<LOD		0.31	
	S006	DPC	20	86.3	11.1		0.66		0.45		0.39	
	S007	Guard	21	26.6	1.93		0.78		0.44		0.41	
	S008	Maintenance	4	21	3.32		0.82		<LOD		<LOD	
Direct contact	S009	Pumping oil	11	28.4	3.76	17.2 [± 13.0]	0.59	0.74 [± 0.11]	0.45	0.47 [± 0.05]	0.47	0.36 [± 0.07]
	S010	DPW	15	62.8	9.41		0.51		<LOD		0.48	
	S011	DPW	6	73.1	6.53		0.88		0.51		0.37	
	S012	DPW	9	70.1	13.2		0.88		<LOD		0.29	
	S013	DPW	3	27.7	3.37		0.87		0.57		0.44	
	S014	DPW	11	246	36.2		0.87		0.43		0.21	
	S015	DPW	14	163	20.6		0.82		<LOD		<LOD	
	S016	DPW	9	138	14.4		0.79		0.47		0.32	
	S017	DPW	16	121	19.4		0.72		0.43		0.30	
	S018	DPW	17	353	38.5		0.66		0.46		0.35	
	S019	DPW	4	16.8	3		0.60		<LOD		0.33	
	S020	DPW	20	300	37.7		0.87		<LOD		<LOD	
	S021	DPW	9	95.2	14.2		0.74		<LOD		0.37	
	S022	DPW	20	231	39.3		0.67		0.47		0.35	
	S023	DPW	15	62.5	10.4		0.67		0.43		0.35	
	S024	DPW	6	37.5	4.16		0.82		0.51		0.32	
	S025	DPW	5	23.4	4.05		0.66		<LOD		0.44	
	S026	DPW	9	134	27.3		0.66		<LOD		0.44	
	S027	DPW	14	149	21.2		0.77		<LOD		<LOD	
Unknown	S028	Unknown	unknown	26.1	3.55	13.7 [± 14.6]	0.82	0.78 [± 0.15]	0.52	0.55 [± 0.048]	0.36	0.35 [± 0.08]
	S029	Unknown	unknown	187	30.4		0.91		0.59		0.27	
	S030	Unknown	unknown	50.7	7.14		0.61		<LOD		0.43	

3.2 Statistical evaluation of PCB signatures

Fifty four PCBs were consistently detected in > 60% of the samples. These were quantified, percent normalised and presented as bar charts to show the PCB signature in each participant (Supplementary Information 1). Only three different Aroclor blends were understood to have been used in transformers; these were A1242, A1254 and A1260 (Johnson et al., 2006). As the dismantling plant was in Europe, transformers were likely to have contained a variety manufacturers products from the European market, including Aroclors, Phenoclor, Pyralenes and Clophens, however there is a very high degree of similarity in the signature from blends with an equivalent chlorine content (Johnson et al., 2006). The signature for the majority of workers was visually similar to the signature of A1260 (Supplementary Information 1) which provides further evidence to suggest that occupational exposure had occurred. However, further assessment was undertaken to confirm if this was the source of contamination or if other potential sources were important.

The PCB signatures of the workers were assessed using principal component analysis (PCA). Where a PCB was not detected it was included in the dataset as a '0'. As part of the data quality check, other values such as LOD/2 were substituted for '0', but these had no observable effect on the data output and so the '0's were retained. To reduce any bias from a high proportion of non-detects for a specific congener, PCBs that were not detected in over 60% of samples (i.e. PCBs present in less than 18 out of the 30 samples) were removed from the analysis following the guidance of Helsel (2006). This resulted in a dataset of 54 PCBs in 30 participants. Before performing PCA the data were normalised by transformation to a percent metric to remove concentration/dilution effects. The data were then mean centred and scaled using a Z-transform (autoscale transform) to prevent high concentration variables from dominating the analysis (Johnson et al., 2007). Principal component 1 accounted for 33.1% of

the variation and principal component 2 accounted for a further 18.2%. The scores plot is presented as Figure 2 and the loadings plot as Figure 3.

The scores plot (Figure 2) displayed a three end member system, showing that there were three groups of workers, each with a different PCB signature. Two of the three groups were linked to the duration that the participant had been working at the plant and their job function, i.e. involving either direct contact with PCBs or only indirect contact. One group may therefore represent participants with prolonged occupational exposure to A1260 and the other group exposure to background levels or a recent minimal exposure to A1260. However, the third group was comprised of participants who all had different ages, jobs and years at the company and may therefore be linked to an additional source(s) of contamination. Bar charts were produced to identify differences in the PCB signature between the three groups and aid the source identification process. Figure 4 displays the signature of A1260 along with the signatures of three participants (selected using the PCA scores plot) to represent the three groups. These were; participant number S020 who had worked at the plant for 21 years (representing a prolonged exposure to A1260), participant number S013 who had been at the company for 3 years (representing a recent minimal exposure to A1260), and participant number S021 (representing a suspected additional exposure source).

