Evaluation of the effectiveness of different indicator PCBs to estimating total PCB concentrations in environmental investigations

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Abstract

Polychlorinated biphenyls (PCBs) are one of the most widely studied group of persistent organic pollutants (POPs). There are 209 different PCBs, however not all 209 can currently be individually quantified in one analytical run. This means that a subset of PCBs congeners are often determined and reported. Some of the most commonly reported subsets are the 7 indicator PCBs (28, 52, 101, 118, 138, 153 and 180) and the WHO 12 PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189). The WHO 12 congeners are co-planar 'dioxin like' PCBs that are effective for establishing health risks. The 7 indicator PCBs were selected as some of the most common PCBs across the compositional range of the most common technical mixtures (such as Aroclors), and are used to give an indication of the total PCB concentrations. These groups of indicator PCBs were established several decades ago. However, in the environment commercial mixtures are subject to weathering and fractionation processes, and additional sources of non-Aroclor PCBs are also becoming more important. In this manuscript we use existing large scale comprehensive congener

specific datasets to evaluate the effectiveness of indicator PCBs to predict total concentrations and establish if they are still fit for purpose. The results indicate that while these traditional indicators are a useful tool to estimate total concentrations in humans with background exposure there are many instances where they are not fit for purpose and can lead to significant under predictions in total PCB concentrations in environmental matrices.

Keywords

Polychlorinated biphenyls, PCBs, Sediments, Water, Aroclors, Humans

Introduction

There is no universal method for the analysis of polychlorinated biphenyls (PCBs). In fact, there are 10s to 100s of published and certified methods for PCB analysis, many of which have been summarised (Guo and Kannan, 2015, Reiner et al., 2014, Megson, 2019, Clement et al., 2012, Muir and Sverko, 2006). Methods typically involve separation by gas chromatography (GC) and detection by electron capture detectors (ECD) or using mass spectrometry (MS). One of the simplest forms of these methods is based on the approach of determining a "technical mixture equivalent total PCB value", which employs a commercial product or mixtures of products as a standard to quantitate the matching congeners contained in the product/mixture and sample (e.g. EPA Method 8082). An intermediate method aims to measure concentrations of various PCB homologues based on the number of chlorines in the congeners (e.g. EPA Method 680), while a more detailed approach targets various individual congeners with a goal of measuring as many of them as practically possible (e.g. EPA Method 1668C) (Muir and Sverko, 2006).

In many environmental investigations a detailed approach is required, and therefore specific congeners need to be determined rather than a total PCB concentration (e.g. EU regulation 117 (2017) which requires analysis of the WHO12 congeners). The rationale for undertaking an environmental investigation should influence which PCBs are targeted. There can be many reasons for undertaking an investigation, from

commissioning brief scoping studies, to establishing potential health effects, or understanding patterns for source apportionment. The number of reported congeners in investigations can range from a few indicator (i) PCBs such as the i7 PCBs (28, 52, 101, 118, 138, 153 and 180). These have been selected as they are some of the most abundant congeners across the compositional range of the most common technical mixtures and the environment and so are often used as indicators for the magnitude of PCB contamination (Johnson et al., 2006, Battelle Memorial Institute et al., 2012). To assess human health risks, the World Health Organisation (WHO) proposes the analysis of a specific subset of 12 dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) (Van den Berg et al., 2006). These PCBs were selected due to their co-planar structure which results in them having the same mode of action as 2,3,7,8-TCDD against the aryl hydrocarbon receptor. Some studies aim to track sources of contamination and so they have attempted to determine as many PCBs as possible. This can result in the detection of over 130 different congeners in an environmental sample (Megson et al., 2013, Megson et al., 2018). With so many different recommendations and with analytical techniques for the analysis of one sample ranging from $50 US to greater than $2000 US (Reiner et al., 2014), it can be difficult to know how many and which PCBs to target, especially with such a variety of standard accredited methods (e.g. EPA 1668C) and non-accredited methods are available. It is important to note that these non-accredited methods should not always be discounted as they could provide higher quality data, although this comes at a cost (e.g through analysis by multidimensional chromatography or ultra high resolution mass spectrometry).

One of the first aims of many environmental investigations is to quantify the amount of PCBs in a given sample to establish if further work or investigation is required. It can be very time and cost intensive to accurately quantify all 209 PCBs, and equally as intensive to also process this data. Therefore, simpler screening methods such as EPA Methods 8082 and 608 are used to estimate total PCB concentrations. These involve the analysis of several individual PCBs which can then be used to calculate a total PCB concentration or estimate an Aroclor equivalent concentration. In humans, various attempts have been made to estimate total PCB concentrations from only a few congeners (Longnecker et al., 2003, Vanderven et al.,

1992, Wicklund Glynn et al., 2000, Barr et al., 2006, Needham et al., 2005, Wolff et al., 2005, Fitzgerald et al., 2005, Jain and Wang, 2010). Wicklund Glynn et al. (2000) showed that CB 153 alone was an excellent predictor for total PCB concentrations with a coefficient of determination (R^2) value of 0.96, however this study was undertaken on a racially homogenous Swedish male sample population. Using the 2001-02 National Health and Nutrition Examination Survey (NHANES) data which represents the exposure of the United States general non-exposed population, Jain and Wang (2010) obtained an R^2 value of 0.78 indicating there is more variability in a wider sample set. The i3 PCB congeners (PCB 138, 153 and 180) have been used to estimate total PCB concentrations (Wicklund Glynn et al., 2000, Barr et al., 2006, Needham et al., 2005, Jain and Wang, 2010). While these three congeners work well at predicting the total PCB concentrations for a localised population, demographic variables such as race/ethnicity can lead to poorer predictions (Jain and Wang, 2010). Similar conclusions were also reached by Gandhi et al. (2015) who investigated the use of a variety of different indicator compounds to calculate total PCB concentrations in fish. They proposed that, alternate i3 PCBs (PCB 95, 118, and 153), i4 PCBs (i3 PCBs plus 138) and i5 PCBs (i4 PCBs plus 110), and existing i6 PCBs (28, 52, 101, 138, 153 and 180) and i7 PCBs are the most optimal indicators, while use of the current i3 PCBs (PCB 138, 153 and 180) should be avoided.

While there is a lot of available literature on estimating PCB totals in animals there are less data available for matrixes such as water and sediment. Fikslin and Santoro (2003) assessed the use of a subset of 18 PCBs (proposed by the National Oceanic and Atmospheric Administration (NOAA)) to estimate total PCB concentrations in estuarine water and sediment. They concluded that total PCBs would be overestimated based on 2× the sum of the 18 congeners for effluent samples during dry weather periods and influent samples at higher PCB levels (>35 ng L^{-1}). However, total PCB estimates for wet weather effluent concentration showed good agreement. Sediment samples show poor agreement for the 18 congener × 2 estimate and clearly underestimated the total PCB value, suggesting biases in estimation of totals may be driven by environmental transformation and/or factors related to the matrix analysed.

This paper aims to evaluate the strength of commonly used indicator groups of PCBs such as CB153, i3 PCBs, i7 PCBs in providing an estimate of the total PCB concentration in different matrices. It also provides recommendations and highlights limitations of the different available methods.

Methodology

An inherent issue with nearly all PCB datasets is that they will have already undergone some degree of "analytical filtering". Currently, no single method can determine all 209 congeners in one run. Therefore, data analysts have to constantly deal with co-elutions (eg CB-28 and CB-31 for most methods) and data that is limited to what the authors deem as the major congeners of importance (or the ones their method targeted). This means that in many cases the total PCB concentration is not actually known as some PCBs go unrecorded or reported. In this assessment, we acknowledge this limitation and have focused our assessment on available datasets which contain as many PCBs as possible with methods targeted towards quantifying the more prevalent PCBs in that matrix. This includes congener specific PCB data from technical mixtures (Aroclors) previously quantified by Frame et al. (1996), hundreds of sediment and water samples collected from North America, and thousands of U.S. human serum samples from the National Health and Nutrition Examination Survey (NHANES). To provide a balanced assessment, samples from "background sites" and known contaminated sites were assessed separately for sediments, water and humans. These data were collated and examined using the popular PCB indicator estimation methods noted above (CB153, i3 PCBs, i7 PCBs) to predict "total PCB concentrations". This was assessed by linear regression calculating the coefficient of determination (R^2) along with significance testing using Spearman rank correlations (data provided in Supplementary Information, Tables S2-S7). The source of these different datasets and analytical methods used for their creation are provided in this section.

