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Identification of 4-Hydroxyheptachlorostyrene in Polar Bear Plasma and Its Binding Affinity to Transthyretin: A Metabolite of Octachlorostyrene?

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A new compound, 4-hydroxyheptachlorostyrene (4-OH-HpCS), was identified as a major component in the chlorinated phenolic compound fraction of polar bear plasma. The structure was hypothesized to be 4-OH-HpCS based on mass spectral interpretation, the assumption that it was a metabolite of octachlorostyrene, and the similarity of the structure to hydroxylated polychlorinated biphenyls (OH-PCBs) identified in plasma. High-resolution, electron impact (EI) ionization mass spectrometry of the methylated compound indicated a molecular formula of $C_9H_3OCl_7$ and major fragment ions of $[M - 15]^+$, $[M - 35]^+$, and $[M - 43]^+$, which was a mass spectral pattern identical to a synthesized and methylated 4-OH-HpCS standard. The identity was further confirmed by matching gas chromatography (GC) retention times on three different GC columns of differing polarity. Levels of 4-OH-HpCS ranged from 2.89 to 22.9 ng/g wet weight in polar bear plasma ($N = 30$) and constituted between 3.8 and 24.8% of the total quantified level of chlorinated phenolic compounds. The mean ratio of 4-OH-HpCS to CB153 concentrations in polar bear plasma samples was 0.712 (± 0.580 SD), which suggests selective retention of the 4-OH-HpCS in plasma. The presumed mechanism of retention involves 4-OH-HpCS binding to transthyretin (TTR). The presence of TTR was confirmed for the first time in polar bear plasma by binding of ^{125}I-thyroxine (T_4), the natural ligand of TTR, to separated plasma proteins. The binding affinity of 4-OH-HpCS to human TTR was tested and found to be 1.1 relative to T_4. This suggests that 4-OH-HpCS has the potential to disrupt T_4 and retinol transport, by analogy to OH-PCBs with similar structure. Metabolism of octachlorostyrene (OCS) is the most likely source of 4-OH-HpCS. OCS was shown to be present at low concentrations in polar bear tissues as well as in plasma of ringed seal, the principal prey species of polar bears. The ratio of 4-OH-HpCS to OCS and 4-OH-HpCS to CB153 concentrations were 150- and 44-fold higher in polar bear plasma than in ringed seal plasma. This study indicates that the phenolic metabolites of relatively minor contaminants possess the capacity to bind to circulating proteins, and their significance as potential endocrine-disrupting agents may be underestimated.

Introduction

Polyhalogenated aromatic hydrocarbons (PHAHs) have been studied extensively due to their ubiquitous presence in biota. Phenolic metabolites of PHAHs isolated from plasma have received particular interest (*1*). Phenolic compounds are capable of modulating hormone-mediated processes in vitro and in vivo, via processes dependent on the estrogen receptor (*2*), and the disruption of thyroid hormone (*3*) and vitamin A transport (*4*). For example, OH-PCBs, phenolic metabolites formed in wildlife and humans and retained in plasma (*5, 6*), have demonstrated potential estrogenic activity (*7*) but especially thyroidogenic effects in vitro and in vivo (*8*).

The prohormone, T_4, is transported to target tissues by thyroid hormone transport proteins, such as TTR. The deiodinase family of enzymes present in these target tissues converts T_4 to the active hormone, triiodothyronine (T_3) (*9*). Aromatic compounds that have OH-groups with adjacent halogen substituents can bind with high affinity to TTR (*8*). The binding potency of pentachlorophenol (PCP) and 4'-OH-3,3',4,5'-tetrachlorobiphenyl toward TTR was 2 and 4 times greater, respectively, than the natural hormone, T_4 (*10,11*). Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) with T_3- and T_4-like structures (i.e. bromine atoms adjacent to the phenolic OH-group) have been reported to bind to the human α- and β-thyroid hormone receptor (*12*) and compete with T_4 for binding with TTR in vitro (*13*).

Biotransformation has been shown to generate other phenolic compounds that may be capable of binding to TTR. In rats, hexachlorobenzene is biotransformed in vivo to PCP (*14*), and OCS is converted to a heptachloro metabolite and a pentachlorophenyldichloroacetic acid metabolite (*15*). The presence of a heptachloro metabolite suggests that the pathway for biotransformation of OCS may be via dechlorination and possibly hydroxylation.

Polar bears are the top predators in the arctic marine food chain, eating primarily ringed seal. Consequently, they bioaccumulate high concentrations of several PHAHs, most notably polychlorianted biphenyls (PCBs) and methylsulfonyl-PCBs (*16−18*). Thus, polar bears are an important biomonitoring species of PHAH exposure in the arctic (*17*).

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The high body burden of contaminants can induce enzymes of the hepatic cytochrome P450 system, which are responsible for the metabolism of both endogenous compounds and xenobiotics (*19*). The polar bear has demonstrated a high capability to metabolize several PHAHs, including 4,4′-DDE and many PCB congeners (*20*). It is reasonable to suppose that persistent OH-PCB metabolites would be formed in polar bear, as has been found for other mammals (*6*) and humans (*5*). Therefore, a study was initiated to examine the presence and identity of OH-PCBs in polar bear plasma and their possible effects on circulating vitamin A and thyroid hormone levels. The results of this study will be reported elsewhere.