3.3 Source identification and age dating exposure

3.3.1 Occupational exposure to A1260

Participants with a negative score on PC1 and PC2 were comprised of workers who had been working at the dismantling plant for more than 10 years, with direct contact through dismantling transformers. The signatures from these samples is similar to the profile of A1260 but with depleted proportions of several of the less chlorinated biphenyls, CB-88 & 95, CB-90 & 101 and CB-151 (Figure 4). These are congeners predominantly containing a

phenyl group with un-chlorinated *meta* and *para* positions (i.e. 2,5- chlorine substitution), which are particularly susceptible to metabolic attack by P450 cytochromes (Letcher et al., 1999). The signature also contained elevated proportions of CB-74, CB-153, CB-138 and CB-180 (Figure 4). These are congeners containing a phenyl group with 2,4,5- substitution which are particularly resistant to biotransformation and elimination (Megson et al., 2013a). Interestingly this group also included a chief of operations who had low PCB concentrations (2.49 $\mu\text{g g}^{-1}$ lipid) but a signature similar to A1260. The signature shows that this individual appears to have been exposed to PCBs through occupational exposure, although the total PCB concentrations indicate exposure was only minimal.

3.3.2 Background or recent minimal occupational exposure

Participants with a positive score on PC2 comprised of workers who had been working at the plant for a relatively short period of time (< 6 years), along with those with jobs that did not involve direct exposure, such as a maintenance worker, cleaner and guard. It also included a chief of operations who had been working at the plant for 10 years but had the lowest PCB concentration of all participants (1.26 $\mu\text{g g}^{-1}$ lipid). All of these individuals contained significantly lower (P-value 0.000) EC7 PCB concentrations (mean value of 3.2 $\mu\text{g g}^{-1}$ lipid (± 1.0 ; 1σ)) than the rest of the samples (mean value of 19.1 $\mu\text{g g}^{-1}$ lipid (± 12.2 ; 1σ)). The signature from this group contained higher proportions of several episodic congeners such as CB-8, CB-18, CB-31, CB-52 and CB-151 (Figure 3), indicating a recent exposure. The signature also displayed slightly elevated proportions of many of the lower chlorinated PCBs that were not present in high concentrations in A1260 such as CB-28, CB-74, CB-99 and CB-118 (Figure 4), indicating a background exposure (Figure 2).