Aroclors

Aroclor data was obtained directly from Frame (2001), which included the results from Frame et al. (1996), and used as the reference dataset for this exercise. Frame et al. (1996) diluted Aroclor standards and

analysed them using three high resolution gas chromatography systems with mass spectrometry. Samples covering the range of Aroclors 1221 through 1262 were analysed on 3 HRGC systems, which between them enabled unambiguous measurements of all 209 congeners. (Frame, et al., 1996; Frame, 2001)."

Water samples – Project Trackdown

Two sets of water samples were collated to represent background sites and contaminated sites. All water samples were collected by the Ontario Ministry of the Environment and Climate Change (MOECC) as part of investigative monitoring programs aimed at tracking PCBs in the environment (Benoit et al., 2016). The background sites were composed of 394 samples obtained from across Ontario and the contaminated site comprised 18 samples, which were identified next to significant contamination sources. Water samples were collected in two litre glass containers and PCBs determined by congener-specific analysis using method E3459 (Ontario Ministry of the Environment, 2008). Briefly, the method identifies 75 of a possible 209 PCB samples in aqueous samples using internal standard/isotope dilution. Samples were fortified with isotopically labelled PCBs and extracted by filtering through a C18 solid phase extraction disk. Particulate bound PCBs and PCBs trapped to the disc are extracted using acetone/hexane. Extracts are cleaned using silica prior to analysis with gas chromatography with high resolution mass spectrometry (GC-HRMS).

Sediment samples

Two sets of sediment samples were collated to represent background sites and contaminated sites. Sediment samples that comprised the background data were collected by the MOECC as part of investigative monitoring programs in the Ontario Great Lakes Basin. Polychlorinated biphenyl concentrations were determined for 55 PCBs in 374 samples taken across Ontario's Great Lakes Basin. Sample preparation and extraction was conducted as described in Muscalu et al. (2011). Briefly, samples were air-dried, crushed and sieved. A 5 g aliquot of sample was extracted using accelerated solvent extraction (ASE) with 25% dichloromethane/75% hexane (v/v), followed by silica cartridge clean-up, and treated with copper granules to remove sulphur interferences. Samples were analysed gas chromatography with electron capture detection (GC-ECD) as detailed in method MOE2008.

Sediment samples that comprised the contaminated data were obtained from Magar et al. (2005) and included 107 PCB congeners in more than 280 samples from 18 sediment cores and surface samples obtained from the Lake Hartwell Superfund site (Pickens County, SC). Analyses was conducted at the Battelle Ocean Science Laboratories (Duxbury, MA) using modified U.S. EPA SW-846 Method 8270. Methods are described in detail by Brenner et al. (2004) and involved extraction with hexane, clean up using alumina, granular copper and gel permeation chromatography and analysis by gas chromatography with mass spectrometry.

Human samples

Two data sets were collated which included a background exposure cohort and an occupationally exposed cohort. The background exposure data were retrieved from the 2003-04 United States National Health and Nutrition Examination Survey (NHANES). 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 determination of a range of contaminants including a total of 37 PCBs in serum. Data from the NHANES surveys are publicly available from the Centres for Disease Control and Prevention (CDC, 2011b). PCBs were extracted using a C18 solid phase extraction (SPE) procedure followed by extraction through neutral silica and Florisil columns with hexane and 1:1 dichloromethane/hexane. Extracts were analysed using high resolution gas chromatography / high resolution mass spectrometry (HRGC-HRMS). Further information regarding the collection and analysis of serum samples together with data quality procedures are available from the CDC (CDC, 2011a).

The data for occupationally exposed humans were obtained from a study conducted by Megson et al. (2015). This included the determination of 209 PCBs in 30 participants. PCBs were extracted using a C18 solid phase extraction (SPE) procedure and processed using EPA method 3665A sulphuric acid/permanganate clean-up followed by EPA method 3620 Florisil clean-up. Extracts were analysed using comprehensive two dimensional gas chromatography with time of flight mass spectrometry (GCxGC-ToFMS).

The full congener specific datasets for each investigation are provided for reference in the supplementary information.

Results and Discussion

Commercial mixtures (Aroclors)

Theoretically 209 PCBs exist, but only approximately 130 of these have been detected in commercial mixtures (Frame, 2001). Five Aroclors (A1016, A1242, A1248, A1254 and A1260) accounted for >95% of sales between 1957 and 1974 (Durfree et al., 1976). There have been a variety of proposed methods to estimate total PCB concentrations using a subset of congeners, including the i7 PCBs, i3 PCBs and CB-153. The analysis of these congeners is often performed as an initial screening method during an environmental investigation to identify if PCBs contamination has occurred. The proportions of these groups of congeners in each of the 5 main Aroclors are presented in Table S1. The best predictor for total concentrations were the i7 PCBs although the total proportions present varied substantially between the different Aroclors. Total proportions ranged from approximately 30% of the total PCBs in the more chlorinated Aroclors (A1254 and A1260), but in A1242 nearly 90% of the total PCBs would go un-reported. The variation would be even greater for CB-153 and i3 PCBs: if only these congeners were analysed no PCBs would be detected in a technical mixture of A1016. The large variation in proportions of the indicator compounds in the different Aroclors could result in false conclusions being drawn when analysing samples that contain fresh inputs of Aroclors. No correction factors can be applied to any of the three indicators to calculate total PCB concentrations.

Most environmental samples rarely contain high proportions of fresh Aroclor inputs. They usually contain a mixture of inputs that have undergone some degree of weathering by physical, chemical and biological processes. These processes result in a relative enrichment in recalcitrant congeners including the i7 PCBs (Hansen, 1999). The relative proportions of these key congeners in water, sediment and humans was investigated along with a regression against the “total” PCB concentration.