During methodological development for the extraction and quantification of OH-PCBs, an unknown phenolic compound was detected in polar bear plasma (*21*). The unknown compound was frequently the major peak in the methylated phenolic compound fraction chromatogram.

We report on the identification of the unknown phenolic compound in polar bear plasma, which we hypothesized to be a hydroxylated metabolite of OCS based on preliminary mass spectral evidence. The identification involved the bulk extraction of polar bear plasma and purification of the unknown compound for mass spectral analysis using high and low-resolution mass spectrometry. The hypothesized compound was then synthesized. Comparisons of the mass spectra and gas chromatographic retention times on three GC columns with varying polarities were used to determine if the synthesized compound was the hypothesized phenolic OCS metabolite. To attempt to resolve the significance of biotransformation as a source of this phenolic compound in the polar bear, its concentration and the concentration of OH-PCBs, OCS, and PCBs were determined in polar bear and ringed seal plasma. OCS and PCBs in polar bear adipose tissue and liver were also quantitated. The presence of TTR in polar bear plasma was determined by polyacrylamide gel electrophoresis (PAGE) separation of proteins and protein binding of ^{125}I-labeled T_4. High affinity binding to TTR is the presumed mechanism of selective retention of phenolic compounds in plasma (*8*). A competitive binding assay using purified human TTR and ^{125}I-labeled T_4 was used to study the TTR binding affinity of the phenolic OCS metabolite relative to the natural thyroid hormone, T_4.

Materials and Methods

Samples. Polar bear plasma samples used in this study were collected from 30 polar bears captured between April and May 1997 by Dr. Malcolm Ramsay (University of Saskatchewan, Saskatoon, SA) around the Resolute Bay area (Nunavut Territory, Canada). Whole blood was drawn into 50 mL heparinized vacutainers and stored on ice and out of light until processed. Blood samples were centrifuged, and the plasma was drawn off, frozen at −40 °C, and stored until further analysis.

Plasma samples ($N = 5$) from ringed seal (age and gender unknown), the main prey species of the polar bear, were similarly collected in 1999 from around Kuujjuaq, Québec by Inuit hunters (Anguvigaq Hunting, Trapping and Fishing Association, Kuujjuaq, Québec) and organized by Daniel Leclair (Nunavik Research Centre, Makivik Corporation, Kuujjuaq, Québec). The plasma samples were stored frozen at −40 °C until analysis.

Polar bear adipose tissue and liver samples ($N = 8$) were collected by Dr. Malcolm Ramsay (University of Saskatchewan, Saskatoon, SA) in legally controlled hunts by Inuit in the Canadian Arctic during the spring of 1992, 1993, and 1994 near Resolute Bay, Nunavut Territory. Details of the sampling and sample preparation are given by Letcher et al. (*19, 20*).

Contaminant Analysis. Prior to extraction, polar bear and ringed seal plasma samples were spiked with the following mixture of $^{13}C_{12}$-labeled OH-PCBs standards acquired from Wellington Laboratories (Guelph, ON, Canada): 4'-OH-2,3',4,5,5'-pentachlorobiphenyl, 4'-OH-2,3,3',4,5,5'-hexachlorobiphenyl, 4'-OH-2,2',3,3',4,5,5'-heptachlorobiphenyl, and 4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl (20 μL at 100 pg/μL). PCP ($^{13}C_6$) (20 μL at 100 pg/μL) and $^{13}C_{12}$-CB28, 52, 118, 153, 180, and 194 (10 μL at 2.0 ng/μL) (Cambridge Isotope Laboratories, Andover, MA) were also added to the plasma samples. The ^{13}C-labeled standards served as recovery standards.

The plasma samples were extracted using the method described by Hovander et al. (*22*) as modified by Sandau et al. (*5*). A performance standard, 4'-Me-4-MeO-2,3,3',5,6-pentachlorobiphenyl (10 μL at 1.0 ng/μL), was added to all phenolic fractions, and $^{13}C_{12}$-CB138 (10 μL at 4.0 ng/μL) was added to all neutral fractions prior to mass spectral analysis. The final volume of both fractions was 200 μL.

OH-PCBs and PCP were quantified by GC-electron capture negative ionization mass spectrometry (ECNI-MS) employing authentic standards when available or by using group response factors for unidentified compounds as described previously (*5*). PCBs were quantitated by GC-electron impact ionization mass spectrometry (EI-MS) using a characterized secondary polar bear quantitation standard (PBQ) (*17*). Concentrations of 4-OH-HpCS were determined separately by GC equipped with an electron capture detector (ECD) using a calibration curve of authentic standard and a response factor relative to the performance standard.

The three types of GC columns used in this study were as follows: DB-5 ([5%-phenyl]methylpolysiloxane from J&W Scientific Inc., Folsom, CA, 30 m × 0.25 mm i.d., 0.25 μm film thickness), DB-1701 ([14% cyanopropylphenyl]methylpolysiloxane from J&W Scientific Inc., Folsom, CA, 30 m × 0.25 mm i.d., 0.25 μm film thickness), and Rtx-2330 ([90% biscyanopropyl−10% cyanopropylphenyl]polysiloxane from Restek Corporation, Bellefonte, PA, 30 m × 0.25 mm i.d., 0.20 μm film thickness).