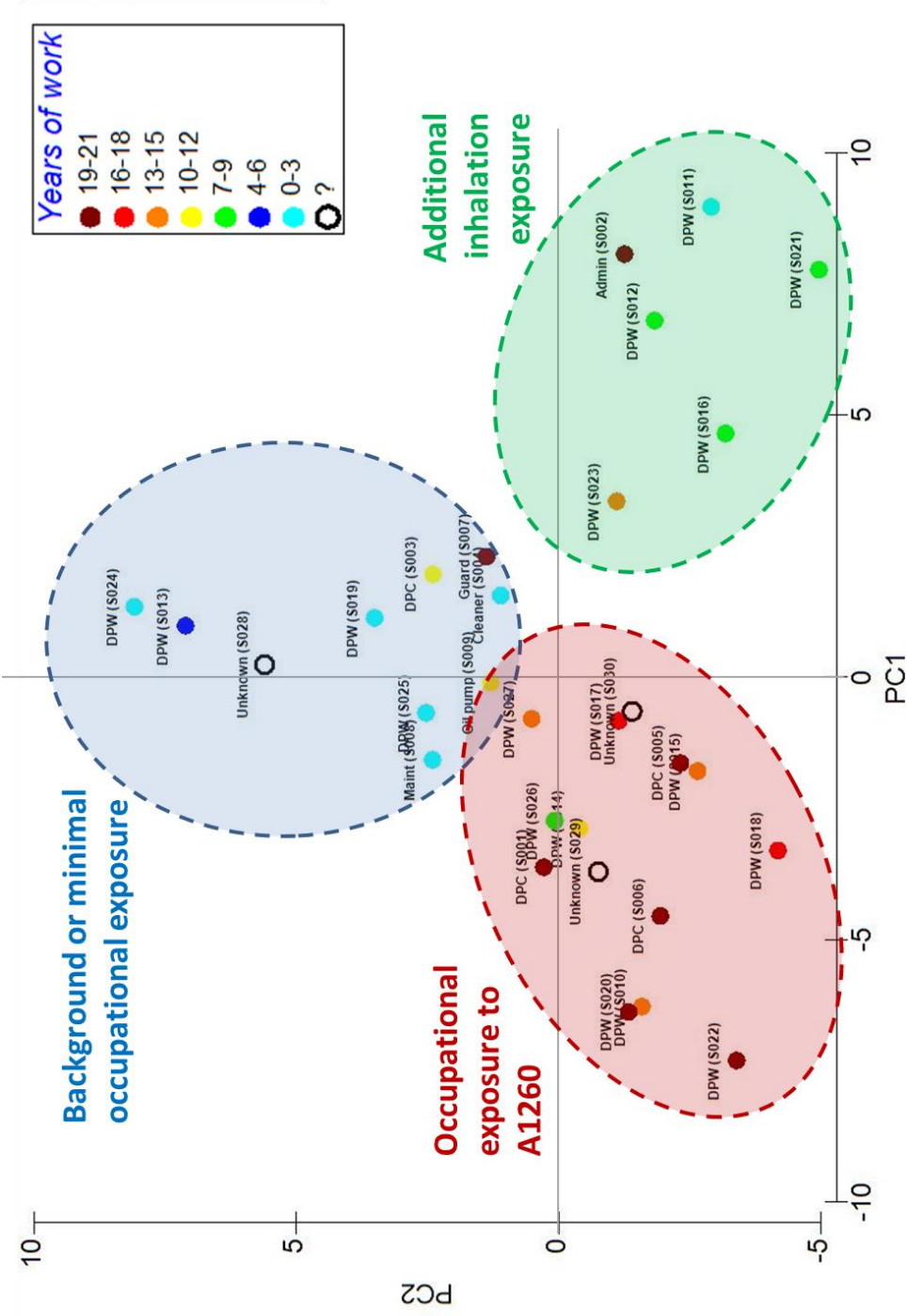


Figure 2. PCA scores plot produced from the PCB signature of workers at the dismantling plant. Data are displayed to show the number of years a worker had been at the plant. Sample identification numbers and job roles are also displayed in short hand; DPW = dismantling plant worker, DPC = dismantling plant chief. For a full list of job roles refer to Table 1

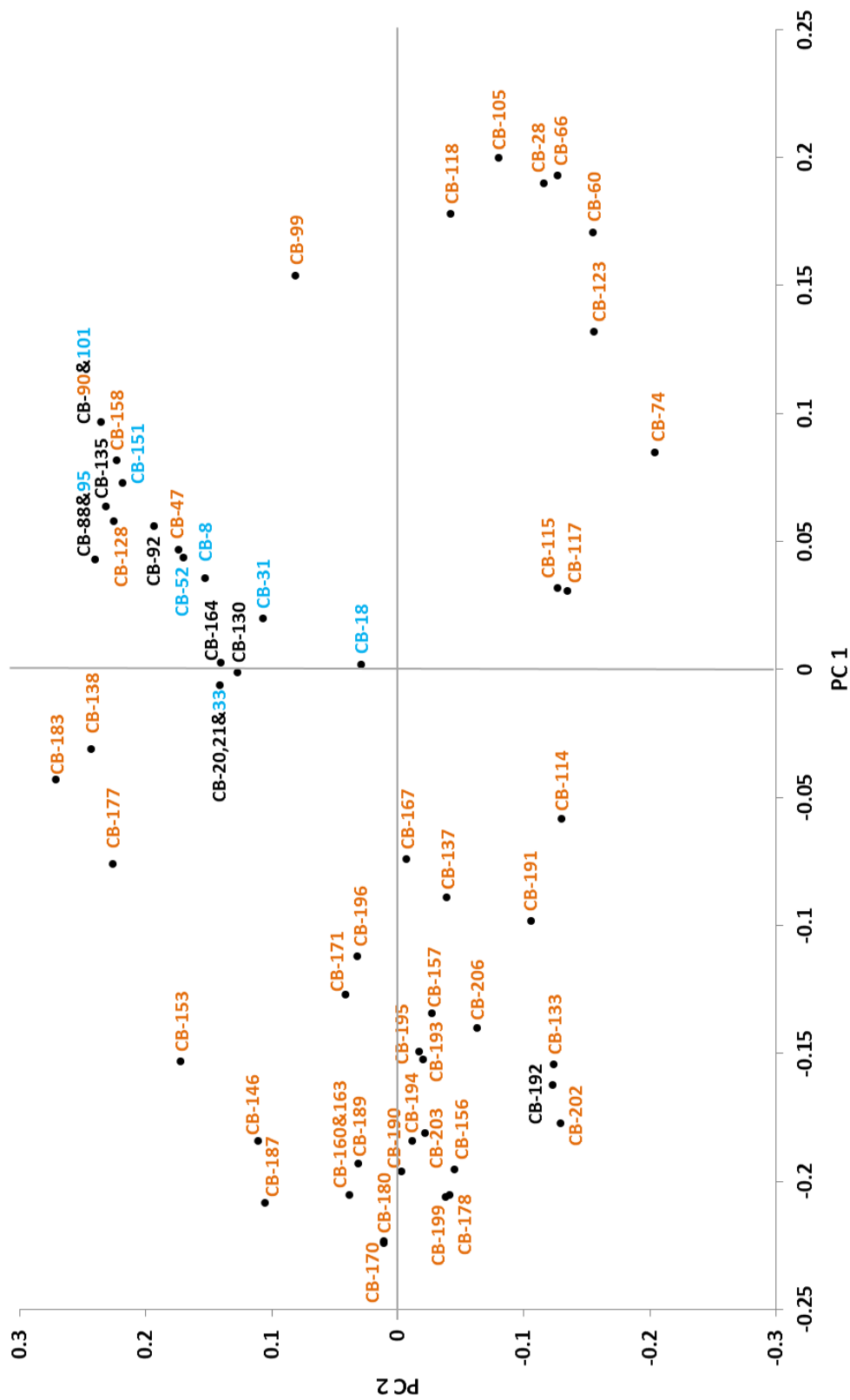
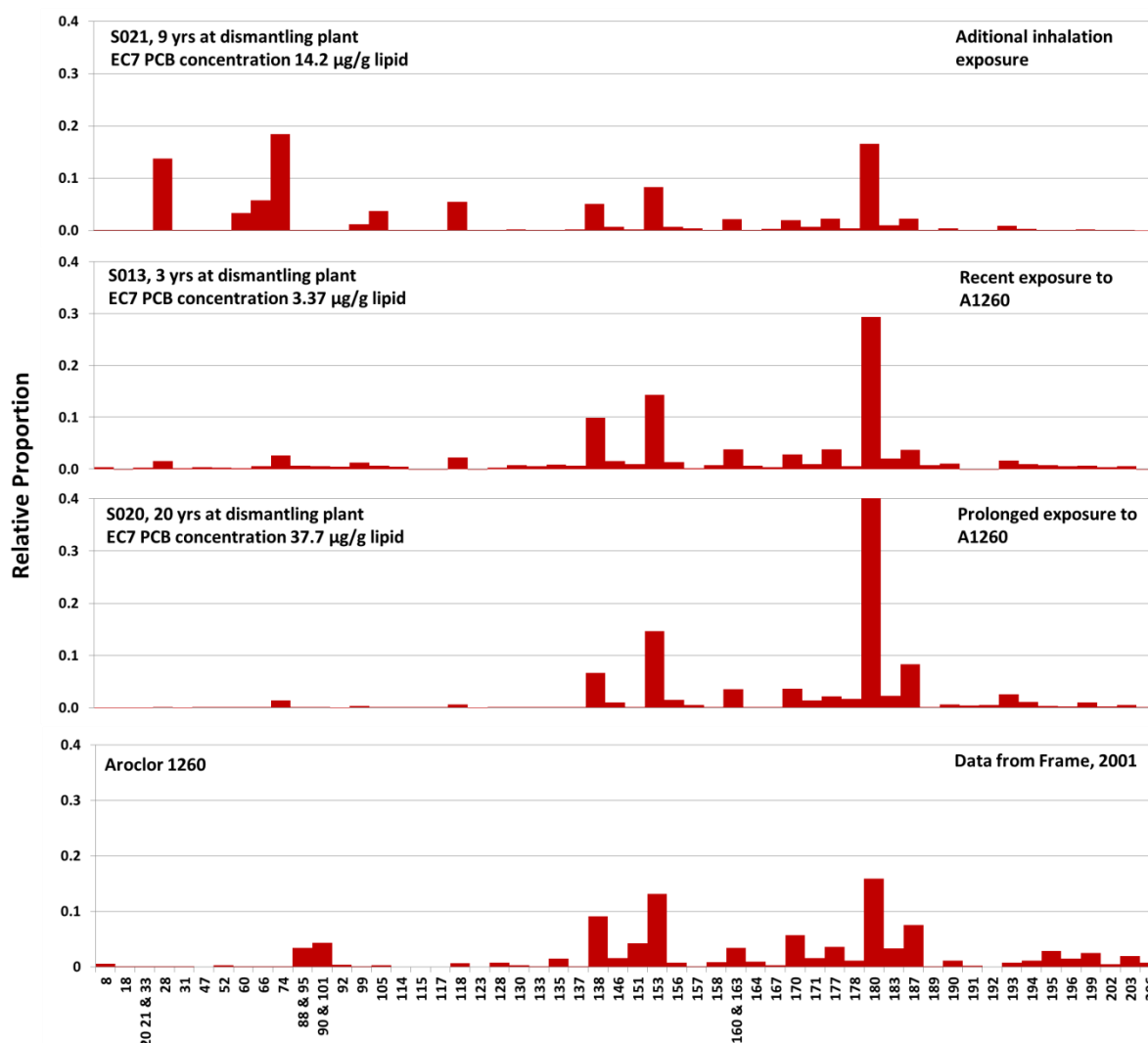


