Hydroxylated PCBs in abiotic environmental matrices: Precipitation and surface waters

Colin Darling[1], Mehran Alaee[1], Linda Campbell[1], Grazina Pacepavicius[1], Daisuke Ueno[1], Derek Muir[1]

[1]National Water Research Institute, Burlington ON

Introduction

Hydroxylated PCBs (OH-PCBs) are of great interest environmentally because of their potential thyroidogenic effects [1,2,3]. OH-PCBs can compete with thyroxine for binding sites on transthyretin, one of the three main thyroid hormone transport proteins in mammals[1]. The chemical structures of some OH-PCBs with a para OH group and adjacent chlorine atoms, particularly 4-OH-CB109, 4-OH-CB146, and 4-OH-CB187, share a similar structure to the thyroid hormones (T3 and T4), which have a para OH with adjacent iodine atoms. A number of OH-PCBs have been identified in the blood of humans and biota during the last 5 to 10 years, however, reports on the identity, presence and levels of OH-PCBs are limited. OH-PCBs are formed biologically via cytochrome P450 mediated oxidation. Congeners with unsubstituted *meta-para*-carbons are susceptible to metabolism. Oxidation can yield a hydroxy- group by direct insertion in a *meta*-position, or via formation of an arene oxide that subsequently rearranges to a hydroxy-group. OH-PCBs are susceptible to further metabolism, i.e. conjugation reaction with glucuronic acid or sulfate, which increases the water solubility and facilitates excretion [3,4]. These phenolic conjugates can potentially be deconjugated by microbial action in waste treatment plants. All vertebrates appear to be capable of forming OH-PCBs although extent of formation in poikilotherms varies with temperature [5].

OH-PCBs can also be formed abiotically by reaction of PCBs with OH radical. Brubaker and Hites[6] found small amounts of OH-PCBs were produced on reaction of gaseous PCBs with OH radical generated by UV degradation of ozone, however, the major products of this reaction were chlorinated benzoic acids. Although reaction with OH radical is thought to be the major removal pathway for PCBs in the atmosphere[7], to our knowledge OH-PCBs have not been identified directly in atmospheric samples. OH-PCBs could also be formed in the aquatic environment by reaction of PCBs with OH radical generated by reactions of dissolved organic matter with sunlight. There are 837 possible mono-hydroxylated PCB congeners compared to 209 PCB congeners. Only 47 commercial standards of OH-PCBs are currently available. Thus identification of specific OH-PCBs in environmental samples is challenging.

This presentation describes preliminary studies on the presence of OH-PCBs in abiotic samples and comparisons of congener patterns with biological samples. We have previously shown that OH-PCBs were present in lake trout from the Great Lakes and nearby large lakes as well as in nearshore environments [8,9]. We hypothesized that some of the OH-PCB present in fish might be from abiotic formation in water or the atmosphere, or from microbial oxidation of PCBs and/or deconjugation of PCB metabolites in waste treatment plants.

Methods

Sample collection: Large volume water samples were collected from various sites in southern Ontario (Canada), including the Detroit River, Hamilton Harbour, and Toronto Harbour near the Ashbridges Bay waste treatment plant, with a Westfalia Field Centrifuge System. Suspended particulates are retained in the centrifuge bowl while the centrifuged water was collected in pre-cleaned 20-stainless steel containers. Flow rate of lake water through the centrifuge was held at 4 L/min to allow for sufficient retention time for suspended particulates in the centrifuge bowl. Suspended particulates were removed from the centrifuge bowl in the laboratory and are being held for future analyses pending method development.

Large volume snow samples were collected at various locations across southern Ontario from north of Sault Ste Marie to south of Hamilton in February 2002 using pre-cleaned aluminium boxes. The entire snow column was sampled to obtain samples representative of winter precipitation. Approximately 100L of snow meltwater or centrifuged lake water were pumped through XAD2 resin to extract OH-PCBs and other hydrophobic organic compounds. The XAD resin was spiked with ^{13}C OH-PCB standards (4-OH PCB 12 (di), 4-OH PCB 29 (tri), 4-OH PCB 61 (tetra), 4-OH PCB 120 (penta), 4-OH PCB 187) just prior to elution with methanol and dichloromethane. Recovery studies were conducted by spiking blank resin columns (N=5) with the ^{13}C standards, 10L of water was pumped through, and the resin subsequently eluted, with good recovery results. Percent recoveries averaged 98 ± 18% for the 5 standards.