GC-ECD was performed on a Hewlett-Packard (Palo Alto, CA) 5890 instrument equipped with an HP7673A automatic injector, DB-5 column, and a ^{63}Ni ECD detector. The carrier gas was helium set with a head pressure of 80 kPa, and the ECD makeup gas was 5% methane−95% argon. All injections were 2 μL in volume and made in splitless mode. The GC temperature program was as follows: 80 °C for 2 min, ramp 10 °C/min to 250 °C hold for 5 min, then 5 °C/min to 300 °C. The injector port and interface temperatures were 250 and 280 °C, respectively.

GC-MS (low-resolution fullscan) analysis was performed with a Hewlett-Packard 5987B instrument. For fullscan ECNI mode, the GC and MS conditions are described in detail elsewhere (*5*). Methane (99.99% pure) was the reagent gas at a source pressure of 2.5×10^{-4} Torr. The source temperature was 140 °C. For EI mode, the source temperature was set at 120 °C and the electron energy was 70 eV. Fullscan spectra for the ECNI and EI modes were obtained from 75 to 650 and 250 to 600 amu, respectively.

High-resolution spectra were obtained with a VG AutoSpec double-focusing mass spectrometer (resolution 10 000) in fullscan EI mode. The instrumental conditions are described in Tittlemier et al. (*23*).

Isolation and Mass Spectral Characterization of the Unknown Chlorinated Phenolic Compound. To obtain clean fullscan spectra, a large pooled sample of polar bear plasma (~65 g) from three bears was prepared. The plasma was extracted, and the phenolic compounds were isolated as described above. The OH-containing compounds were methylated to their corresponding methoxy-derivatives by treatment with diazomethane (*24*). After further clean up steps, which included gel permeation chromatography,

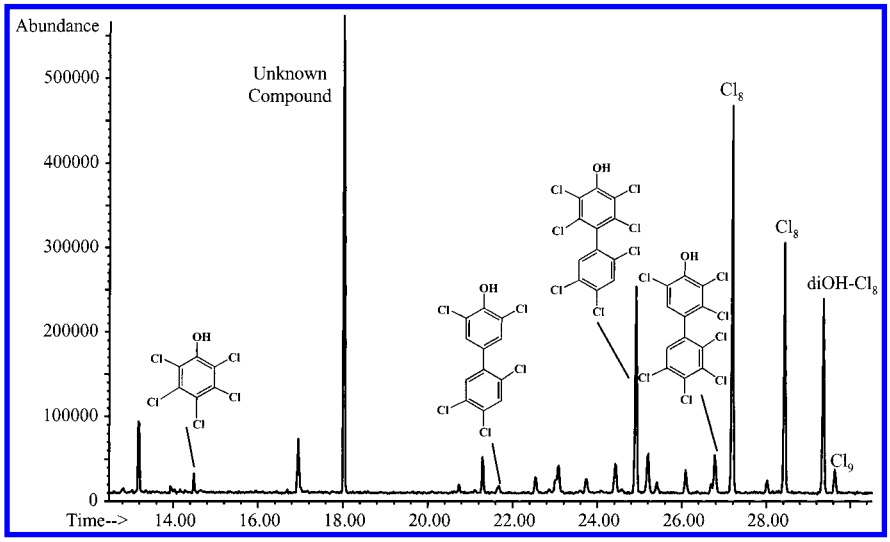


FIGURE 1. Electron-capture negative ionization mass spectra (fullscan) chromatogram of the phenolic compound fraction from polar bear plasma. The compounds have been methylated but are shown as hydroxylated precursors.

multiple sulfuric acid partitioning (*22*), silica:sulfuric acid column treatment (*5*), and florisil column chromatography (*25*), the methylated unknown compound was isolated by applying the methylated fraction to a florisil column (8.0 g, 1.2% deactivated) and eluting with hexanes. Fractions of 5 mL were collected and analyzed for the presence of the unknown compound using GC-ECD as described above. Fractions from 10 to 25 mL contained the majority of the unknown compound. These were pooled and concentrated for analysis using both high- and low-resolution fullscan mass spectrometry.

The methylated phenolic compound fraction was initially analyzed using GC/ECNI-MS because of interfering biogenic residue still present following the initial extraction procedure (*5*). Interferences were minimized using ECNI since biogenic compounds generally have low electron-capturing capability compared to halogenated compounds. A GC/ECNI-MS chromatogram of the methylated phenolic compound fraction of a polar bear plasma sample is shown in Figure 1. In addition to several OH-PCB congeners, there was a large unknown compound eluting nearly 3.5 min after PCP and about 3.5 min before the first eluting OH-PCB. The retention time indicated that the compound could either be a chlorinated monocyclic aromatic compound with a side chain or a lower chlorinated OH-PCB.