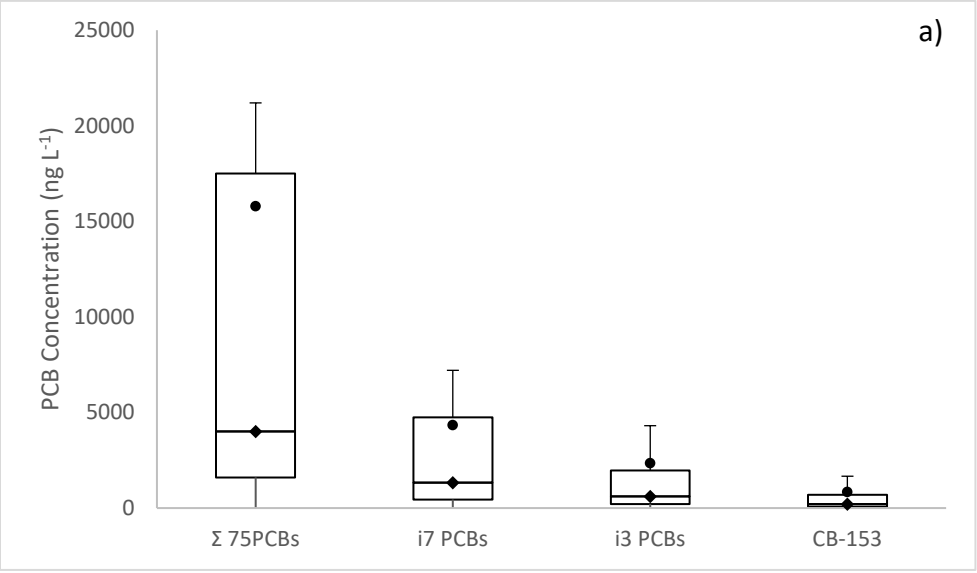
Water samples

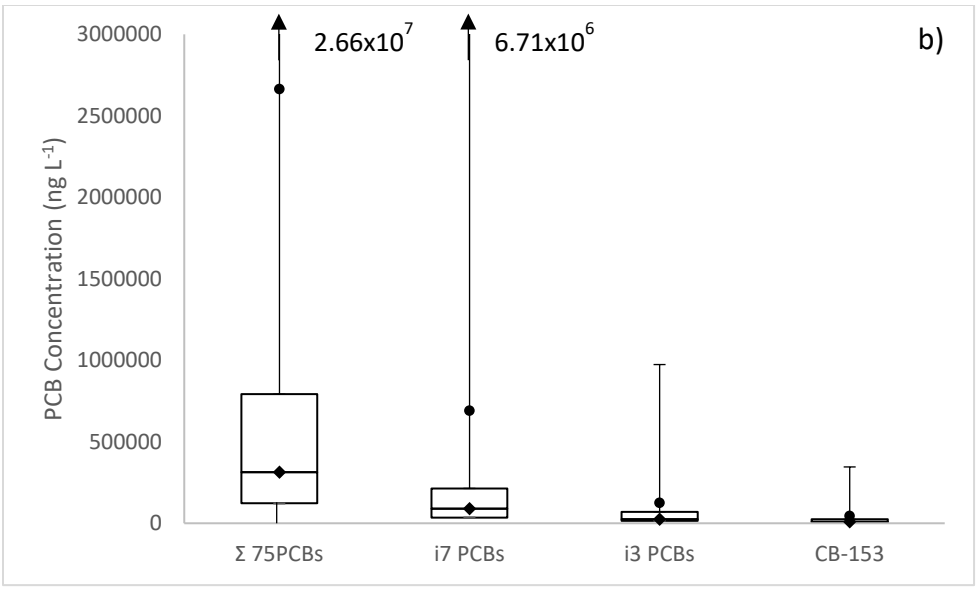
Three hundred and ninety four water samples were collected from background sites across Ontario as described in Benoit et al. (2016). The i7 PCBs accounted for approximately 28% of the total detected PCB concentrations whereas the i3 PCBs and CB-153 accounted for 15% and 5% respectively (Figure 1a). The relative proportions of the i7 PCBs, i3 PCBs and CB-153 to the total concentration all showed a relatively poor correlation to the total PCB concentration (Figure 2a). A coefficient of determination (R^2) of approximately 0.91 was recorded for the i7 PCBs, but the i3 PCBs and CB-153 were below 0.6. The coefficient of determination was calculated for each of the 75 PCBs against the total concentration and is presented in the Supplementary Information as Table S2. The 5 PCBs with the best correlation were CB-99 (R^2 of 0.81), CB-156 (R^2 of 0.80), CB-110 (R^2 of 0.80), CB-118 (R^2 of 0.79), and CB-123 (R^2 of 0.79). PCBs 99, 110 and 118 are abundant in technical PCB mixtures which may explain the good correlation for these congeners, however PCBs 156 and 123 are not as common (<0.5% of total Aroclor PCBs).

A separate targeted study was undertaken which involved the collection of eighteen water from a known contaminated site in Ontario (Figure 1b); the i7 PCBs accounted for approximately 26% of the total whereas the i3 PCBs and CB-153 accounted for 4.7% and 1.7% respectively. The relative proportions of the i7 PCBs, i3 PCBs and CB-153 had an excellent correlation with the total PCB concentration (Figure 2b), these correlations are partly attributable to a couple of samples with very high concentrations. A coefficient of determination (R^2) of approximately 1.0 was recorded for the i7 PCBs, with the correlations for the i3 PCBs and CB-153 both greater than 0.95. The coefficient of determination was calculated for each of the 75 PCBs against the total concentration and is presented as Supplementary Information Table S3. Fifty of the 75 congeners had a R^2 value of greater than 0.95 suggesting that the samples were likely from a single (or similar) sources with limited alteration/degradation).

The results for the contaminated site showed the best correlation between the indicator compounds and total concentrations (Figure 2b). This strong correlation can be explained because these samples were all obtained from an area with a known source of contamination, with a relatively unaltered congener profile

(Benoit et al., 2016). In .the background sites, the PCB load will have been more impacted by post depositional factors such as areal deposition, mixing, biodegradation, volatilisation, etc. and therefore have a poorer correlation. The results indicate that the i7 PCBs, i3 PCBs and CB-153 can be good predictors for total PCBs from technical mixture sources with a relatively unaltered congener profile, but they are likely to under-predict concentrations in other environmental samples. When considering aquatic environments, the indicator compounds should not be considered in isolation. There will be partitioning between sediment, biota and the atmosphere which will alter the PCB congener profile over time (Hansen, 1999). The i3 PCBs and CB-153 have been shown to preferentially accumulate in biota and sediment and so over time, their proportions will be depleted from the aquatic environment. Therefore, when assessing PCBs from aquatic environments, care should be used when just considering a small subset of PCBs. There may be added benefit to considering an additional selection of mid and lower chlorinated congeners from the supplementary information (SI1) to estimate total PCBs.





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232 **Figure 1.** Box and whisker diagram of PCB concentrations in of "total PCBs", i7, PCBs, i3 PCBs and CB-
233 153 for a) water samples obtained from streams in Ontario (n = 394) and b) water samples obtained from
234 contaminated sites in Ontario (n = 18) Dots represent the mean value and diamonds represent the median,
235 boxes represent the interquartile range and whiskers the minimum and maximum reported values

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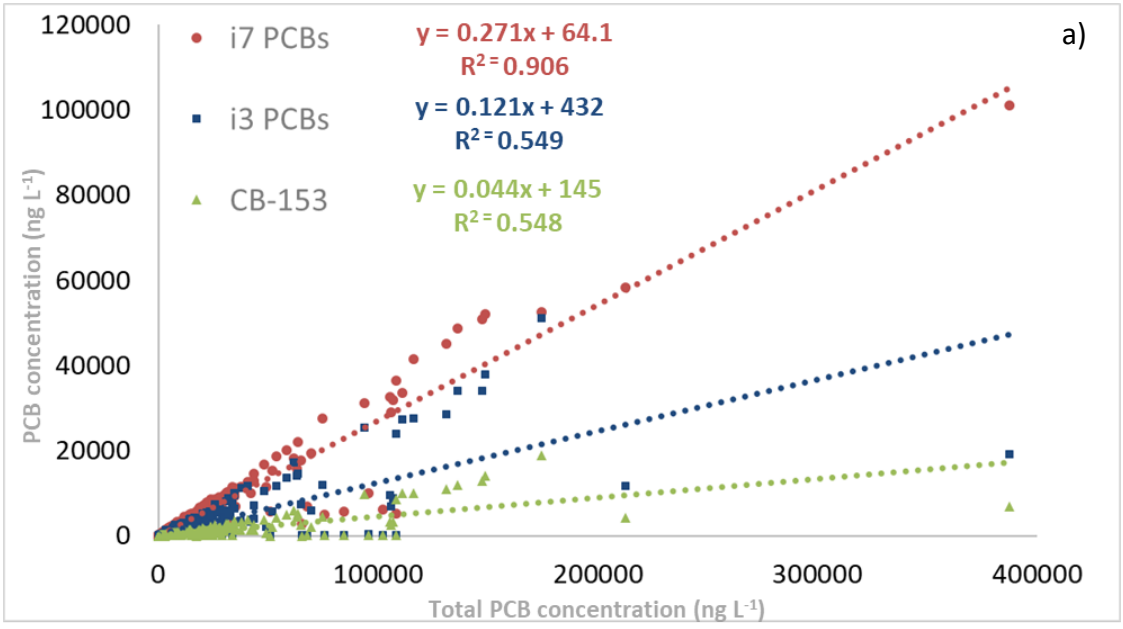

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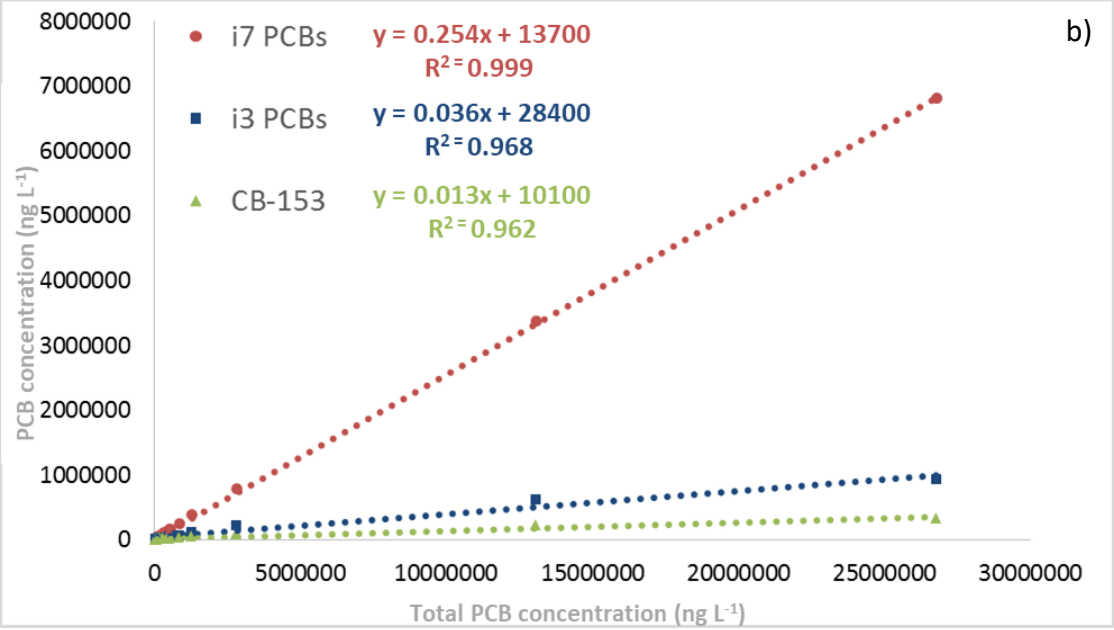