Figure 3. PCA loadings plot, PCBs identified as episodic (Megson et al 2013a) are presented in blue and PCBs identified as steady state (Megson et al 2013a) are presented in orange.



241

242 **Figure 4. PCB signature for the serum samples obtained from participants S020, S013**
 243 **and S021 compared with the signature from A1260.**

244 3.3.3 Additional inhalation exposure

245 In some instances inhalation has proven to be an important PCB exposure pathway
 246 (DeCaprio et al., 2005; Herrick et al., 2011). In a school in Boston (U.S.) inhalation of PCBs
 247 leaching from caulking materials and sealants was determined as the main route of exposure
 248 for teachers. This exposure resulted in the teachers having a distinctive PCB signature, with
 249 proportions of the less chlorinated PCBs such as CB-8, CB-33, CB-37, CB-41, CB-47 and
 250 CB-136 up to five times higher than the control group (Herrick et al., 2011). In this current

study, participants with a positive score on PC1 and negative score on PC2 had a signature similar to A1260, but with the addition of high proportions of several less chlorinated PCBs such as CB-28, CB-60, CB-66 and CB-74, along with CB-105 and CB-118. This group was comprised of participants who all had different ages, jobs and years at the company. The signatures all contained higher proportions of more volatile PCBs which have been previously linked to indoor air sources such as leaching from sealants and caulking materials (Harrad et al., 2005; Herrick et al., 2011; Kohler et al., 2005). Several of the congeners present in higher proportions (CB-28, CB-60 and CB-66 and CB-74) have also been linked to exposure from capacitors in electrical equipment (Luotamo et al., 1993). Concentrations of PCBs in individuals from this group were similar to those from the group with prolonged exposure to A1260. Therefore, the results indicate that this group was exposed to an additional source of PCBs through inhalation, possibly originating from leaching of materials at the home rather than from their workplace (Figure 2).