The ECNI spectrum of the methylated unknown phenolic compound is shown in Figure 2a. The base peak at 322 amu had an isotope pattern indicative of a hexachlorinated species. There was only one detectable isotope cluster at higher mass (338 amu). The 338 amu isotope cluster was also characteristic of a compound or fragment with six chlorines and must contain a methoxy group because only methylated phenolic compounds are present in this fraction. The formula best matching this fragment was $[C_9H_4Cl_6O]^-$, suggesting that the unknown compound was most likely a monocyclic aromatic compound with a side chain. Although this formula was consistent with a methoxyhexachlorostyrene (MeO-HxCS), loss of $[M-16]^-$ to form the fragment at 322 amu was unlikely even though addition of $H^\bullet$ (i.e. $[M-CH_3+H]^-$) to molecular and fragment ions frequently occurs in ECNI-MS (*26*). Therefore, MeO-heptachlorostyrene (MeO-HpCS, no molecular ion) or MeO-hexachlorostyrene (MeO-HxCS) isomers were consistent with the ECNI spectrum. No significant low-mass fragments were detected, and no other significant structural information could be inferred from the ECNI spectra except fragment ions corresponding to the successive loss of chlorines.

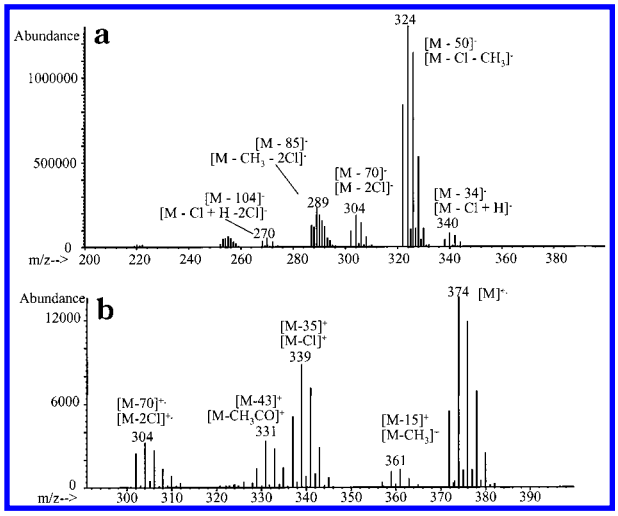


FIGURE 2. Low resolution (A) electron-capture negative ionization (200−400 amu) and (B) electron impact (290−400 amu) ionization mass spectra of the unknown methylated phenolic compound isolated from polar bear plasma (see Figure 1).

The molecular ion of the unknown phenolic compound was discovered by EI-MS. The low-resolution EI spectrum indicated that the molecular ion was a heptachlorinated species (Figure 2b). The $[M]^+$ of 372 amu corresponded to $[C_9H_3Cl_7O]^+$, which was most consistent with a MeO-HpCS isomer. In Figure 2, fragments are labeled for loss of mass units and their corresponding structures based on this assignment. The EI spectrum showed the distinctive loss of a methyl group, a chlorine, and an $[M-43]^+$ ion, which represented an $[M-CH_3CO]^+$ fragment ion. All these fragmentation patterns have been observed previously for MeO-PCBs (*27*). The $[M-CH_3CO]^+$ fragment is indicative of a para-substituted, aromatic compound when accompanied by an $[M-CH_3]^+$ fragment (*28*). The fragmentation is thought to entail a two-step process—the loss of a methyl radical followed by the loss of carbon monoxide and subsequent formation of a nonaromatic cyclopentadienyl ring. The formation of a $[M-43]^+$ ion was demonstrated previously for polymethoxybiphenyls (*29*). Para-substitution of the aromatic ring was the only molecular structure that allows for charge delocalization to the ethylene side chain and electronic stabilization of the proposed $[M-CH_3]^+$ fragment ion. The $[M-CH_3CO]^+$ fragment is stabilized by charge delocalization through conjugation of the cyclopen-

TABLE 1. Elemental Compositions Derived from High Resolution EI-MS of the Four Major Ions (See Figure 2) of the Methylated Unknown Phenolic Compound and the Methylated 4-OH-HpCS Synthesized Standard[a]

fragment	most probable elemental composition	sample			standard	
		theoretical mass	exptl mass	difference	exptl mass	difference
$M^{\bullet+}$	$C_9H_3OCl_7$	371.8004	371.7999	0.0005	371.7976	0.0027
$[M - 15]^+$	C_8OCl_7	356.7769	356.7802	−0.0033	356.7762	0.0007
$[M - 35]^+$	$C_9H_3OCl_6$	336.8315	336.8323	−0.0008	336.8303	0.0012
$[M - 43]^+$	C_7Cl_7	328.7833	328.7863	−0.0029	328.7820	0.0014

[a] The sample and standard were analyzed at a resolution of 10 000.

tadienyl group to the ethylene side chain and may be further stabilized by forming a heptachlorotropylium cation.

The elemental composition of the molecular ion and the first four major ion clusters in the EI spectrum (Figure 2b) were confirmed using high-resolution EI-MS. The most probable elemental compositions were calculated by software provided as part of the OPUS operating system of the VG AutoSpec using the technique described by Tittlemier et al. (*23*). The exact mass of the molecular ion and fragments of the unknown compound were compared relative to the most probable theoretical compositions (Table 1). The mass differences between the theoretical masses and those of the unknown compound agreed to within 3‰ or better, confirming that the molecular formula was $C_9H_3OCl_7$, and that the first three fragment ions in the EI spectrum resulted from a loss of CH_3, Cl, and CH_3CO.