Fish plasma from brown bullhead (*Ictalurus nebulosus*) were collected as part of a study of Great Lakes Areas of Concern (AOC) which focused on documenting the effects on fish and distribution of persistent organic pollutants at several highly contaminated locations in the Great Lakes [9].

OH-PCB extraction and isolation: PCBs and OH-PCBs were extracted and separated using liquid-liquid solvent extraction and pH adjustment[10]. In the case of serum approximately 2 g was denatured with concentrated HCl, and extracted with 2-propanol and a 1:1 methyl tert-butyl ether (MTBE):hexane mixture. The samples were washed with KCl, and then the pH was raised using KOH to force the OH-PCBs into an aqueous fraction, and the PCBs into the organic fraction. Concentrated sulfuric acid was added to the aqueous fraction, and this solution was then extracted with 1:1 MTBE:hexane. This extract was evaporated, derivatized using diazomethane, and then cleaned up using an acidified silica gel column. The organic fraction was also cleaned using acidified silica gel, and then put through a non-acidified silica gel column. The derivatized fractions containing methoxylated PCBs were evaporated to a final volume of 100 µL, and the organic fractions to 1 mL. XAD extracts were subjected to KOH treatment and then processed as described for serum.

Quantification by GC-High-Resolution Mass Spectrometry: GC-HRMS in electron ionization (EI) mode was selected for quantification of the methoxy-PCBs. The higher specificity of the HRMS was required to detect and quantify these lower-chlorinated biphenylols. GC-MS was carried out on a MicroMass Ultima HRMS in selected ion mode at mass resolution of 10,000, following separation on a Agilent 6890 GC using a 30 m DB5-MS column. Data files were processed using MassLynx® 4.0 software. Recoveries were calculated against a derivatized standard made up of ^{13}C-labelled 4-OH-PCB 12, 4-OH-PCB 29, 4-OH-PCB 61, 4-OH-PCB 120, and 4-OH-PCB 187. Calibration curves were generated using 46 OH-PCBs ranging from monochloro to nonachloro congeners. Peaks that matched primary and secondary ions and retention times of compounds in the standards were quantified directly using the calibration curve. Unknown peaks were quantified using a response factor (concentration/peak area), which was based on identified peaks within the same mass spectrometry function and the same homolog group (i.e. with the same number of chlorines). Parent PCBs were determined by GC-ECD as described by Campbell et al.[8].