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239 **Figure 2.** Scatter plot and regression of i7, PCBs, i3 PCBs and PCB-153 against "total PCBs (∑75)" for a)
240 water samples obtained from waterways in Ontario (n = 394) and b) 18 water samples obtained from
241 contaminated sites in Ontario

242

243 ## Sediment samples

244 PCB concentrations were determined in 374 sediment samples obtained from across Ontario. The i7 PCBs
245 accounted for approximately 30%, of the total whereas the i3 PCBs and CB-153 accounted for 14% and
246 5% respectively (Figure 3a). The relative proportions of the i7 PCBs, to the total concentration showed a
247 good correlation whereas the i3 PCBs and CB-153 correlation was poor. A coefficient of determination of
248 0.98 was recorded for the i7 PCBs, and an R2 value of approximately 0.8 recorded for the i3 PCBs and CB-
249 153 (Figure 4a). The coefficient of determination was calculated for each of the 55 PCB congeners against
250 the total concentration and is presented as Supplementary Information Table S4. The 5 PCB congeners with
251 the best correlation were CB-101 (R^2 of 0.93), CB-168 (R^2 of 0.91), CB-138 (R^2 of 0.90), CB-110 (R^2 of
252 0.87), and CB-118 (R^2 of 0.85). PCBs 101, 110, 118 and 138 are abundant in technical PCB mixtures

253 (Frame et al. (1996)) which may explain the good correlation for these congeners, however PCB 168 is not
254 as common (<0.5% of total Aroclor PCBs).

255 Data for 280 sediment samples collected from the Lake Hartwell Superfund site were obtained from Magar
256 et al. (2005). The i7 PCBs accounted for approximately 7%, of the total whereas the i3 and CB-153
257 accounted for 2% and 0.8% respectively. The relative proportions of the i7 PCBs, i3 PCBs and CB-153 to
258 the total concentration all showed a very poor correlation to the total PCB concentration (Figure 4b). A
259 coefficient of determination of less than 0.7 was recorded for the i7 PCBs, i3 PCBs and CB-153. The
260 coefficient of determination was calculated for each of the 107 PCBs against the total concentration and is
261 presented as Supplementary Information Table S5. The 5 PCBs with the best correlation were CB-19 (R^2
262 of 0.97), CB-16/32 (R^2 of 0.95), CB-24/27 (R^2 of 0.95), CB-51 (R^2 of 0.94), and CB-4/10 (R^2 of 0.93).

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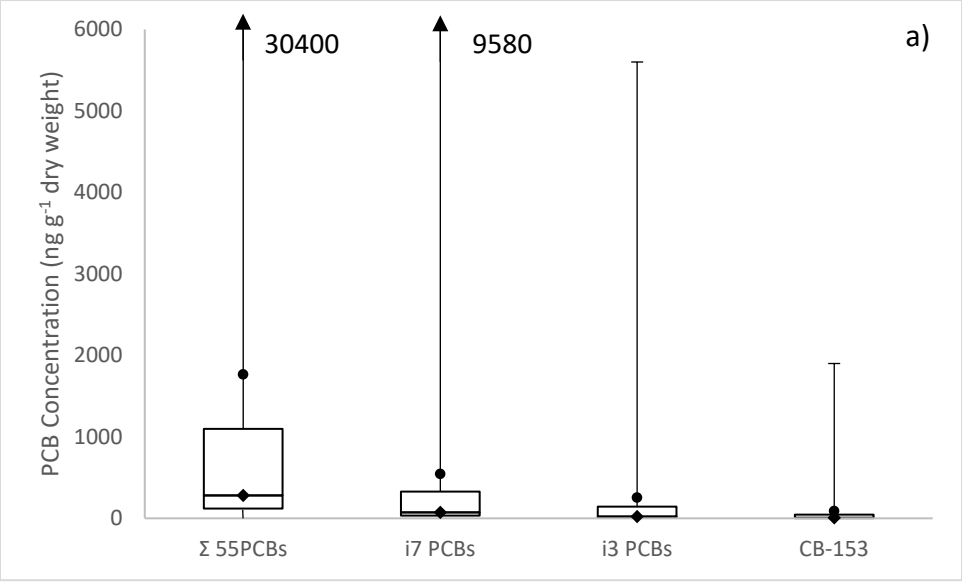

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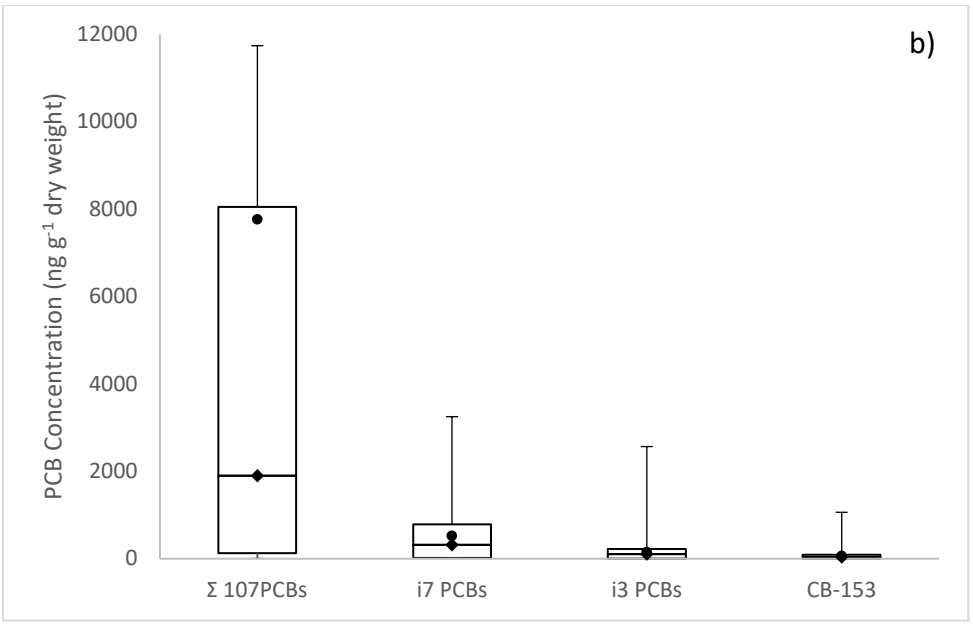
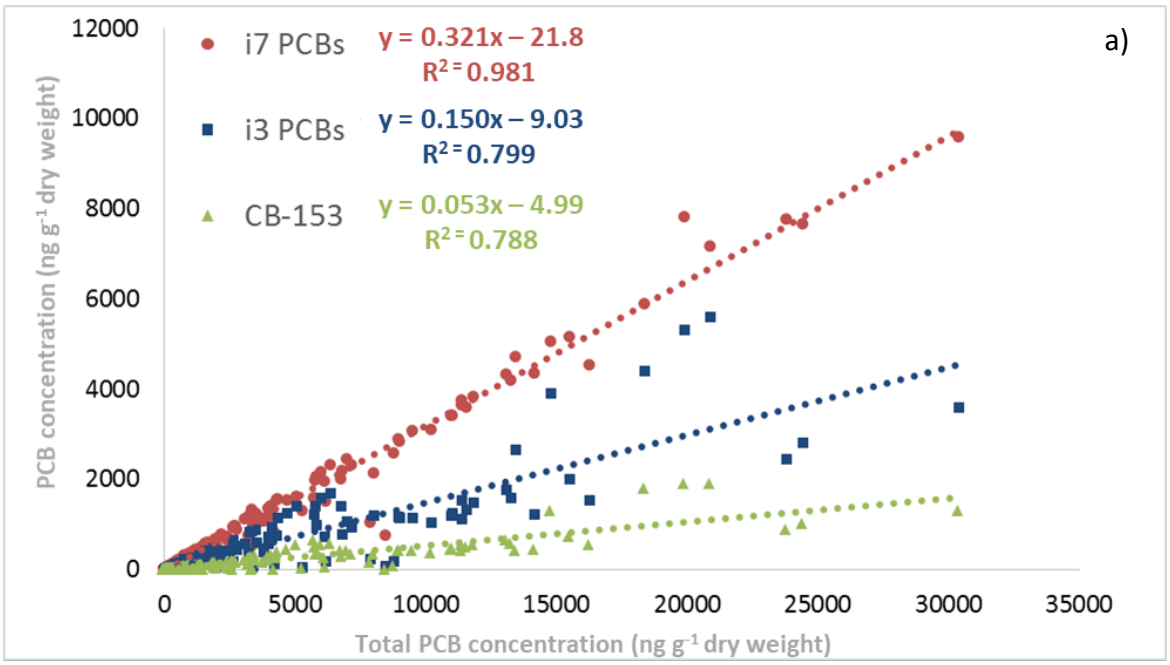