3.3.4 Enantiomeric fractions

To further elucidate the potential sources of exposure to these individuals we also examined the enantiomer signatures of three chiral PCBs (CB-95, CB-136 and CB-149) in the samples to ascertain if there were any trends in enantio-specific processing of these congeners that could be related to variations in the exposure of the workers, as well as potentially providing further insight into the pharmacokinetics of these pollutants in humans. To our knowledge this is the first time that analysis of this type has been done for human serum samples despite the widespread use of this matrix in human bio-monitoring programs (e.g. Canadian Health Measures Survey, United States Human Bio-monitoring Program).

The enantiomeric fractions of CB-95 and CB-149 recorded in the workers varied considerably (0.41 to 0.91 and 0.21 to 0.48, respectively), whereas fractions of CB-136 remained close to racemic (0.43 to 0.59) (Table 1). For CB-95 sera from most participants contained higher

proportions of the *E1* or (+) enantiomer than the *E2* or (-) enantiomer, indicating that significant enantioselective metabolism or excretion of this enantiomer has occurred. In one worker (S029) the proportion of *E1* enantiomer was over 10 times greater than the *E2* enantiomer. This mirrors the work of Chu et al. (2003) who found similar results for CB-95 in human liver samples. However in this study the extent of change was much greater than was reported in Chu et al. (2003), with the results showing evidence of near complete removal of the *E2* enantiomer in some participants. For CB-149 sera from all participants contained higher proportions of *E2*. In one worker the proportion of *E2* was over 3.5 times greater than *E1* (S014).

No clear trends in enantiomeric signatures with the exposure type or duration of work were identified in this study (Figure 5). However there was a weak correlation between the EFs of CB-95 and CB-149 with EC7 PCB concentrations (Figure 5). Participants with higher PCB concentrations tended to display a greater degree of enrichment of the *E1* enantiomer of CB-95 (P-value 0.139) and *E2* enantiomer of CB-149 (P-value 0.026). This suggests that there may be a concentration dependent element to the metabolism of these congeners in humans. The high variability in the data for these two congeners also indicates that there is significant intra-individual variation in the enantiospecific processing of these contaminants.

Importantly these data also indicate that PCB profiles measured in sera may not fully match those found in other bodily tissues, as the data recorded here are in contrast to those recorded by Chu et al. (2003) for CB-95, CB-132 and CB-149 in human muscle, kidney and brain samples that were all racemic or nearly racemic. However they do match those recorded by Chu et al. for liver samples which were largely non-racemic in nature. This suggests that sera may in fact reflect liver profiles only, rather than whole body signatures. This has potentially important implications for sera based human biomonitoring programs such as the Canadian Health Measures Survey (CHMS) and the National Health and Nutrition Examination Survey

(NHANES) as it suggests that they may underestimate the true PCB burden and profiles of subjects. However further work is needed to confirm this hypothesis. These data also suggest that the enantiomeric profile of the PCBs should be taken into account when assessing the toxicity of any potential exposure as the persistence and consequent effects of the enantiomers and their metabolic products may be significantly different (Kodavanti and Curras-Collazol, 2010).