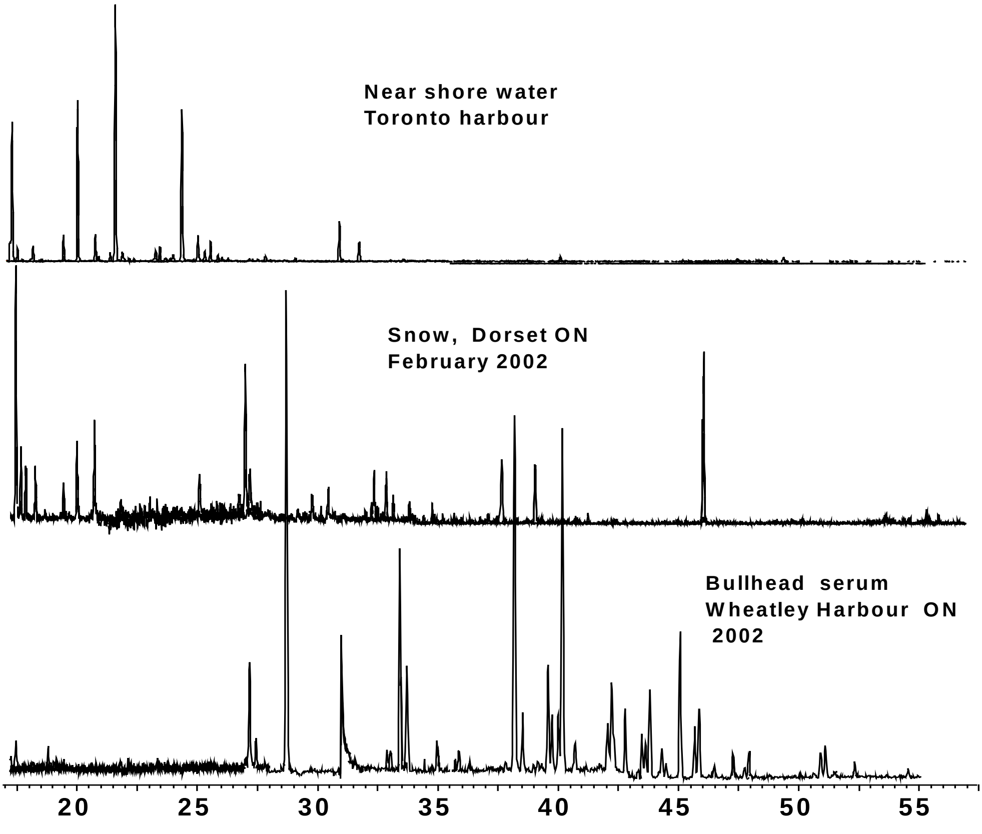


Figure 1: GC-HRMS combined selected ion chromatograms of monochloro- to nonachloro-(methoxy)-PCBs in water from Toronto harbour, snow from a boreal forest site, and fish serum.

Results and Discussion

GC-HRMS analysis of the snow, surface water and fish extracts revealed a large number of OH-PCBs, ranging from mono- to nonachloro- as illustrated in Figure 1. Surface water collected in

Toronto Harbour near a municipal sewage treatment outfall contained mainly mono- and dichloro-OH-PCBs, most of which could not be structurally identified with the standards available. A more detailed chromatogram of the dichloro-congeners in near shore surface water sample is shown in Figure 2. Only 2 congeners could be identified by retention time, while at least 14 were detectable. No penta-, hexa-, hepta or octachloro-OH-PCBs were detectable (< 0.1 pg/L) in the surface water. Blanks contained very low levels of methylated dichloro OH-PCBs (Figure 2).