Figure 3. Box and whisker diagram of PCB concentrations in of "total PCBs", i7 PCBs, i3 PCBs and CB-153 for a) sediment samples obtained from across Ontario (n = 374) and b) sediment samples obtained from Lake Hartwell Superfund site (n = 280) (data from Magar et al. (2005)). Dots represent the mean value and diamonds represent the median, boxes represent the interquartile range and whiskers the minimum and maximum reported values



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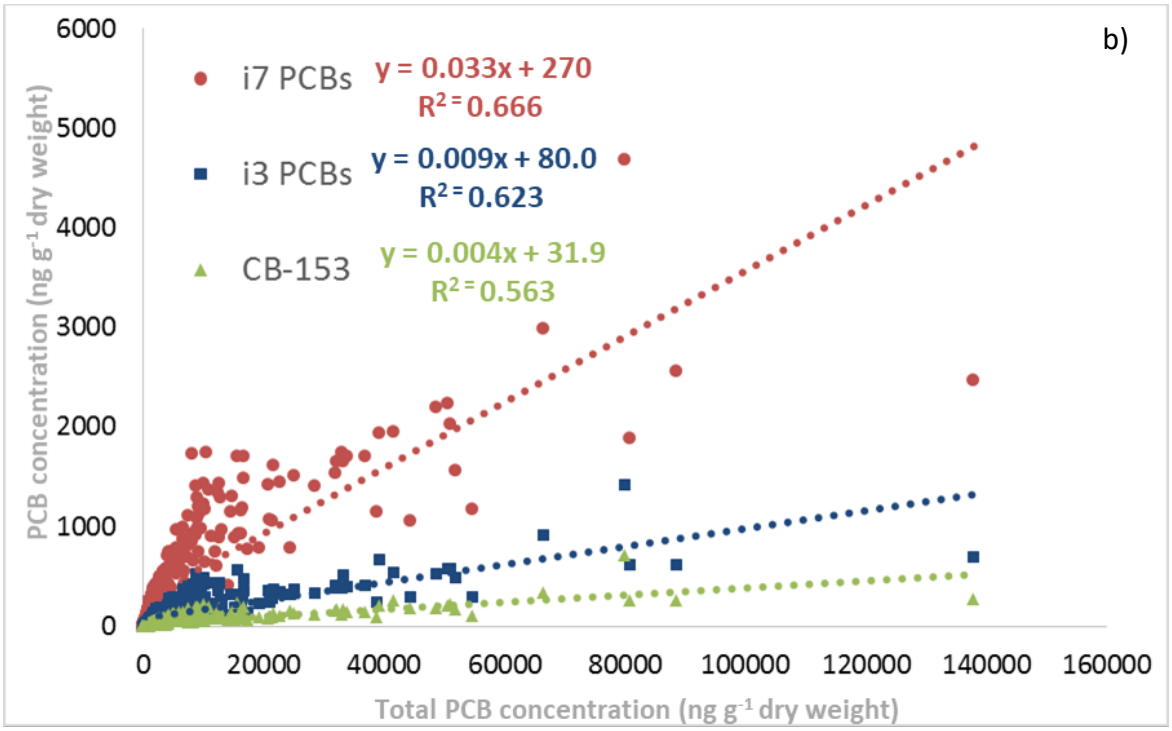

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274 **Figure 4.** Scatter plot and regression of i7, PCBs, i3 PCBs and PCB-153 against "total PCBs" for a)
275 sediment samples obtained from across Ontario (n = 374) and b) sediment samples obtained from Lake
276 Hartwell Superfund site (n = 280) (data from Magar et al. (2005))

277 The results from the background sediments in Ontario indicate that the i7 PCBs are a fairly good predictor
278 for total PCB concentrations. However, the results for the contaminated Superfund data set showed that the
279 i7 PCBs, i3 PCBs and CB-153 concentrations were very poor predictors of total PCB concentrations.
280 Unusually high concentrations of the lower chlorinated congeners were observed in the Superfund site data
281 set. The authors noted that this was due to dechlorination from microbial degradation. The net accumulation
282 of CB-1, the di-PCBs CB-4/10 & 5/8 and tri-PCBs CB-17/19 matched characteristic PCB dechlorination
283 products for Processes M, Q, and C; whereas the persistence of tetrachlorobiphenyls (TeCBs) that contained
284 24- and 25-congener groups resembled dechlorination Processes H or H'(Magar et al., 2005, Bedard and
285 Quensen III, 1995) . Detailed discussions of these and other known dechlorination processes have been
286 presented earlier by Bedard and Quensen (1995) and Johnson, et al. (2006). Many of these congeners show
287 the best R^2 value (Table S5) again indicating the dominance of a dechlorination pattern. Figure S1 shows
288 the effect of the dechlorination which is targeting the i7 PCB resulting in relative decreasing concentrations
289 of these congeners against the total. When the most dechlorinated samples were removed from the dataset
290 the R^2 value was greater than 0.95. This highlights the limitation of only using the i7 PCBs to estimate total
291 PCB concentrations: if a correction factor was to be applied to a site where PCBs were undergoing
292 dechlorination due to microbial activity then it would drastically under predict the total concentration.

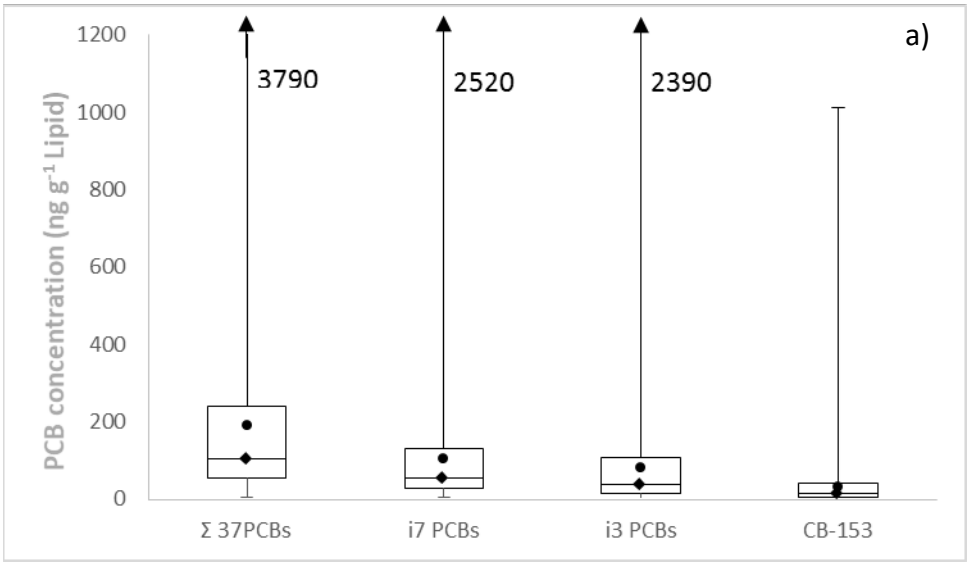
293

294 ## Human samples

295 The NHANES data contains results for approximately 2000 participants and included 37 PCBs. The i7
296 PCBs accounted for approximately 54% of the total whereas the i3 and CB-153 accounted for 37% and
297 14% respectively (Figure 5a). Although Figure 5a appears to show a large range in PCB concentrations, the
298 relative proportions of the i7 PCBs, i3 PCBs and CB-153 to the total concentration all remained relatively
299 constant and produced a coefficient of determination (R^2) of approximately 0.95 or greater (Figure 6a). The
300 coefficient of determination was calculated for each of the 37 PCBs against the total concentration and is
301 presented as Supplementary Information S6. The 5 PCBs with the best correlation were CB-153 (R^2 of

302 0.95), CB-138 (R^2 of 0.91), CB-146 (R^2 of 0.88), CB-172 (R^2 of 0.87), and CB-180 (R^2 of 0.87). This is
303 not surprising as all of these congeners contained chlorine substitution patterns (-234, -245, or -2345) on
304 one or more of the phenyl rings that were shown by Megson et al. (2013) to be resistant to biotransformation
305 and elimination in humans.