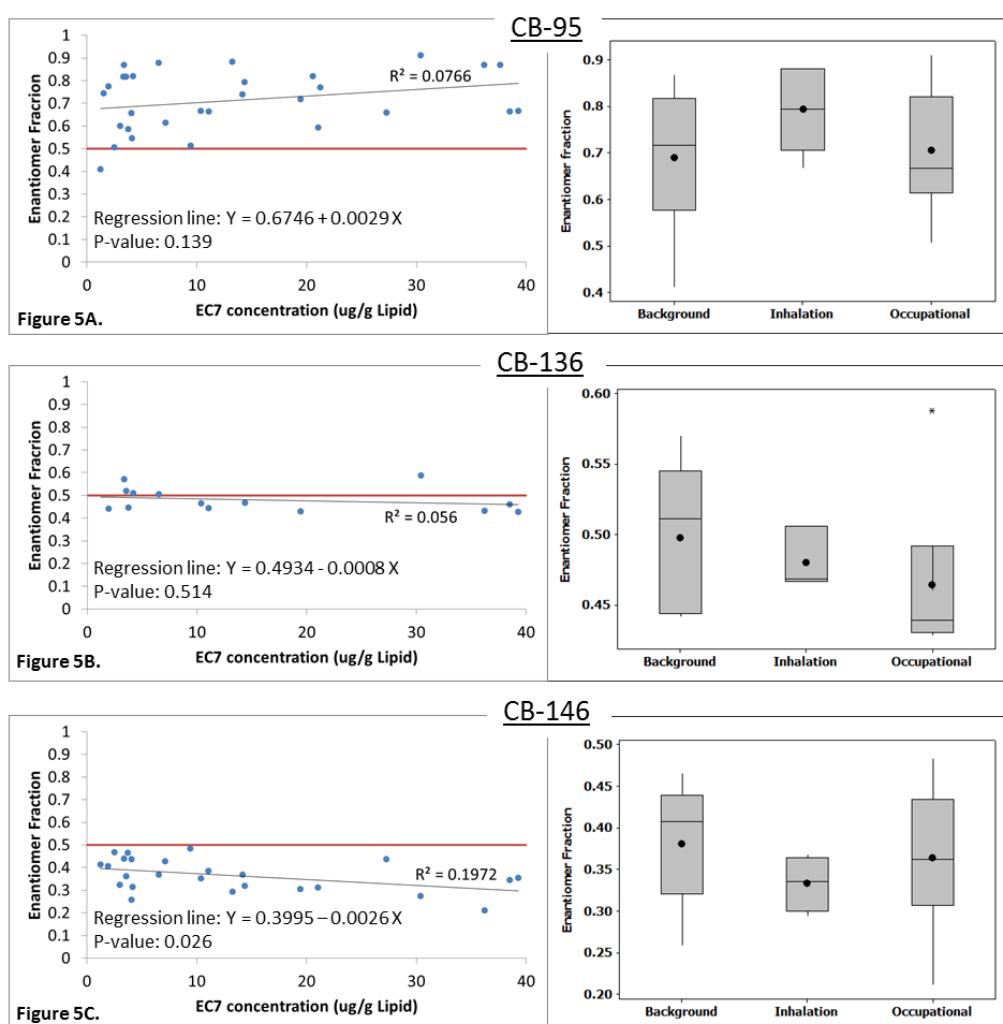


Figure 5. Enantiomer Fraction of CB-95 (Figure 5A), CB-136 (Figure 5B) and CB-149 (Figure 5C), and their relationship with EC7 PCB concentrations and the groups identified by PCA. The red line on the scatter plots represents an EF of 0.5 (i.e. a racemic mixture). Box plots display the interquartile range, median and mean (•).

4 Conclusions

Identifying the source of contamination and age dating human exposure to PCBs is a highly complex task. This is due to the wide range of PCB sources that humans are exposed to along with different exposure pathways and processes such as volatilization, dissolution, biodegradation and post uptake processes that can all alter the original PCB signature. However, by considering PCB concentrations along with detailed congener specific signatures it was possible to identify different sources of contamination and gain an insight into both the magnitude and duration of exposure. Occupationally exposed individuals had a similar PCB profile to Aroclor A1260. Individuals with prolonged exposure had depleted proportions of several PCB congeners that are susceptible to metabolism (CB-95, CB-101 and CB-151), and elevated proportions of PCBs that are resistant to metabolism (CB-74, CB-153, CB-138 and CB-180). A group of workers was also identified with a suspected additional source of exposure through the inhalation of PCBs, as their sera contained elevated proportions of CB-28, CB-60, CB-66, CB-74, CB-105 and CB-118.

Whilst there were no clear trends in enantiomer signatures with the exposure type and duration identified in this study, there was a weak correlation between the EFs of CB-95 and CB-149 with EC7 PCB concentrations, suggesting that there may be a concentration dependent element to the metabolism of these congeners in humans. The extent of enantioselective metabolism or excretion in one worker (S029) was so great it resulted in the near complete removal the E2 enantiomer.

Acknowledgements

The authors would like to thank; Ann Tanderitispolle and Chris Gallagher (University of Strathclyde) for their assistance with sample analysis and the Scottish Funding Council and

336 EPSRC Grant EP/D013739/2 for funding associated with the University of Strathclyde
337 Laboratory. Corina Brimacombe and Terry Kolic for their help with the HRMS analysis of
338 chiral PCBs. Alec Kettle (Leco) for his help and support with the research. All the volunteers
339 who gave blood used in this study. Finally, David Megson would like to thank Plymouth
340 University for funding this project as part of his PhD research.

References

- Akins JR, Waldrep K, Bernert JT. The estimation of total serum-lipids by a completely enzymatic summation method. *Clinica Chimica Acta* 1989; 184: 219-226.
- Axelrad DA, Goodman S, Woodruff TJ. PCB body burdens in US women of childbearing age 2001-2002: An evaluation of alternate summary metrics of NHANES data. *Environmental Research* 2009; 109: 368-378.
- Aylward LL, Collins JJ, Bodner KM, Wilken M, Bodnar CM. “Intrinsic” elimination rate and dietary intake estimates for selected indicator PCBs: Toxicokinetic modeling using serial sampling data in US subjects, 2005–2010. *Chemosphere* 2014; 110: 48-52.
- Brown JF, Lawton RW. Factors Controlling the Distribution and Levels of PCBs after Occupational Exposure. In: Robertson LW, Hansen LG, editors. *PCBs recent advances in Environmental Toxicology and Health Effects*. The University Press of Kentucky, 2001.
- Chu S, Covaci A, Schepens P. Levels and chiral signatures of persistent organochlorine pollutants in human tissues from Belgium. *Environmental Research* 2003; 93: 167–176.
- DeCaprio AP, Johnson GW, Tarbell AM, Carpenter DO, Chiarenzelli JR, Morse GS, Santiago-Rivera AL, Schymura MJ, Akwesasne Task Force E. Polychlorinated biphenyl (PCB) exposure assessment by multivariate statistical analysis of serum congener profiles in an adult Native American population. *Environmental Research* 2005; 98: 284-302.
- Durfree RL, Contos G, Whitmore FC, Barden JD, Hackman EEI, Westin RA. *PCBs in the United States - industrial use and environmental distribution*. 1976.
- Environment Agency. Updated Technical Background to the CLEA Model, Science Report SC050021/SR312. 2009.
- Erickson MD. Introduction: PCB properties, uses, occurrences and regulatory history. In: Robertson LW, Hansen LG, editors. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. University of Kentucky Press, 2001.
- Focant JF, Sjodin A, Patterson Jr DG. Improved separation of the 209 polychlorinated biphenyl congeners using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A* 2004; 1040: 227 - 238.
- Frame GM. The Current State-of-the-Art of Comprehensive, Quantitative, Congener-Specific PCB Analysis, and What We Now Know about the Distributions of Individual Congeners in Commercial Aroclor Mixtures. In: Robertson LW, Hansen LG, editors. *PCBs Recent Advances in Environmental Toxicology and Health Effects*. The University Press of Kentucky, 2001.
- Hansen LG. *The ortho side of PCBs*: Kluwer Academic Publishers, 1999.