The molecular formula is indicative of a compound with five units of unsaturation. In conjunction with the low molecular weight, this suggests a chlorinated monocyclic aromatic compound. A benzene ring accounts for four units of unsaturation, leaving the fifth unit of unsaturation to reside in a side chain double bond. The molecule has seven chlorine atoms and must contain a methoxy substituent. Thus, the most likely structure is a MeO-HpCS isomer. The *para* position is the most likely position on the phenyl ring for a hydroxyl group according to the mass spectrometry data. This position is also the most common location for the OH-group in OH-PCB compounds retained in plasma (*1, 5, 6*).

Synthesis of 4-Hydroxyheptachlorostyrene and Confirmation of Unknown Compound Identity. Based on the mass spectral evidence, it was probable that the unknown compound was 4-OH-HpCS, which has not been reported previously in the literature. Therefore, synthesis of 4-OH-HpCS was carried out.

N-Butyllithium in tetrahydrofuran was reacted with 2,3,5,6-tetrachloroanisole at −78 °C to generate the 4-methoxy-2,3,5,6-tetrachlorophenyllithium. This was reacted immediately with chloral and allowed to warm to room temperature to generate the 4-methoxyheptachlorostyrene oxide. The yield for the reaction was approximately 31% after preparative thin-layer chromatography (TLC, silica gel, 25% dichloromethane/hexanes) which was applied to remove the remaining tetrachloroanisole and a pentachloroanisole impurity. The 4-methoxyheptachlorostyrene oxide was treated with excess phosphorus pentachloride (1.5−2.0 equiv) in dichloromethane and allowed to react overnight at room temperature. The products of the reaction included 4-(1,2,2,2-tetrachloroethyl)-2,3,5,6-tetrachloroanisole and MeO-HxCS. This mixture was heated for 4 h on a steam bath with 1 equiv of 1,5-diaza[5.4.0]undecane in acetonitrile to give 4-MeO-HpCS and two other impurities. The MeO-HxCS was no longer detected. 4-MeO-HpCS was isolated by TLC and demethylated by refluxing with excess boron tribromide (6−7 equiv, 1 M in dichloromethane) in 1,2-dichloroethane for 20 h. The resulting compound, 4-OH-HpCS, was separated from unreacted 4-MeO-HpCS using preparative TLC. The final purity of 4-OH-HpCS was greater than 99%.

TABLE 2. Retention Times of 4-OH-HpCS on Three Different GC Columns Relative to the Retention Time of 4-OH-2,2',3,4',5,5',6-Heptachlorobiphenyl, One of the Major OH-PCBs in Polar Bear Plasma (See Figure 1)

GC column	relative retention time		
	standard	sample	difference
DB-5	0.7239	0.7245	−0.0006
DB-1701	0.6075	0.6079	−0.0004
Rtx-2330	0.5914	0.5923	−0.0009

Low-resolution EI and ECNI spectra for the 4-OH-HpCS were identical to those determined in the polar bear plasma samples (Figure 2). Furthermore, the exact mass assignments agreed with the unknown compound within 3‰ amu (Table 1). The retention time of the standard and the unknown compound were then compared relative to the retention time of a major OH-PCB in polar bear plasma (4-OH-2,2',3,4',5,5',6-heptachlorobiphenyl) on three different GC columns of varying polarity. The relative retention time of the standard and compound present in the samples matched within experimental error on all three columns (Table 2).

The unknown phenolic compound in polar bear plasma was therefore confirmed to be 4-OH-HpCS.

Transthyretin (TTR) Determination in Polar Bear Plasma and Binding Affinity of 4-OH-HpCS to Human TTR. TTR is a thyroid transport protein that is highly conserved among most mammals, birds, and some reptiles (*30*). To confirm that polar bears possess TTR, polar bear plasma proteins ($N = 4$) were separated by PAGE, as described previously by Brouwer and van den Berg (*4*). Determination of $^{125}I-T_4$-competitive binding to specific proteins was performed as described by Lans et al. (*31*) and Darnerud et al. (*32*). Plotting $^{125}I-T_4$-radioactivity against migration distance on the gel made the PAGE gel profile shown in Figure 3.

Three peaks showing TTR-bound radioactivity were identified in polar bear plasma after PAGE (Figure 3). Identification of the peaks was based on comigration of the reference proteins and by comparing R_f-values (i.e. the position of a protein on the gel (in mm) divided by the position of the front of the gel (in mm)) of the peaks containing radioactivity with R_f-values of reference samples. R_f-values of the two peaks containing radioactivity ($R_f = 0.45-0.49$ and $0.62-0.63$) are in accordance with R_f-values of the reference proteins, bovine serum albumin ($R_f = 0.41-0.42$) and human TTR (0.59−0.61), respectively. Thus, polar bears likely possess TTR. The last peak in Figure 3 represents free T_4. As with other mammals, albumin is also present in polar bear plasma, and binding of T_4 with albumin is higher than with TTR.