306 The NHANES data set represents a group of samples with predominantly background / low level PCB
307 contamination. The data also contains fewer PCBs than the other matrixes investigated which could explain
308 why correlations between the indicator compounds and total PCBs were so high. Data from Megson et al.
309 (2015) was used to investigate if the indicator PCBs are still good at predicting total PCB concentrations in
310 an exposed cohort in an investigation where all 209 PCBs were targeted. The coefficient of determination
311 was calculated for each of the 54 PCBs detected > LOD against the total concentration and is presented as
312 Supplementary Information S7. Figure 6b shows that the coefficient of determination for the i7 PCBs and
313 i3 PCBs was similar in the exposed cohort as it was for the NHANES data. The R^2 value for CB-153 was
314 lower for the exposed cohort; this was likely due to a subset of the cohort being exposed to a different
315 source of PCBs with elevated proportions of some less chlorinated PCBs including CB-28, CB-60, CB-66,
316 and CB-74. Gandhi et al. (2015) recommended that the use of the i3 PCBs and CB-153 to calculate total
317 PCB concentrations in fish should be avoided, however for humans they appear to be fairly good indicators
318 for basic screening assessments to estimate total PCB concentrations. The best results were achieved using
319 the i7 PCBs and the regression equation for Figure 6a&b was similar in each instance. The results indicate
320 that total PCB concentrations in humans can be estimated by multiplying the i7 PCBs by a factor of 1.75.
321 However, for more detailed assessments involving risks to human health and source tracking, the
322 determination of more congeners (preferably all 209) is strongly recommended.



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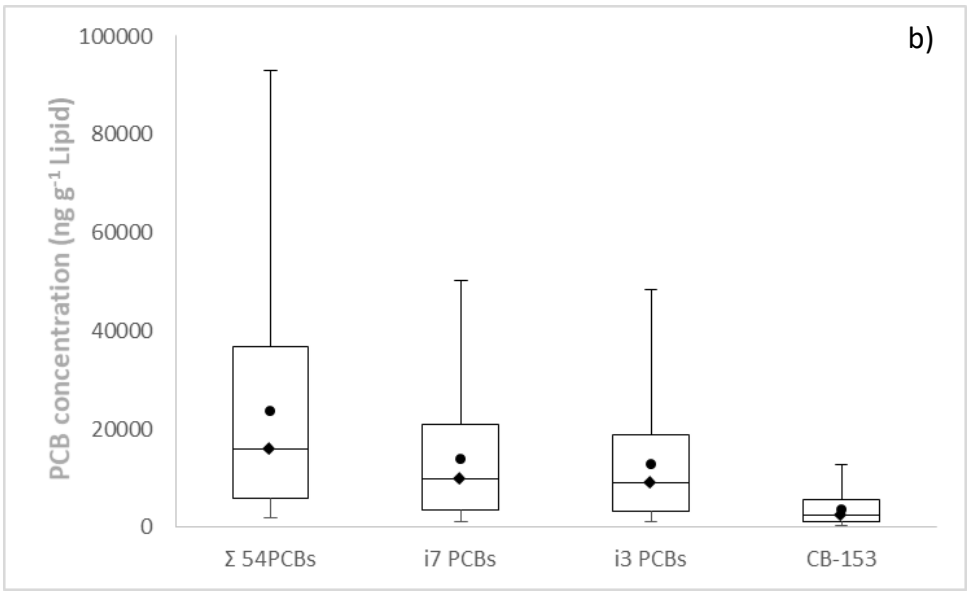

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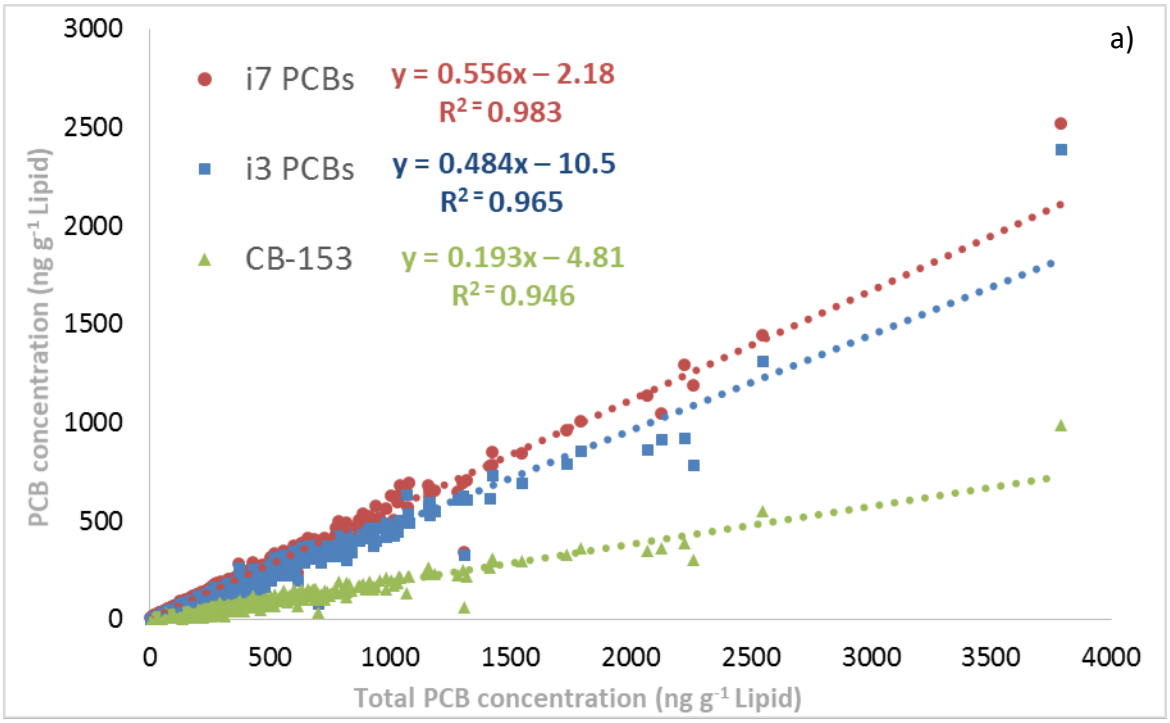
325 **Figure 5.** Box and whisker diagram of PCB concentrations in of "total PCBs", i7, PCBs, i3 PCBs and CB-
326 153 for **a**) a background cohort (n = 1900) (NHANES 2003-04 participants) (n = 1900), and b) an exposed
327 cohort (n = 30) (data from Megson et al. 2015). Dots represent the mean value and diamonds represent the
328 median, boxes represent the interquartile range and whiskers the minimum and maximum reported values



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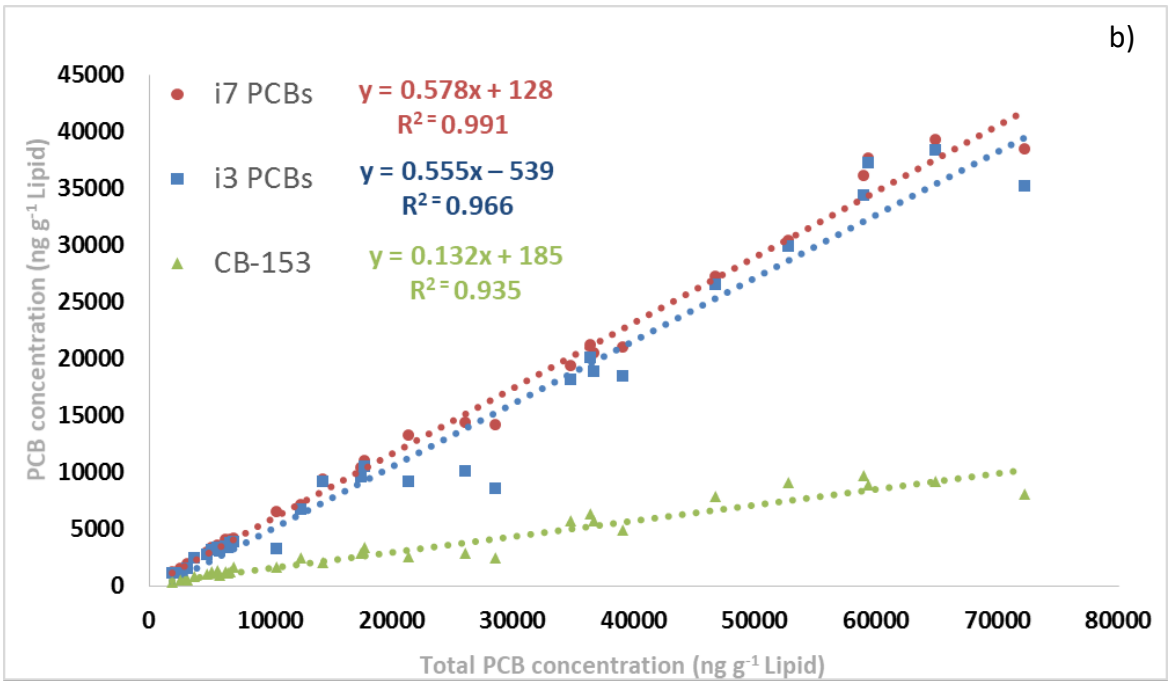