379 Harju M, Danielsson C, Haglund P. Comprehensive two-dimensional gas chromatography of
380 the 209 polychlorinated biphenyls. *Journal of Chromatography A* 2003; 1019: 111-
381 126.

382 Harner T, Wiberg K, Norstrom R. Enantiomer fractions are preferred to enantiomer ratios for
383 describing chiral signatures in environmental analysis. *Environmental Science &*
384 *Technology*. 2000; 34: 218-220.

385 Harrad S, Ren JZ, Hazrati S. Chiral signatures of PCB#s 95 and 149 in indoor air, grass,
386 duplicate diets and human faeces. *Chemosphere*. 2006; 63: 1368-1376

387 Harrad SJ, Hazrati S, Ibarra C. Concentrations of polychlorinated biphenyls in indoor air and
388 polybrominated diphenyl ethers in indoor air and dust in Birmingham, United
389 Kingdom: implications for human exposure. *Environmental Science & Technology*
390 2005; 40: 4633-4638.

391 Helsel DR. Fabricating data: How substituting values for nondetects can ruin results, and
392 what can be done about it. *Chemosphere* 2006; 65: 2434-2439.

393 Herrick RF, Meeker JD, Altshul L. Serum PCB levels and congener profiles among teachers
394 in PCB-containing schools: a pilot study. *Environmental Health* 2011; 10.

395 Holoubek I. Polychlorinated biphenyl (PCB) contaminated sites worldwide. In: Robertson
396 LW, Hansen LG, editors. *PCBs: Recent Advances in Environmental Toxicology and*
397 *Health Effects*. University of Kentucky Press, 2001, 17-26.

398 Jain RB, Wang RY. Association of caffeine consumption and smoking status with the serum
399 concentrations of polychlorinated biphenyls, dioxins, and furans in the general U.S.
400 population: NHANES 2003-2004. *Journal of Toxicology and Environmental Health-*
401 *Part a-Current Issues* 2011; 74: 1225-1239.

402 James MO, Sacco JC, Faux LR. Effects of food natural products on the biotransformation of
403 PCBs. *Environmental Toxicology and Pharmacology*, 2008; 25: 211–7.

404 James MO. Polychlorinated Biphenyls: Metabolism and Metabolites. In: Robertson LW,
405 Hansen LG, editors. *PCBs Recent Advances in Environmental Toxicology and*
406 *Health Effects*. The University Press of Kentucky, 2001.

407 Jaspers VLB, Megson D, O'Sullivan G. Chapter 7, POPs in the Terrestrial Environment. In:
408 O'Sullivan G, Sandau CD, editors. *Environmental Forensics for Persistent Organic*
409 *Pollutants*. Elsevier, 2013.

410 Johnson GW, Ehrlich R, Full W, Ramos S. Chapter 7: Principal components analysis and
411 receptor models in environmental forensics. In: Morrison R, Murphy BL, editors. *An*
412 *Introduction to Environmental Forensics*. 2nd Edition. Elsevier, Amsterdam, 2007,
413 pp. 207-272.

414 Johnson GW, Quensen III JF, Chiarenzelli JR, Coreen Hamilton M. Polychlorinated
415 Biphenyls. In: Morrison RD, Murphy BL, editors. *Environmental Forensics*
416 *Contaminant Specific Guide*. Academic Press, 2006.

417 Kodavanti PRS, Curras-Collazo MC, Neuroendocrine actions of organohalogenes: Thyroid
418 hormones, arginine vasopressin, and neuroplasticity. *Frontiers in*
419 *Neuroendocrinology* 2010; 31: 479-496

420 Kohler M, Tremp J, Zennegg M, Seiler C, Minder-Kohler S, Beck M, Leinemann P,
 421 Wegmann L, Schimi P. Joint sealants: an overlooked diffuse source of
 422 polychlorinated biphenyls in buildings. *Environmental Science & Technology* 2005;
 423 39: 1967-1973.

424 Korytar P, Haglund P, de Boer J, Brinkman UAT. Comprehensive two-dimensional gas
 425 chromatography for the analysis of organohalogenated micro-contaminants. *Trac-*
 426 *Trends in Analytical Chemistry* 2006; 25: 373-396.