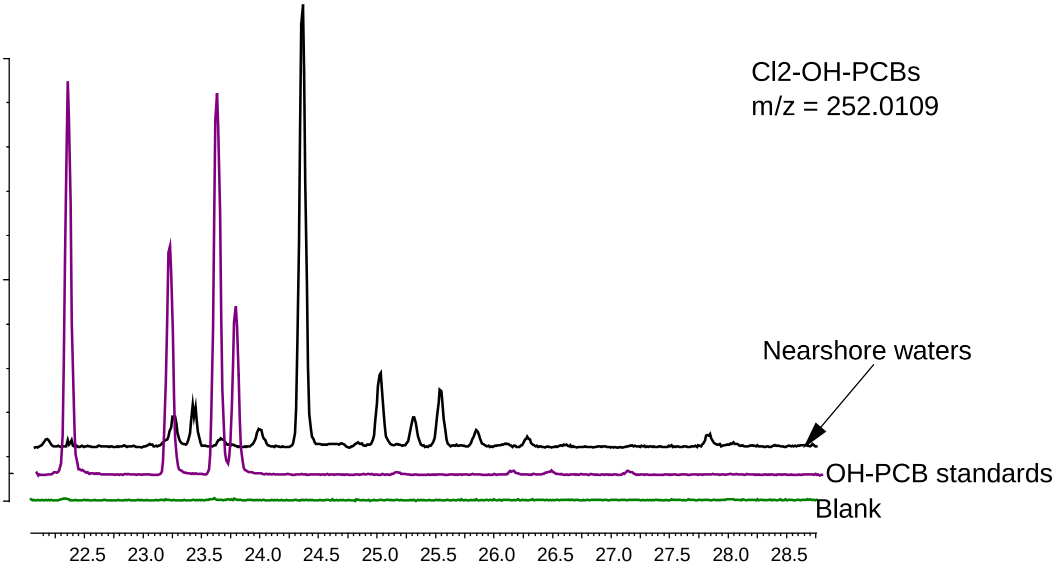


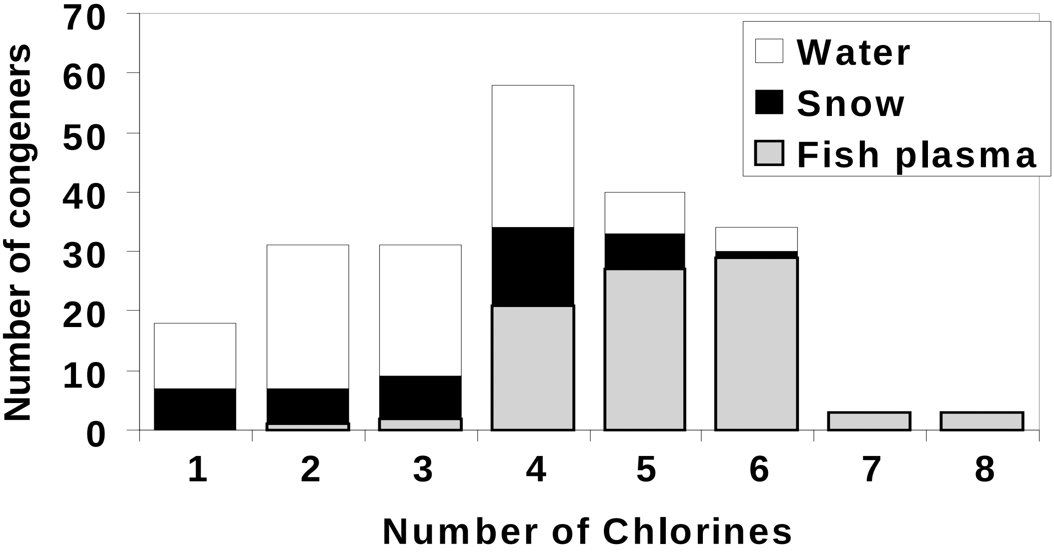
Figure 2: GC-HRMS ion chromatograms of dichloro-methoxy PCBs (m/z 252.0109) in surface water collected in Toronto Harbour near a municipal sewage treatment combined with methylated OH-PCB standards and a blank all run under the same conditions

Snow extracts had several of the dichloro-OH-PCBs that were detected in surface water, however, the extracts also contained sub-pg/L concentrations of several tri- to heptachloro- congeners. Hydroxy-PCB substituted at both the 2-, 3- and 4-positions were detected (e.g. 2-OH-PCB 69, 4-OH-PCB 69, 3-OH-PCB 65, 2-OH-PCB 112). Lab blanks processed with the snow samples contained several higher chlorinated congeners, e.g. 4-OH-PCB 187 and 4-OH-PCB 159. The source of these contaminants is unclear. Total PCB levels is the snow samples ranged from 0.1-4 ng/L range similar to previous reports for the Great Lakes basin [11] while total OH-PCBs ranged from 1-7 pg/L. Thus the OH-PCBs appear to present only as a minor contaminant compared to the parent compounds.

In contrast to the water and snow samples, brown bullhead plasma contained mainly hexa- to nonachloro congeners particularly, 4-OH-PCB 130, 3-OH-PCB 138, 4-OH-PCB 146, 4-OH-PCB 172, 3-OH-PCB 180, and 4-OH-PCB 187, as previously reported for Great Lakes fishes by Campbell et al. [8] and Li et al. [12]. Hexachlorobiphenylols were the dominant homolog group in all bullheads. No mono or dichloro- OHPCBs were found in any fish analysed.

This study has shown that there are numerous OH-PCB congeners present in environmental samples at low concentrations. The number of congeners detected in surface waters, snow and in fish plasma is shown in Figure 3. We can positively identify only a fraction of the congeners because of a lack of commercially available standards. The use of ^{13}C-labelled congeners can be used to quantify, but not definitively identify, unknown congeners. Nevertheless, these preliminary results suggest that the highly chlorinated OH-PCB congeners that are detectable in Great Lakes fishes are formed from metabolism of PCBs by the fish. Indeed, recent work by Buckman et al [5] has shown that rainbow trout can produce a wide range of hydroxylated PCBs although amounts of the metabolites in plasma are dependent on water temperature. Uptake from water and from prey may also occur. However, fish may be able to readily metabolize and excrete the lower chlorinated biphenylols.

Figure 3: Number of individual OH-PCB congeners detected by GC0HRMS in surface water from Toronto Harbour, snow from a boreal forest site in central Ontario and in brown bullhead plasma.



The results also suggest that OH-PCBs are being formed in the atmosphere and are also entering aquatic environments from waste treatment plants, albeit at low concentrations. Further work is needed to understand the sources, trends and environmental cycling of these compounds. In the case of waste treatment plants, OH-PCBs could be formed from microbial oxidation of PCB entering the plants or from reactions with OH radical if ozone is used to treat the final effluent. Lower chlorinated congeners are more likely to be in the dissolved phase and thus available for oxidation. The possibility that these mono- and dichloro-biphenylols are impurities in other industrial compounds also cannot be ruled out.

Acknowledgements

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