330

331 **Figure 6.** Scatter plot and regression of i7 PCBs, i3 PCBs and PCB-153 against "total PCBs" for **a**) a
332 background cohort (n = 1900) (NHANES 2003-04 participants) and **b**) an exposed cohort (n = 30) (data
333 from Megson et al. 2015)

The results of this investigation show large differences in correlation coefficients between the indicator PCBs investigated and total PCB concentrations in the different case studies. This highlights the limitations of relying on just a few indicator compounds to make informed decisions. There are larger groups of indicator compounds (e.g. (Ishikawa et al., 2007)) which can be used to provide a more reliable estimate of total PCBs and toxic equivalents. However, these are not flawless and so it is up to investigators to balance cost and time constraints against the quality and quantity of data they require.

Additional (non-Aroclor) PCB sources

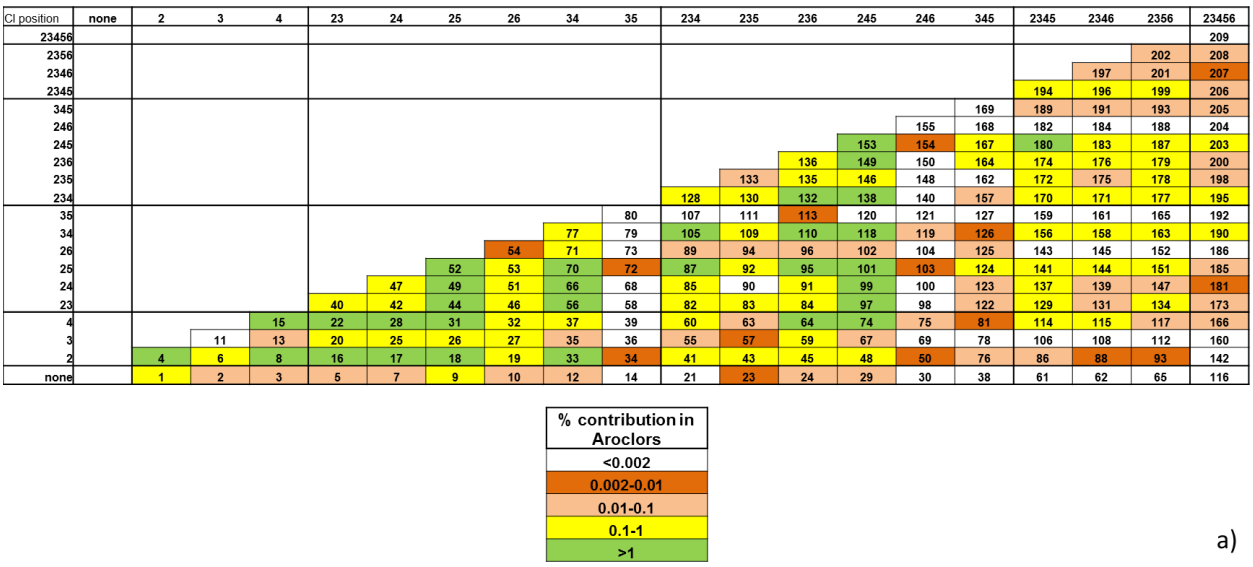
One significant limitation with the existing analytical methods that only target specific PCBs is that these methods were established to focus solely on co-planar PCBs (e.g. WHO 12) or on PCBs that are abundant in commercial PCB mixtures (e.g. i7 & i3 PCBs). Therefore, they often miss PCB contamination from non-Aroclor sources. Non-Aroclor PCBs, also referred to as non-legacy PCBs, unintentional PCBs (u-PCBs), incidental-PCBs (i-PCBs). These are PCBs that are produced inadvertently as biproducts of chemical and industrial processes and should be distinguished from secondary sources of technical mixtures (e.g. inadvertent release of PCBs from the recycling of carbonless copy paper which contained technical PCB mixtures such as Aroclor 1242). Historically, PCBs were primarily used as coolants and lubricants in electrical equipment (e.g., capacitors & transformers), however the direct production of PCBs for these or any other uses ceased in the 1970s. Since then, the importance of other sources of PCBs has increased to the extent that non-Aroclor PCBs are now considered ubiquitous contaminants in the environment (Hu et al., 2011, King et al., 2002, Hu et al., 2008, Rodenburg et al., 2010, Megson et al., 2018).

A review of the available peer-reviewed literature showed most papers focus on pigments as a source of non-legacy PCBs, with azo, phthalocyanine and titanium dioxide pigments being the main contributors. Many of these pigments are used in everyday consumer products and packaging with their impact on the environment gaining more attention. Other industrial processes that can produce unintentional PCBs include chlorinated benzenes, phenols or quinones used as solvents or reaction feeds along with the waste

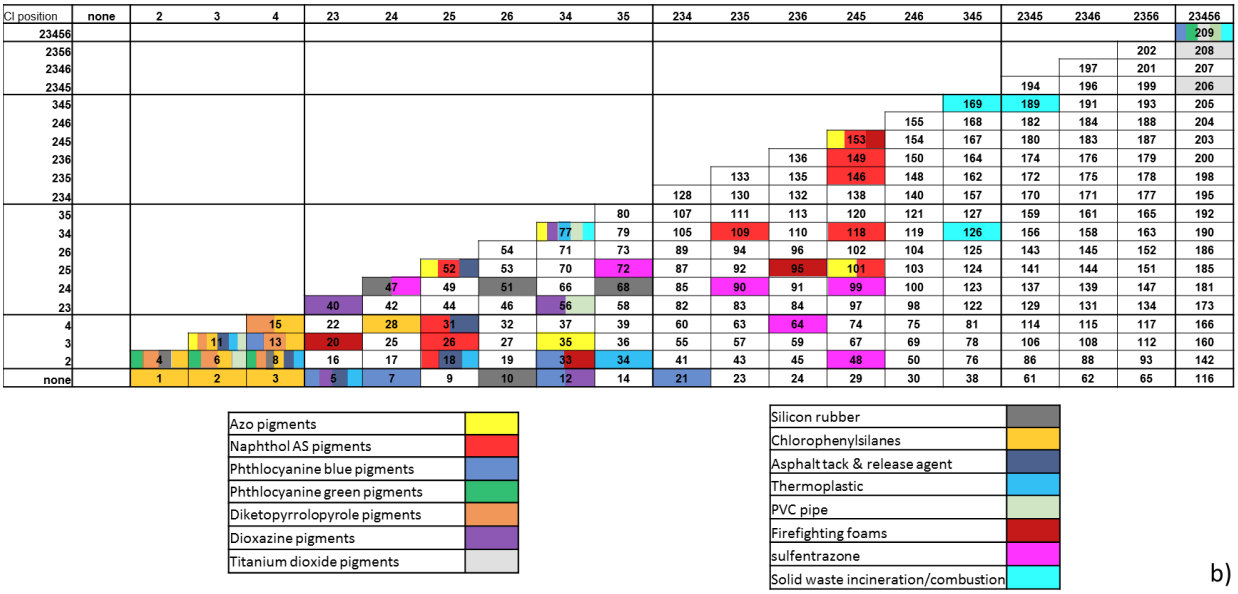
358 incineration industry. Most studies that identified non-legacy PCBs focused on the determination of either
359 PCB11 or PCB209. This is likely because these two congeners were not present in detectable concentrations
360 in many Aroclors and so their presence in environmental samples is easier to spot. There have been many
361 instances where PCB11 was found to make up a significant portion of the total PCB concentrations in
362 environmental samples (Hu and Hornbuckle, 2010, Law, 1995, Khairy et al., 2015, Rodenburg et al., 2010,
363 Du et al., 2008, Vorkamp, 2016, Hu et al., 2008, King et al., 2002, Mohrherr et al., 2012, Megson et al.,
364 2018). In several other instances PCB209 was found to contribute a significant proportion of the total PCBs
365 in environmental samples (Hu et al., 2011, Huo et al., 2017, Martinez and Hornbuckle, 2011, Martinez et
366 al., 2016). In these cases, the contamination was attributed to non Aroclor sources (Martinez and
367 Hornbuckle, 2011, Martinez et al., 2016) paint pigments (Hu et al., 2011, Martinez et al., 2016) or
368 specifically to phtalocyanine-type pigments (Huo et al., 2017). It is important to note that while Frame et
369 al. (1996) could not measure PCB 209 in any eight Aroclors he examined, it is an important congener in
370 three less common, more highly chlorinated Aroclors. PCB-209 is the dominant congener in Arolclors
371 (1270 & 1271) which were produced by Monsanto from 1938 to 1949 (Hermanson et al., 2016). It also
372 accounts for approximately 6% of Aroclor 1268(Rushneck et al., 2004) which accounted for just 0.36% of
373 Monsanto Aroclor sales from 1957 to 1974 ((Durfree et al., 1976) Johnson, et al., 2006). As such the
374 presence of PCB-209 in field studies does not necessarily confirm a non technical mixture source (e.g.
375 (Hartmann et al., 2004)).

