The effect of co-occurring polychlorinated biphenyls on quantitation of toxaphene in fish tissue samples by gas chromatography negative ion mass spectrometry

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Abstract

Determinative methods based on gas chromatography-negative chemical ionization mass spectrometry (GC-NCI/MS) provide improved sensitivity and specificity for toxaphene in environmental samples, but are subject to misidentification due to oxygen reaction in the presence of polychlorinated biphenyls (PCBs). The goal of this study was to quantify the impact of co-occurring PCBs in fish tissue samples when utilizing single quadrupole instruments to implement this method. Mixtures of PCB congeners and technical toxaphene, and extracts of fish tissue with varying concentrations of PCBs were analyzed for individual congener and total toxaphene concentrations by GC-NCI/MS. The contribution of co-injected PCB 204 ranged from 23 to 88% of the total peak area for the Cl-9 toxaphene homolog quantitation ion, a contribution that increased as the ratio of technical toxaphene to PCB 204 decreased. PCB interferences in fish tissue extracts, including a standard reference material, were subtracted using a three-step procedure featuring spectral analysis of isotopic patterns for target peaks. Total toxaphene concentrations without PCB subtraction in three fish tissue samples with low, intermediate and high co-occurring PCBs were overestimated by 33, 55 and 750 %, respectively, underscoring the need for practical strategies to account for PCB interferences in GC-NCI/MS based protocols. In contrast, no appreciable interference or resulting positive bias in concentrations was observed for quantitation of eight common toxaphene residue congeners.

Introduction

Technical toxaphene (TTX) is a complex mixture of polychlorinated bornane and camphene congeners produced and used as a biocide in the U.S. for the better part of three decades before its use was severely restricted domestically and abroad during the 1980s. The complex composition of TTX along with the hydrophobic nature and environmental persistence of several of its components poses several analytical challenges since weathered residue patterns observed in environmental samples can be matrix specific (e.g. sediment vs. fish tissue) and deviate from the pattern associated with unmodified TTX (Alder and Vieth 1996, Maruya *et al*. 2001). A second major complicating factor is the co-occurrence of a host of other commonly detected compounds, including polychlorinated biphenyls (PCBs) and structurally-related legacy cyclodiene pesticides (e.g. chlordanes).

Various analytical approaches based on gas chromatography (GC) have evolved to quantify the concentration of toxaphene residues in complex environment samples. Manual, pre-separation of toxaphene from other common co-occurring persistent organic pollutants (e.g. PCBs) prior to instrumental analysis has been shown to minimize the potential for bias and/or misidentification, a step that is particularly important when using non-specific methods based on electron capture detection, or ECD (Krock *et al*. 1997, Maruya *et al*. 2001). Isolation of co-eluting GC peaks using heart-cutting technology and two-dimensional separations (de Geus *et al*.

1998, Bordajandi *et al*. 2008), MS/MS (Chan and Yeboah 2000, Gouteux *et al*. 2002, Skopp *et al*. 2002, Xia *et al*. 2009), GC× GC-time-of-flight/MS (Korytar *et al*. 2003), and high resolution mass spectrometry (Santos *et al*. 1997, Fowler 2000, Veyrand *et al*. 2008) have all been attempted, many with a high degree of success. Yet, each of these approaches require expensive and/or specialized instrumentation, highly trained personnel and/or time consuming preparatory steps to achieve high quality results that, in the end, limit their utility as tools for cost-effective monitoring.

Negative chemical ionization-low resolution mass spectrometry (NCI/MS) has emerged as the leading candidate to replace ECD as the standard determinative technique for toxaphene. It serves as the basis of the recently promulgated EPA method 8276 for analysis of toxaphene and toxaphene congeners (EPA 2010). This technique affords greater selectivity than ECD as well as greater sensitivity for homologs with ≥5 chlorine atoms than, e.g. electron ionization mass spectrometry (EI-MS). Single quadrupole bench-top instruments that are relatively simple to operate and maintain are now widely available to perform GC-NCI/MS analyses. Investigators have utilized such instrumentation to measure toxaphene concentrations in sediment and fish tissue samples from highly contaminated environments, including Terry-Dupree Creek, a former salt marsh Superfund site in coastal Georgia, USA (Maruya *et al*. 2001).