OH-PCBs have been shown to bind with high affinity to TTR and not other thyroid hormone transport proteins, such as thyroxine binding globulin (*11, 33*). Most OH-PCB congeners detected in plasma have an OH-group in the *para*-position relative to the phenyl−phenyl bond and have adjacent chlorines in both meta-positions (*1, 5, 6*). Since 4-OH-HpCS is structurally similar to other chlorinated

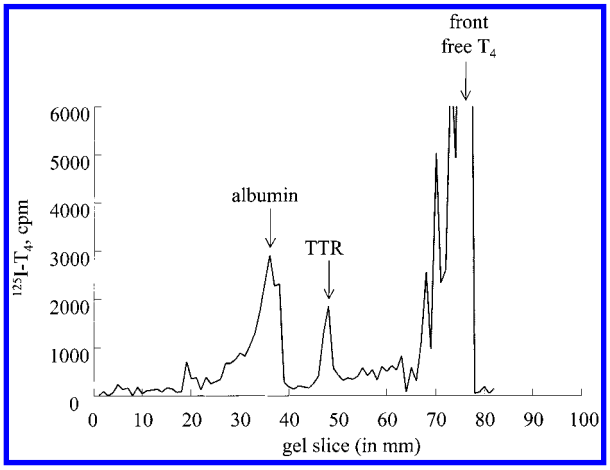


FIGURE 3. Separation by polyacrylamide gel electrophoresis (PAGE) and radioactive detection of proteins in polar bear plasma after incubation with ^{125}I-thyroxine (T_4).

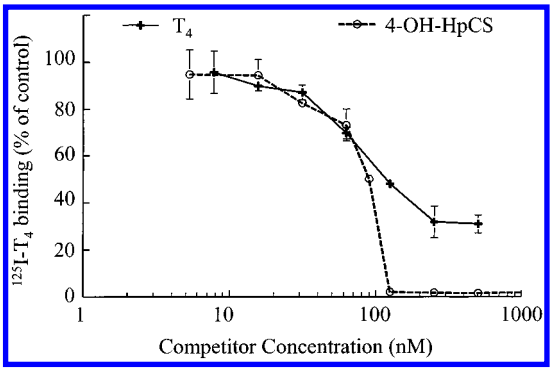


FIGURE 4. The concentration-dependent, competitive binding of 4-hydroxyheptachlorostyrene (4-OH-HpCS) and thyroxine (T_4) relative to ^{125}I−T_4 for human transthyretin (TTR). Assays were performed in duplicate.

phenolics that bind with high affinity to TTR and TTR was found in polar bear plasma, it was of interest to determine the relative binding affinity of 4-OH-HpCS and T_4 to TTR.

The assay for determining competitive binding of 4-OH-HpCS and ^{125}I-labeled T_4 to human TTR was performed as described elsewhere (*31*). Concentration-dependent, competitive binding curves of T_4 and 4-OH-HpCS relative to ^{125}I−T_4 (% of control) for TTR protein are shown in Figure 4.

IC_{50} values (concentration of competitor at half-maximal specific binding) and relative binding affinities (RBA) were calculated as described by Meerts et al. (*13*). RBA was calculated by dividing the IC_{50} of T_4 by the IC_{50} of 4-OH-HpCS. The competitive binding assay was done in duplicate, and within the assay each concentration was tested in triplicate.

The RBA and IC_{50} values of 4-OH-HpCS were 3.96×10^7 M^{-1} and 71.74 nM, respectively. The relative potency of 4-OH-HpCS versus T_4 for TTR binding was 1.1. This is within the range of potencies of other halogenated phenolic compounds reviewed by Brouwer et al. (*8*) and Brucker-Davis (*34*) and indicates that circulating levels of 4-OH-HpCS in blood have the potential to disrupt thyroid hormone transport and possibly retinol transport in vivo. The displacement of the natural ligand (T_4) from TTR by halogenated phenolic compounds is hypothesized to result in an increased clearance of plasma T_4 in vivo. This has been observed in animals exposed to PHAHs, such as PCBs (*32*) or OH-PCBs (*3*). In another example, Sinjari et al. (*35*) demonstrated that administration of 4'-OH-3,3',4,5'-tetrachlorobiphenyl and 4-OH-2,3,3',4',5-pentachlorobiphenyl to pregnant mice reduced total T_4 levels in both maternal and fetal plasma.

Concentrations of 4-OH-HpCS Compared to Other Chlorinated Phenolic Compounds, OCS and PCBs. Thirty polar bear plasma samples (3−5 g) were analyzed for chlorinated phenolic compounds, PCBs and OCS. The polar bears included both males and females (15 each) and ranged from 1 to 27 years of age. Mean recoveries of the ^{13}C-labeled OH-PCBs, PCBs, and PCP were 102% ± 9%, 99 ± 19%, and 110 ± 9% CV, respectively. Therefore, recovery correction was not required. The total concentration of chlorinated phenolic compounds was calculated from the sum of OH-PCBs (ΣOH-PCBs, 37 congeners), PCP, and 4-OH-HpCS. ΣPCBs comprised the sum of 23 congeners (*17*). The OH-PCB data is presented only to illustrate the significance of the 4-OH-HpCS with respect to the other major chlorinated phenolic compounds. Further interpretation of the OH-PCBs found in polar bear plasma will be discussed in another publication.