376 Non-Aroclor sources can contain a wider range of PCBs other than just PCB11 and PCB209 thus
377 differentiating these from traditional commercial PCB sources in an environmental sample can be
378 challenging. Figure 7 presents chlorine substitution matrices to allow a comparison of which of the i7 PCBs
379 and WHO12 PCBs are present in Aroclors and a variety of non-Aroclor sources. Aroclor data was obtained
380 from (Frame, 2001) and non-Aroclor data from the following sources: pigments (Hu and Hornbuckle, 2010,
381 Shang et al., 2014, Anezaki and Nakano, 2014, Guo et al., 2014, Anezaki et al., 2015, Rodenburg et al.,
382 2015, Forstinger et al., 2015, City of Spokane, 2015, Buchta et al., 1985, Rodenburg, 2012, Vorkamp,

2016), Silicone rubber (Perdih and Jan, 1994), Anezaki et al. (2015), chlorophenylsilanes (Anezaki et al., 2015), asphalt tack and release agent, Thermoplastic, PVC pipes, firefighting foams and Sulfentrazone (City of Spokane, 2015), waste incineration (Yasuhara et al., 2003, Jiang et al., 2015, Li et al., 2017, Shin et al., 2006, Anezaki et al., 2016). There is not the same level of information on the composition of PCBs in non-Aroclor sources as there are for different technical mixtures and so figure 7b is an incomplete picture. However, it is useful as it highlights the large degree of overlap between PCBs found in technical mixtures and non-Aroclor sources.



a)



b)

Cl position	none	2	3	4	23	24	25	26	34	35	234	235	236	245	246	345	2345	2346	2356	23456
23456																				209
2356																			202	208
2346																		197	201	207
2345																	194	196	199	206
345																169	189	191	193	205
246															155	168	182	184	188	204
245														153	154	167	180	183	187	203
236													136	149	150	164	174	176	179	200
235												133	135	146	148	162	172	175	178	198
234											128	130	132	138	140	157	170	171	177	195
35										80	107	111	113	120	121	127	159	161	165	192
34									77	79	105	109	110	118	119	126	156	158	163	190
26								54	71	73	89	94	96	102	104	125	143	145	152	186
25							52	53	70	72	87	92	95	101	103	124	141	144	151	185
24						47	49	51	66	68	85	90	91	99	100	123	137	139	147	181
23					40	42	44	46	56	58	82	83	84	97	98	122	129	131	134	173
4				15	22	28	31	32	37	39	60	63	64	74	75	81	114	115	117	166
3			11	13	20	25	26	27	35	36	55	57	59	67	69	78	106	108	112	160
2		4	6	8	16	17	18	19	33	34	41	43	45	48	50	76	86	88	93	142
none		1	2	3	5	7	9	10	12	14	21	23	24	29	30	38	61	62	65	116

i7 PCBs	
WHO12 PCBs	

c)

Figure 7. Chlorine matrices illustrating a) the PCB congeners present in Aroclors and their % abundance (taken from data from Hansen 1999 based on Aroclor production) , b) the major PCB congeners present in a variety of non-Aroclor sources and c) the i7 PCBs and WHO12 PCBs

The only PCBs that were present in non-Aroclor sources but not in Aroclors were PCBs 11, 68, 90, 189 and 209. There were 40 different PCBs that were present in both Aroclor and non-Aroclor sources and differentiating these in environmental samples may prove a significant challenge. However, Bartlett et al. (2019) did attribute unusually high levels of the indicator PCB-52 in artic snow samples, to non-Aroclor sources. There are likely to be many similar instances where non-Aroclor sources of PCBs have been missed as they have been falsely attributed to a PCB technical mixture. The results highlight that while the i7 PCBs cover some of the most abundant PCBs in Aroclors they were only present in one third of the non-Aroclor sources investigated (Azo pigments – excluding those made with 3,3'-dichlorobenzidine, Naphthol AS pigments, chlorophenylsilanes, Asphalt tack & release agents, and firefighting foams). The i7 PCBs are therefore not an effective screening tool as they completely miss contributions from many non-Aroclor sources.

Conclusions

When investigating commercial mixtures, the i7 PCBs, i3 PCBs and CB-153 provide different estimates for total PCB concentrations depending on the Aroclor tested. Comparing total PCBs estimated from the

410 indicator compounds in different sources could therefore lead to significant errors. In water, the i7 PCBs,
411 i3 PCBs and CB-153 were all fairly poor indicators of total PCB concentrations in background
412 environments. Where a significant, single, relatively unaltered source of PCBs was identified, the indicator
413 compounds were good predictors. In sediment i7 PCBs, i3 PCBs and CB-153 were all poor indicators of
414 total PCB concentrations due to microbial dechlorination biotransforming the indicator compounds into
415 less common isomers. When the most heavily dechlorinated samples were removed from the dataset, there
416 was a good correlation but this was because the samples were linked to a known source of PCBs. Therefore,
417 for sediment and water samples use of indicator compounds (i7 PCBs, i3 PCBs or CB-153) to predict totals
418 is not recommended. For humans however, the i7 PCBs proved to be excellent predictors for total PCB
419 concentrations in those with both occupational and background exposure to PCBs. Good correlations were
420 also observed for the i3 PCBs and individual congeners such as CB-153, and CB-180. Due to the variation
421 in congener profiles in the different environmental samples there are no subset of indicator PCBs that can
422 be reliably used to estimate PCB totals in every sample. Cost constraints on projects will inevitably mean
423 that not all 209 PCBs will be analysed for every sample taken, however scientists and consultants who are
424 relying on datasets with a reduced number of PCBs should appreciate the limitations this causes. While the
425 use of common indicator PCBs can give unreliable estimates of total concentrations they can provide quick
426 and useful comparisons between datasets.

427 Such variations in the level of prediction of the indicator PCBs underlines the need for a larger number of
428 congeners to be analysed that represent eventual breakdown products that may improve the predictability
429 of a subset of congeners when looking at total concentrations. When assessing human health risks, the focus
430 is solely on the WHO12 PCBs but many of these studies include a basic screen for just the indicator
431 compounds. This study highlights the limitations of relying on investigations that only determine the
432 indicator PCBs alone. Under most scenarios, these compounds give a poor estimate of total PCBs
433 concentrations, and are a poor indicator to compare total PCB levels between different sources and media.
434 They are also not an appropriate tool to identify non-Aroclor sources of PCBs.

For environmental investigations aiming to quantify total PCBs, establish sources of contamination or model fate and transport, the term "more is better" still stands. The results of this assessment show that there is not one method, or group of PCBs that can accurately predict total PCB concentrations in all matrices. Environmental samples are not well served by the indicator compounds due to complexities of weathering in the environment and the presence of non-Aroclor PCB sources. While there are obvious cost implications with performing a full comprehensive congener specific analysis the quality of data generated and the ability to identify sources of PCBs is likely to be far superior. Studies relying on only a subset of congeners need to understand the limitations and not over interpret their limited datasets.

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