On the other hand, GC-NCI/MS is subject to interference resulting from the formation of oxygen adducts of PCB and chlordane-related compounds in vacuo using low resolution MS instrumentation (Kucklick and Helm 2006). The characteristic masses of $[M\text{-}Cl+O]^{-1}$ and $[M\text{-}Cl_2+O]^{-1}$ adducts, where M is the parent PCB congener, are often indistinguishable from the preferred quantitation ions of toxaphene homologs; moreover, PCBs elute within the same retention time windows as residues of toxaphene on non-polar GC stationary phases. PCB congeners are also used as internal standards, fortified into sample extracts prior to analysis. As a result, guidelines to minimize and monitor the impact of oxygen reaction, e.g. incorporating analysis of an "oxygen reaction standard", are necessary to minimize bias and implement more robust GC-NCI/MS determinative methods. However, limited trials to assess the effectiveness of such guidelines have been performed to date using samples with varying degrees of PCB co-contamination.

The goal of this study was to characterize the effect of co-occurring PCBs on the quantitation of individual toxaphene congeners and total toxaphene by GC-NCI/MS using single quadrupole instrumentation. First, the effect of oxygen reaction on quantitation of total toxaphene was investigated by analyzing PCB-spiked solutions of technical toxaphene. Second, a stepwise data processing method was illustrated to eliminate PCB interferences. Third, the stepwise quantitation method was applied to different fish tissues representing a range of toxaphene and PCB contamination levels, including a standard reference fish tissue material. The results of this study will be used to create guidelines, as necessary, to increase the robustness of GC-NCI/MS based determinative methods for toxaphene.

Methods

Chemicals and Materials

Eight toxaphene congeners commonly referred to as Hx-Sed, Hp-Sed, P26, P41, P40, P44, P50 and P62 (see Table 1 for International Union of Pure and Applied Chemistry (IUPAC) names) were purchased from RT Corp (Laramie, WY, USA). Technical toxaphene was obtained from Restek (Bellefonte, PA, USA). PCB congeners and 4,4'-dibromooctafluorobiphenyl (DBOFB) were purchased from Accustandard (New Haven, CT, USA). Hexanes (95% n-hexane) and dichloromethane (DCM, HR-GC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Isooctane (high purity) was obtained from Burdick & Jackson (Muskegon, MI, USA). Silica gel (60 - 200 mesh, J.T. Baker) and alumina (60 - 325 mesh, Fisher Scientific) were activated overnight at 160 and 250ºC respectively, deactivated with deionized water (3% by weight) and stored in hexane. Sodium sulfate (Mallinckrodt, Phillipsburg, NJ, USA) was baked at 500ºC for 4 hours before use. Standard Reference Material 1946 (SRM 1946), a homogenate of lake trout (*Salvelinus namaycush namaycush*) from Lake Superior was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). A certified reference material prepared from carp (*Cyprinus carpio*) tissue (CARP-2) was purchased from National Research Council of Canada (Ottawa, Canada). A composite of white

Table 1. Analytical parameters and nomenclature for the GC-NCI/MS analysis of toxaphene homologs (TOX) and congeners, polychlorinated biphenyl (PCB) homologs and recovery surrogates.

SIM Group	Retention Time (minutes)	Target Compounds	Monitoring Ions	IUPAC Name
1	8.00 - 29.00	e-HCH[a]	253, 254, 257	
		DBOFB[a]	79, 81, 456	
2	29.00 - 35.06	Hx-Sed	307, 309, 311	2-exo,3-endo,6-exo,8,9,10-Hexachlorobornane
		Cl-5 PCB	324, 326	
3	35.06 - 41.08	Hp-Sed	341, 343, 345	2-endo,3-exo,5-endo,6-exo,8,9,10-Heptachlorobornane
		P26	377, 379, 381	2-endo,3-exo,5-endo,6-exo,8,8,10,10-Octachlorobornane
		P41	377, 379, 381	2-exo,3-endo,5-exo,8,9,9,10,10-Octachlorobornane
		P40	377, 379, 381	2-endo,3-exo,5-endo,6-exo,8,9,10,10-Octachlorobornane
		P44	377, 379, 381	2-exo,5,5,8,9,9,10,10-Octachlorobornane
		Cl-6 PCB	360, 362	
		Cl-7 PCB	394, 396	
4	41.08 - 51.01	P50	411, 413, 415	2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-Nonachlorobornane
		P62	411, 413, 415	2,2,5,5,8,9,9,10,10-Nonachlorobornane
		Cl-8 TOX	377, 379, 381	
		Cl-10 TOX	445, 447, 449	
		Cl-7 PCB	394, 396	
		Cl-8 PCB	428, 430, 432	
5	51.01 - 57.02	PCB209	496, 498, 500	

[a]Recovery Surrogate

croaker muscle (*Genyonemus lineatus*) collected off the coast of southern California (USA) was supplied by the National Oceanic and Atmospheric Administration (NOAA). A muscle filet of Pacific halibut from Alaska (*Hippoglossus stenolepis*) was purchased from a local supermarket.