427 Letcher RJ, Klasson-Wehler E, Bergman Å. Methylsulfone and hydroxylated metabolites of
 428 polychlorinated biphenyls. In: Passivita J, editor. *The Handbook of Environment*
 429 *Chemistry*; Vol. 3, Part K: New Types of Persistent Halogenated Compounds.
 430 Springer-Verlag, Heidelberg, 1999, pp. 315-360.

431 Longnecker MP. Endocrine and Other Human Health Effects of Environmental and Dietary
 432 Exposure to Polychlorinated Biphenyls. In: Robertson LW, Hansen LG, editors.
 433 PCBs recent advances in Environmental Toxicology and Health Effects. The
 434 University Press of Kentucky, 2001.

435 Luotamo M, Patterson Jr DG, Needham LL, Aitio A. Concentrations of PCB congeners in
 436 sera from workers with past and present exposure. *Chemosphere* 1993; 27: 171-177.

437 Megson D, O'Sullivan G, Comber S, Worsfold PJ, Lohan MC, Edwards MR, Shields WJ,
 438 Sandau CD, Patterson Jr DG. Elucidating the structural properties that influence the
 439 persistence of PCBs in humans using the National Health and Nutrition Examination
 440 Survey (NHANES) dataset. *Science of the Total Environment* 2013a; 461-462: 99-
 441 107.

442 Megson D, Kalin RB, Worsfold P, Gauchotte-Lindsay C, Patterson Jr DG, Lohan MC,
 443 Comber S, Brown TA, O'Sullivan G. Fingerprinting polychlorinated biphenyls in
 444 environmental samples using comprehensive two-dimensional gas chromatography
 445 with time-of-flight mass. *Journal of Chromatography A* 2013b; 1318: 276-283.

446 Oki M. Recent Advances in Atropisomerism. *Topics in Stereochemistry* 1983; 14: 1-81

447 O'Sullivan G, Sandau CD. *Environmental Forensics for Persistent Organic Pollutants*:
 448 Elsevier, 2013.

449 Patterson D, O'Sullivan G, Sandau CD. The use and misuse of the National Health and
 450 Nutrition Examination Survey (NHANES) data for assessing human exposure to
 451 environmental chemicals. In: Morrison RD, O'Sullivan G, editors. *Environmental*
 452 *Forensics*. RSC Publishing, Cambridge, 2009, pp. 188-201.

453 Price NO, Young RW, Dickinson Jk. Pesticide residues and polychlorinated biphenyl levels
 454 in diets, urine, and fecal matter of preadolescent girls. *Proceedings of the Society for*
 455 *Experimental Biology and Medicine* 1972; 139: 1280-1283.

456 Quinn, CL & Wania, F. Understanding Differences in the Body Burden- Age Relationships
 457 of Bioaccumulating Contaminants Based on Population Cross Sections versus
 458 Individuals. *Environmental Health Perspectives*, 2012, 120; 4: 554-559

459 Ritter R, Scheringer M, MacLeod M, Moeckel C, Jones KC, Hungerbühler K. Intrinsic
 460 Human Elimination Half-Lives of Polychlorinated Biphenyls Derived from the

461 Temporal Evolution of Cross-Sectional Biomonitoring Data from the United
462 Kingdom. *Environmental Health Perspectives* 2011; 119: 225-231.

463 Robson M, Harrad S. Chiral PCB signatures in air and soil: Implications for atmospheric
464 source apportionment. *Environmental Science & Technology* 2004; 38: 1662-1666.

465 Sandau CD. Analytical chemistry of hydroxylated metabolites of PCBs and other halogenated
466 phenolic compounds in blood and their relationship to thyroid hormone and retinol
467 homeostasis in humans and polar bears. Carleton University, 2001.

468 Seegal RF, Fitzgerald EF, Hills EA, Wolff MS, Haase RF, Todd AC, Parsons P, Molho ES,
469 Higgins DS, Factor SA, Marek KL, Seibyl JP, Jennings DL, Mccaffrey RJ.
470 Estimating the half-lives of PCB congeners in former capacitor workers measured
471 over a 28-year interval. *Journal of Exposure Science and Environmental*
472 *Epidemiology* 2011; 21: 234-246.

473 Weintraub M, Birnbaum LS. Catfish consumption as a contributor to elevated PCB levels in a
474 non-Hispanic black subpopulation. *Environmental Research* 2008; 107: 412-417.

475 Wong CS, Hoekstra PF, Karlsson H, Backus SM, Mabury SA, Muir DCG. Enantiomer
476 fractions of chiral organochlorine pesticides and polychlorinated biphenyls in
477 standard and certified reference materials. *Chemosphere* 2002; 49: 1339-1347.

478 Wu XA, Kammerer A, Lehmler HJ. Microsomal Oxidation of 2,2',3,3',6,6'-
479 Hexachlorobiphenyl (PCB 136) Results in Species-Dependent Chiral Signatures of
480 the Hydroxylated Metabolites. *Environmental Science & Technology* 2014; 48:
481 2436-2444.

482 Zapadlo M, Krupcik J, Kovalczuk T, Majek P, Spanik I, Armstrong DW, Sandra P. Enhanced
483 comprehensive two-dimensional gas chromatographic resolution of polychlorinated
484 biphenyls on a non-polar polysiloxane and an ionic liquid column series. *Journal of*
485 *Chromatography A* 2011; 1218: 746-751.

486