Concentrations of 4-OH-HpCS, ΣOH-PCBs, PCP, OCS, CB153, and ΣPCBs in polar bear plasma are listed in Table 3. Plasma concentrations of 4-OH-HpCS were on average 39 times higher than OCS, although the ratio of 4-OH-HpCS to OCS ranged from 5.19 to 216. The mean ratio of 4-OH-HpCS to CB153 was 0.712 (± 0.580 SD), indicating that 4-OH-HpCS is one of the main contaminants in polar bear plasma.

Concentrations of 4-OH-HpCS constituted on average 12.2% of the total concentration of chlorinated phenolic compounds in polar bear plasma, the remainder being almost entirely OH-PCBs (Table 3, Figure 1). 4-OH-HpCS was similar in concentration to the major OH-PCB congeners. The concentration of Σphenolics was ca. twice that of ΣPCBs and is likely caused by the high binding affinity of the phenolics to plasma proteins. PCP was a small percentage of total chlorinated phenolics, unlike humans, where it is frequently the most important contributor (*5*).

Evidence for Bioaccumulation and Metabolism of OCS as a Source of 4-OH-HpCS in the Polar Bear Food Chain. Long-range transport from source regions and bioaccumulation of 4-OH-HpCS in the polar bear food chain is not likely. First, chlorophenols generally have high water solubility and low volatility. Therefore, they would be prone to remain in areas close to sources (*36*). Halogenated phenols have not been reported in any Arctic biota (*37*). Second, phenolic compounds are readily conjugated and excreted in many higher organisms and are not expected to biomagnify in mammalian food chains (*38*). Thus, the most probable sources of 4-OH-HpCS are through metabolism of OCS or a heptachlorostyrene (HpCS) congener.

Isomers of HpCS with unknown chlorine substitution pattern have been reported in the literature at levels 10-fold less than OCS in the Elbe river in Germany and the Great Lakes where OCS contamination is relatively high (*39, 40*). HpCS isomers have never been observed in Arctic biota and are unlikely sources of 4-OH-HpCS. There can be little doubt that 4-OH-HpCS in polar bear plasma results from CYP450-mediated metabolism of OCS.

OCS was identified in polar bears as part of another study but not reported (*18, 20*). To compare relative accumulation of OCS and CB-153 in plasma with that in adipose tissue and liver, concentrations of OCS and CB-153 from these previous studies were reexamined. A description of analytical methods and concentrations of PCBs, DDE, and their methylsulfonyl-PCB metabolites in polar bear adipose tissue and liver are given in Letcher et al. (*19, 20*). OCS concentrations, which were simultaneously quantitated by GC/EI-MS in that study, are reported in Table 4. OCS was found to be a relatively minor contaminant in liver and adipose tissue, as was the case for plasma. Tissue-specific accumulation of OCS occurred in liver since the ratio of OCS to CB-153 in polar bear liver was four times that of fat ($p < 0.001$). The ratio of OCS to CB-153 in liver was nearly identical to that in plasma of

TABLE 3. Plasma Concentrations (ng/g Wet Weight) and Selected Mean Ratios of Concentrations of Chlorinated Phenolic Compounds, Including 4-Hydroxyheptachlorostyrene (4-OH-HpCS) and Related PHAHs in Polar Bear Plasma ($N = 30$) from the Resolute Bay Area, Nunavut Territory, Canada and Ringed Seal Plasma ($N = 5$) from Kuujjuaq, Québec, Canada

	polar bear plasma				ringed seal plasma	
	mean	min.	max.	SD	mean	SD
4-OH-HpCS	9.11	2.89	22.9	3.85	0.062	0.023
PCP	0.210	0.093	0.531	0.099	0.237	0.136
Σ OH-PCBs	92.6	26.4	576	117	0.081	0.042
Σ phenolics	103	33.2	600	120	0.379	0.181
OCS	0.348	0.106	0.940	0.188	0.266	0.086
CB 153	20.2	4.81	82.0	16.3	5.16	3.71
Σ PCBs	46.9	16.1	161	32.3	27.1	16.5
4-OH-HpCS/ Σ phenolics	0.122	0.038	0.248	0.053	0.176	0.077
4-OH-HpCS/OCS	39.2	5.19	216	41.2	0.260	0.124
4-OH-HpCS/CB153	0.712	0.035	2.43	0.580	0.016	0.009
OCS/CB153	0.024	0.005	0.078	0.018	0.072	0.042

TABLE 4. Mean Concentrations of CB153 and Octachlorostyrene (OCS) (ng/g Lipid Weight) and Mean Ratios in Polar Bear Liver and Adipose Samples (Mean ± SD)

		sample		ratio
tissue	size	OCS	CB-153	OCS:CB-153
polar bear adipose	8	14 ± 12	2670 ± 640	0.005 ± 0.005
polar bear liver	8	156 ± 115	6840 ± 3120	0.022 ± 0.008

bears from the same area (Table 3). Given the high variance in the ratios, this finding is probably a coincidence. Nevertheless, it suggests that plasma concentrations of OCS relative to CB153 in polar bears are reflective of other tissues.