Sample Preparation

Freeze dried aliquots of SRM 1946 (~1 g dry weight), white croaker (~0.5 g), and Alaskan halibut (~2.5 g) were homogenized in duplicate and spiked with DBOFB and PCB209 as recovery surrogates and extracted with DCM using a Dionex Accelerated Solvent Extraction (ASE) 300 system (Sunnyvale, CA, USA). The ASE procedure consisted of four sequential extraction cycles at 100°C and 1500 psi followed by purging (100 seconds) with ultra-high purity nitrogen (> 99.999%). DCM extracts were concentrated using a rotary evaporator (Buchi, Switzerland) or a TurboVap 500 evaporator (Zymark, Hopkinton, MA, USA), exchanged into hexane, and concentrated to ~1 to 5 ml for gravimetric lipid content determination in triplicate (3µl aliquots). On a wet tissue basis, lipid contents for SRM 1946, white croaker and Alaskan halibut were 10, 2.7, and 1.2%, respectively. Moisture content for white croaker and halibut were 78 and 77%, respectively; and was reported as 72% on the certificate of analysis for SRM 1946.

The bulk extract was subjected to gel permeation chromatography (GPC), consisting of 40 g SX-3 "Bio-Beads" (Bio-Rad Laboratories, Hercules, CA, USA) packed in a 50 cm h × 2.5 cm i.d. glass column. Toxaphene and co-occurring organochlorines (including PCBs) were eluted with 1:1 DCM/ hexane (v/v) in the 100 to 220 ml fraction. After concentration and exchange to hexane, the GPC extract was fractionated on a 30 cm h ×10 mm i.d. glass column packed, from the top to bottom, with sodium sulfate (1 cm), 3% water deactivated neutral alumina (6 cm) and silica gel (12 cm). Target organochlorines (PCBs and toxaphene) were eluted in the first two fractions of 15 ml hexane followed by 60 ml of hexane/DCM (70:30, v/v). After addition of PCB 204 as internal quantitation standard, the combined extract was reduced to 0.5 ml in a Kuderna-Danish concentrator and a gentle ultra-high purity (UHP) nitrogen stream.

Instrumental Analysis

GC-NCI/MS analysis of sample extracts was carried out on an Agilent 7890 GC coupled to a 5975C mass selective detector (MSD) via a two-way effluent splitter connected via uncoated, deactivated fused silica capillary tubing. The analytical column was a 30 m × 0.25 mm × 0.25 µm DB-XLB (Agilent J&W Scientific; Folsom, CA) and the carrier gas (UHP helium, >99.999%) flow was programmed for 1.9 ml/minute. One µl of extract was injected splitless at 280°C. The GC temperature program was as follows: 90°C (1-minute hold); ramp to 150°C at 5°C/minute, ramp to 260°C at 3°C/minute, ramp to 320°C at 20°C/minute (5-minute hold). A 10 minute post run backflush step at 330°C with UHP helium was included to remove high-boiling components. The MSD was operated in NCI mode with methane (>99.999%) as the reagent gas at a 40% flow rate setting. The MSD transfer line, ion source and mass analyzer were maintained at 320, 150, and 150°C, respectively. Mass spectra were collected in the selected ion monitoring (SIM) mode targeting $[M\text{-}Cl\text{-}1]^{-1}$, $[M\text{-}Cl]^{-1}$, and $[M\text{-}Cl\text{+}1]^{-1}$ ions for toxaphene homologs and $[M]^{-}$, $[M\text{-}2]^{-1}$ and $[M\text{+}2]^{-1}$ ions for PCBs. Single quantitation ions for toxaphene homologs were m/z 309 (Cl-6); 343 (Cl-7); 379 (Cl-8); 413 (Cl-9); and m/z 447 (Cl-10) (Table 1).

Seven-point calibration solutions of the target toxaphene congeners at nominal concentrations of 0.5, 5, 25, 100, 200, 300, and 500 ng/ml (each congener); and technical toxaphene at 50, 100, 150, 200, 250, 500, and 750 ng/ml were used to compute mean response factors (RFs) for quantitation using the external calibration method. Individual congener concentrations were quantified using the area of a single quantitation ion for the peak corresponding to the retention time recorded from the calibration solutions. Total toxaphene was quantified by summing the total peak area for Cl-6 to Cl-10 homolog $[M\text{-}Cl]^{-1}$ quantitation ions (i.e., m/z 309 (Cl-6), 343 (Cl-7), 379 (Cl-8), 413 (Cl-9), and m/z 447 (Cl-10)) eluting between 29.00 to 51.01 minutes.

To account for PCB oxygen reaction interferences, $[M]^{-1}$, and $[M\text{-}2]^{-1}$ or $[M\text{+}2]^{-1}$ ions of Cl-5 to Cl-8 congeners were monitored by SIM (Table 1). This program also allows for $[M\text{-}Cl]^{-1}$ of PCB congeners to be monitored (e.g., m/z 396 for Cl-8 homologs). Inclusion of m/z 464 for Cl-9 PCBs was deemed unnecessary since only three homologs exist (PCBs 206, 207 and 208) that are readily identified by retention time and their $[M\text{-}Cl]^{-1}$ ions (m/z 430). PCB 204 was spiked at 50 ng/ml as an oxygen reaction standard; however, it was excluded from the fish tissue samples to avoid additional interference. The Ballschmiter and Zell numbering system was used for PCB congeners (Chu and Hong 2004).

Quality Assurance/Quality Control

No target congeners were detected in hexane, DCM, or procedural blanks mimicking the fish tissue extraction protocol. The recoveries of DBOFB and PCB209 added as surrogates (mean ±std dev) were 81.8 ±13.0% (n = 7) and 92.8 ±3.8% (n = 9), respectively. The recoveries of PCBs and organochlorine pesticides (e.g. chlordanes; dieldrin/endrin; OCPs) in CARP-2 averaged 89.0 ±7.2% and 88.3 ±12.0%, respectively; the corresponding values in SRM 1946 were 79.8 ±12.0% and 72.5 ±17.2%.

For calibration of the GC-NCI/MS instrument for toxaphene congeners, the relative standard deviation (RSD) of the mean RFs were <20% and the R^2 values for linear regression of the seven-point calibration series were >0.99. The temporal stability of instrument calibration was assessed by injecting technical toxaphene and congener mixture solutions (100 ng/ml) every 12 hours. Oxygen reaction within the mass analyzer was quantified by monitoring m/z 411/430 of the PCB204 peak. The oxygen reaction values for all calibration standard and fish tissue sample injections were <1%. The lower limit of quantitation (LLOQ) was determined from the lowest standard concentration whose response when re-fitted fell within ±20% of the response established by the calibration curve in accordance with EPA Method 8276 (*http://www.epa.gov/osw/hazard/testmethods/pdfs/8276.pdf*). The resulting LLOQs were 0.5 ng/ml for Hx-Sed, Hp-Sed, P26, P41, P40, P44, and P50; 5 ng/ml for P62; and 25 ng/ml for TTX. The limit of detection (LOD) was estimated by dividing LLOQ by mass of sample extracted. The resulting congener-specific LODs on a wet tissue basis were 0.038 to 0.19 ng/g for SRM 1946, 0.055 to 0.27 ng/g for white croaker, and 0.030 to 0.093 ng/g for Alaskan halibut. The corresponding total toxaphene LODs were 3.6, 5.4, and 1.2 ng/g.

Results

Potential Contribution of PCB Interferences

The formation of $[M\text{-}Cl\text{+}O]^{-1}$ and $[M\text{-}Cl_2\text{+}O]^{-1}$ fragments originating from PCBs in the presence

of trace oxygen within the mass spectrometer results in a contribution to toxaphene quantitation ions for Cl-8 (m/z 379) and Cl-9 (m/z 413) homologs that decreases in relative magnitude as toxaphene concentration increases. For example, the contribution of PCB 204 at 100 ng/ml to quantitation of technical toxaphene injected at 5 to 250 ng/ml ranged from 82 to 3.6% for m/z 379, and from 88 to 23% for m/z 413 (Figure 1). At higher toxaphene concentrations, the relative contribution of $[M\text{-}Cl+O]^{-1}$ was greater than the contribution of $[M\text{-}Cl_2+O]^{-1}$ (Figure 1). The extent of oxygen reaction ranged between 0.22-0.73%, increasing with successive injections of the PCB-spiked toxaphene solutions but never exceeding 1%.