Five ringed seal plasma samples (3−7 g) were analyzed as described above, and the results are shown in Table 3. Mean recoveries of the ^{13}C-labeled OH-PCBs, PCBs, and PCP were 110% ± 8%, 74 ± 4%, and 71 ± 10% CV, respectively. The ringed seals were sampled from a region that is a considerable distance from where the polar bear samples were taken. However, differences in patterns and concentrations of PHAHs (including PCBs) in seals and bears between these areas has been determined to be relatively small (*17*, *41*). Therefore, geographical differences in contamination are not expected to influence the comparison between species.

Concentrations of 4-OH-HpCS were 147 times lower in seals than in polar bears even though OCS plasma concentrations were only slightly lower in seals. In seals, 4-OH-HpCS constituted 17.6% of the total concentration of the chlorinated phenolic compounds, which was similar to the polar bear samples (12.2%). The ratio of 4-OH-HpCS to OCS was 150 times lower in seal than in polar bear. Assuming that that 4-OH-HpCS has similar binding affinities for TTR in both the polar bear and ringed seal, the ratio of 4-OH-HpCS to OCS indicates that seals are capable of metabolizing OCS to 4-OH-HpCS but at a much slower rate than polar bears. The mean concentration of total phenolic compounds was 272 times lower in seals than bears. These findings support previous data that showed ringed seals have a lower capability to metabolize PHAHs (*20*).

Despite a large difference in ratios of 4-OH-HpCS to OCS, the mean ratio of OCS to CB153 was only three times higher for seal than polar bear. This suggests that while formation/retention of 4-OH-HpCS is much more rapid in bears than in seals, it is still slow compared to net bioaccumulation of OCS in the bear. The situation is similar to that for PCBs. Most of the major OH-PCBs in human plasma are believed to be formed by metabolism of highly recalcitrant PCBs, such as CB118, CB138, and CB153, but this rate of metabolism is insignificant compared to rate of accumulation and loss by other mechanisms, such as partitioning into fecal matter (*6*).

Discussion

OCS is an industrial byproduct. The major source of OCS is thought to involve electrolysis of salt solutions using a carbon electrode, especially in the production of sodium hydroxide and chlorine from sodium chloride (*42*). Other possible sources include emissions from the purification of aluminum with gaseous chlorine in graphite vessels (*43*) and industrial processes involving the electrolysis of magnesium chloride (*44*). OCS is generally a low-level environmental contaminant that is usually considered to be of little significance compared to major persistent PHAHs, especially PCBs. Although there are few publications that report OCS concentrations, it is a global contaminant found in arctic fish (*45*) and Antarctic seabirds (*46*). OCS has been found to accumulate to high concentrations in fish from the Frierfjord in Norway (*45*) and the Great Lakes (*40*), close to local sources.

OCS is not a major PHAH in Arctic marine food webs (*37*). Therefore, the relatively high concentration of 4-OH-HpCS in polar bear plasma compared to other phenolic compounds (mainly OH-PCBs) as well as other major contaminants (such as CB153) was unexpected. The presence of 4-OH-HpCS in polar bear and ringed seal plasma is most reasonably explained by metabolism of OCS. At least in some species at higher trophic levels, the findings from the present study suggest that the significance of OCS as an environmental contaminant may have been underestimated.

Phenolic compounds appear to be mainly retained in plasma, and thus bioaccumulation of 4-OH-HpCS from seal to bear is not an influencing factor, since seal fat, not blood, is the important component in the polar bear diet. The concentrations of 4-OH-HpCS and the relative concentrations to CB153 and OCS were much lower in ringed seal relative to polar bear plasma. This is probably because of lower capacity of seals to metabolize PHAHs (*20*). OCS/CB153 ratios were similar in plasma of the two species, suggesting that rate of formation of 4-OH-HpCS in the polar bear is not fast compared to the rate of accumulation of OCS from the diet. The apparent anomaly of relatively high levels of metabolites of slowly metabolized PHAHs in plasma presumably occurs because of specific and high binding affinity of the metabolites to plasma proteins (*6*). If this binding is strong enough, it will compete with conjugation and excretion mechanisms in liver and kidney. Thus, the metabolites are effectively transferred into the plasma compartment and protected from excretion.

TTR is assumed to be the main plasma protein responsible for specific binding of phenolic compounds found in plasma, because most of the compounds, including 4-OH-HpCS, have a similar structure to the natural ligand, T_4 (*31*). We demonstrated that TTR was present in polar bear plasma, so binding to this protein is a plausible explanation for the relatively high levels of 4-OH-HpCS in these species.

The binding affinity of 4-OH-HpCS to human TTR was about the same as T_4. However, several OH-PCBs, PCP, and even PCBs, such as CB153 (*47*), have been shown to bind with even greater relative binding affinity then T_4 (*10, 11*). Little is known about differences in binding affinity of T_4 or metabolites of PHAHs to TTR among species except for humans and laboratory animals. Nor is anything known if other proteins, such as albumin, are participating in plasma binding of these compounds in some species. Therefore, it is unclear how important a role TTR plays in maintaining high concentrations of halogenated phenolic compounds or in the transport of T_4, in the plasma of mammals. These are important factors in understanding possible disruption of thyroid hormone and retinol homeostasis. We are presently studying the effects of contaminants and their metabolites on circulating thyroid hormone and vitamin A levels in the polar bear as well as the distribution of 4-OH-HpCS in plasma of other species, to help resolve some of these questions.

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