The percent contribution of an oxygen reaction standard (e.g. PCB 204) may be estimated via curve fitting in Figure 1 by the following formulas:

$$C_{413} = 152.2\ T^{-0.33}\ (R^2=0.959) \qquad \text{Eq. 1}$$

$$C_{379} = 297.7\ T^{-0.80}\ (R^2=0.998) \qquad \text{Eq. 2}$$

where C_{413} and C_{379} are the percent contributions of PCB 204 oxygenated fragment ions to toxaphene quantitation ions 413 and 379, respectively, and T is the total toxaphene concentration.

It is important to note that the interference illustrated in Figure 1 was produced by a single PCB congener, whereas several if not dozens of such congeners can be found in extracts of fish tissue or other environmental samples. Moreover, Cl-4 to Cl-9 PCB homologs that are commonly found in fish tissue and other matrices (e.g. sediment) elute within the same general retention time window as Cl-5 to Cl-10 toxaphene homologs. Therefore, it is essential to monitor parent and oxygenated fragment ions for all relevant PCB homologs when quantifying toxaphene using GC-NCI/MS-SIM. In contrast, other OCPs such as *cis*- and *trans*-chlordane, *trans*-nonachlor and dieldrin typically elute earlier on commonly used non-polar stationary phases (e.g., 5% phenyl-methylpolysiloxane) so that their potential for interference can be minimized by judicious selection of SIM retention time windows (Skopp *et al*. 2002). One exception is *cis*-nonachlor whose retention time (37.84 minutes in this study) falls within the retention time windows for Cl-7 to Cl-8 toxaphene homologs (Table 1), where it could potentially contribute to the peak area for m/z 379. No such contribution in fish tissue samples analyzed in this study was observed, indicating that concentrations of *cis*-nonachlor were sufficiently low.

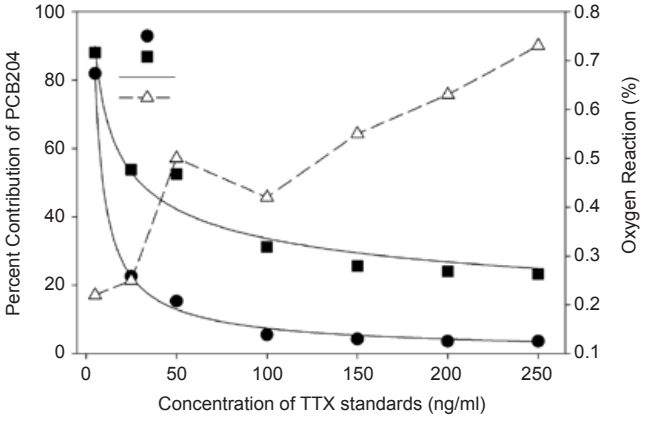

Figure 1. Relative contribution of ions 379 and 413 resulting from PCB204 oxygen reaction to toxaphene quantitation using GC-NCI/MS. Solutions of technical toxaphene (TTX; 5 to 250 ng/ml) spiked with PCB 204 (100 ng/ml) were analyzed.

Subtraction of PCBs Interferences by Isotopic Analysis of Unresolved Peaks

A three-step data processing method was developed to distinguish between toxaphene congeners and PCB oxygen reaction interferences in estimating total toxaphene concentration. Due to elevated concentrations of toxaphene and PCBs, SRM 1946 was used to demonstrate this method. First, GC-NCI/MS ion chromatograms for Cl-6 through Cl-10 toxaphene homologs and the corresponding PCB interference homolog were displayed side-by-side to facilitate peak comparison in the Supplemental Information (SI) Figures SI-1 through SI-3 available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_05SI.pdf, e.g. the Cl-8 toxaphene homolog (m/z 379) vs. the Cl-7 PCB homolog (m/z 396; Figure 2). Second, PCB congeners were confirmed using previously published relative retention times for, e.g. the DB-XLB stationary phase used in this study, to record the expected retention time for potential PCB oxygen reaction adducts (Frame 1997, Cochran and Frame 1999, Frame 1999, Rogers *et al*. 2004). Note that PCBs 206, 207 and 208 were readily identified from the $[M\text{-}Cl]^{-1}$ ion at m/z 430, and PCB 209, used as a spiked recovery surrogate in the present study, eluted outside the retention time window for toxaphene. Third, the isotopic distribution within each peak (with signal/noise ≥3) was examined in the toxaphene

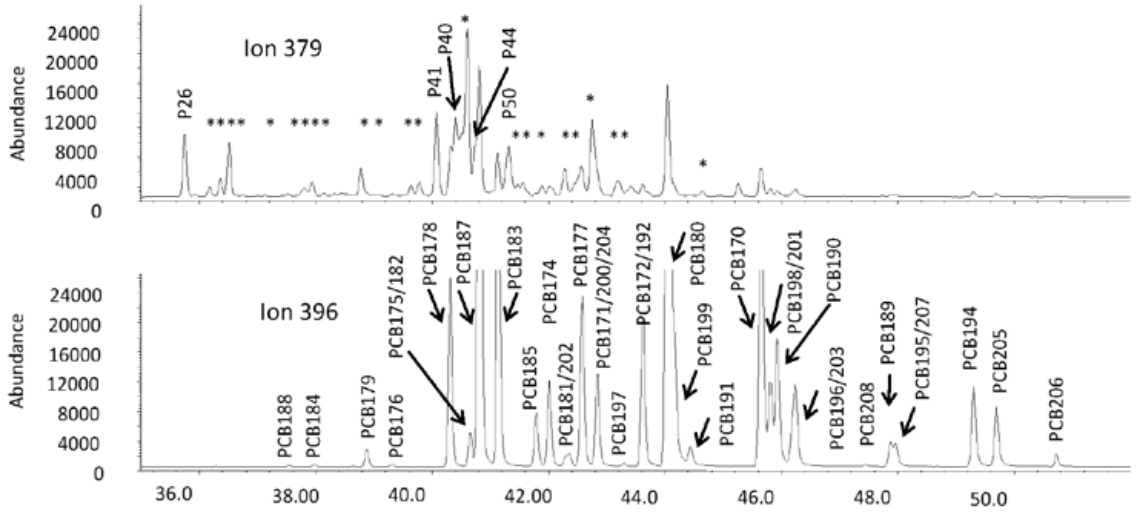

Figure 2. GC-NCI/MS ion chromatograms of Cl-8 toxaphene congeners (m/z 379) and Cl-7 PCBs (m/z 396) in NIST Standard Reference Material 1946, a homogenate of Lake Superior lake trout. *toxaphene congener used for quantitation of total toxaphene.

homolog ion chromatograms. For toxaphene congeners, a characteristic distribution with $[M\text{-}Cl]^{-1}$ as the most abundant ion with lower proportions of $[M\text{-}Cl\text{-}2]^{-1}$ and $[M\text{-}Cl+2]^{-1}$ ions (Figure 3 left panel) was observed. In contrast, the isotopic abundance of PCB oxygen adducts showed a decreasing "stair-stepping" distribution (Figure 3 right panel). Peaks with the latter distribution were flagged as a non-toxaphene interferent (see Table SI-1) and their area contributions subtracted from the total area estimated for that particular toxaphene homolog.

A total of 167 peaks consisting of 98 toxaphene peaks and 69 PCB peaks were identified in SRM 1946 using the above procedure for all Cl-6 through Cl-10 toxaphene homologs (Table 2). The majority of toxaphene congeners yielded $[M\text{-}Cl]^{-1}$ ions, with some also producing $[M]^{-1}$ and $[M\text{-}HCl\text{-}Cl]^{-1}$ ion peaks. For example, P26 (eluting at 35.76 min) contributed to m/z 343 via its $[M\text{-}HCl\text{-}Cl]^{-1}$ fragment ion (Table SI-1). A total of 12 such "redundant" peaks were identified and thus excluded in the estimate of total toxaphene concentration, resulting in a summation of 86 toxaphene peaks. The estimated total toxaphene concentration in SRM 1946 after subtraction of PCB interferences was 1175 ±32.5 ng/g, compared to 1820 ±121 ng/g before the correction (Table 3). Concentrations of the eight target toxaphene congeners ranged from 0.22 to74 ng/g with concentrations for Hx-Sed and Hp-Sed reported in SRM 1946 for the first time (Table 3). In contrast to estimation of total toxaphene, congener-specific quantitation using the GC-NCI/MS protocol described herein did not require subtraction of PCB

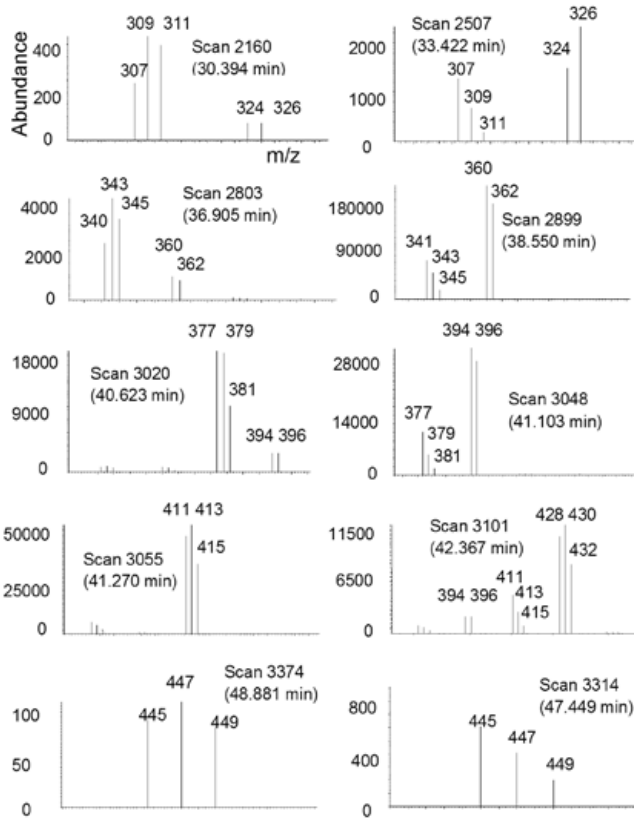

Figure 3. Selected ion monitoring (SIM) mass spectra of toxaphene congeners (left column) and PCB interferences due to oxygen reaction (right column) in SRM 1946.

interferences (Table SI-1). As further evidence of correct identification of PCB congeners in SRM 1946, the sum of Cl-5 to Cl-9 PCBs was 612 ±44.0 ng/g, which compared well to the certified value of 711 ±49.4 ng/g (Table SI-2).

Table 2. The number of resolvable peaks assigned as toxaphene and PCBs by homolog in Standard Reference Material 1946. The GC column was DB-XLB (30 m x 0.25 mm i.d. x 0.25 µm film thickness).

Ion	Tox $[M-Cl]^{-1}$	Tox $[M-HCl-Cl]^{-1}$	Tox $[M]^{-1}$	PCB*
309	11			10
343	10	4		17
379	39	1		24
413	17		5	12
447	9		2	6
Sum	86	5	7	69

* $[M]^{-1}$, $[M-Cl+O]^{-1}$, $[M-Cl2+O]^{-1}$, $[M-Cl3+O]^{-1}$, $[M+15]^{-1}$ where M is the parent ion.

Estimated total toxaphene concentration after subtraction of PCB interferences for the white croaker and Alaskan halibut samples analyzed in this study were <10 ng/g, while all target toxaphene congeners were <1 ng/g for both tissue samples (Table 4). Hx-Sed was barely detectable in white croaker and was not detected in the Alaskan Halibut tissue. The sum of Cl-5 to Cl-9 PCBs were 435 ±10.0 ng/g for the white croaker and 2.45 ±0.64 ng/g for the Alaskan halibut (Table SI-2). The estimated total toxaphene concentration for white croaker was nearly an order of magnitude higher without subtraction of PCB interferences (56 vs. 6.6 ng/g; a difference of 750%), whereas the corresponding difference for Alaskan halibut was <30%.

Table 3. Concentration (mean ± std dev) of toxaphene congeners and total toxaphene (ΣTOX; ng/g wet weight) in Standard Reference Material 1946 with and without subtraction of PCB interference.

	ECNI-MS*	EI-MS/MS†	ECNI-MS‡
Hx-Sed	0.225 ± 0.024		
Hp-Sed	2.49 ± 0.212		
P26	23.7 ± 2.19	29.5 ± 6.4	31 ± 3.7
P41	8.05 ± 0.891	19.7 ±2.4	
P40	14.0 ± 0.849	14.8 ± 2	
P44	14.5 ± 1.14	18.0 ± 1.9	
P50	74.1 ± 7.00	121.4 ± 17.1	86.6 ± 5.6
P62	71.4 ± 5.30	68.4 ± 8.3	54.6 ± 4.1
ΣTOX	1175 ± 32.5@	846 ± 72♀	1960 ± 133

*This study;
†From (Xia et al. 2009);
‡From (Stern et al. 1996)
@1820 ± 121 ng/g without subtraction of PCB interferences;
♀1915 ± 71 ng/g using NCI-MS without subtraction of PCB interferences.

Discussion

Potential Contribution of PCB Interferences

The addition of PCB 204 to quantify the impact of interfering oxygenated adducts in the present study revealed that a significant bias is possible even at very low levels of oxygen reaction (Figure 1). To maintain an acceptably "clean" (i.e., oxygen free) system, regularly scheduled maintenance is required to prevent the buildup of residual oxygen species in the mass analyzer. In the present study, purging of the ion source every 20 to 25 injections, or about once every 24 hours of instrument operation, maintained the extent of oxygen reaction to <1%. In Equations 1 and 2, we illustrated an empirical approach to quantifying the effect of these interferences; however, it should be noted that these relationships were dependent on system configuration and operating conditions.

Table 4. Concentration (average ±1 std dev) of toxaphene congeners, total toxaphene (ΣTOX) and PCBs (ng/g wet weight) in fish tissues with varying levels of co-occurring PCBs.

	Alaskan Halibut (ng/g ww)	White Croaker (ng/g ww)
Hx-Sed	<0.003	0.008 ± 0.002
Hp-Sed	0.009 ± 0.001	0.056 ± 0.006
P26	0.566 ± 0.026	0.086 ± 0.024
P41	0.054 ± 0.005	0.060 ± 0.008
P40	0.090 ± 0.005	<0.075
P44	0.061 ± 0.006	<0.128
P50	0.675 ± 0.047	0.088 ± 0.024
P62	0.167 ± 0.019	<0.272
STOX	9.69 ± 0.650	6.65 ± 0.884
STOX*	12.9 ± 1.19	56.2 ± 1.13
PCBs (Cl_{5-9})	2.45 ± 0.064	435 ± 10.0

*Without subtraction of PCB interferences.

Subtraction of Co-Occurring PCB Interferences in Fish Tissue Extracts

Theoretically, 127 PCB Cl-5 to Cl-9 congeners may create interferences for quantitation of toxaphene in environmental samples. For example, the 12 octachlorobiphenyl congeners (PCBs 195-205) can contribute ions to toxaphene quantitation ions 379 and 413, resulting in a high bias for Cl-8 and Cl-9 toxaphene homologs. In practice, the magnitude and relative congener abundance of PCB contamination may vary widely across environmental samples, necessitating careful peak-by-peak analysis and monitoring of both characteristic toxaphene and interfering adduct and parent ions described herein. Automation of this process with a package to compare mass spectral isotopic ratios is possible, e.g. using a QBasic program (Glassmeyer *et al*. 1999), precluding the need for time-consuming manual inspection and/or data analysis.

Mean total toxaphene concentrations reported for SRM 1946 using NCI/MS without correcting for PCB interferences in the present and previous studies were very similar (1820 ±121 ng/g [this study] vs. 1960 ±133 and 1915 ±71 ng/g in previous studies). After correcting for PCB interferences, however, the mean concentration was 55% lower (1175 ±32.5 ng/g; this study), and was comparable to results based on EI-MS/MS using two different technical toxaphene quantitation standards (Table 3). It should be noted that the detection limit afforded by GC-EI-MS/MS (Xia *et al*. 2009) was approximately 10 times higher than for GC-NCI/MS (this study), a possible source of low bias for EI-MS/MS results. Thus, not accounting for PCB interferences in this case resulted in overestimation of total toxaphene concentration in SRM 1946 by more than 60%.

Agreement between congener-specific concentrations reported for SRM 1946 in the present and previous studies was achieved with two exceptions. Mean concentrations of P26, P40, P44 and P62 determined in this study for SRM 1946 agreed within 22 and 27% of previously published results using GC-EI-MS/MS (Kucklick *et al*. 2004, Xia *et al*. 2009), respectively (Table 3). Concentrations for P41 and P50 were substantially lower in the present study compared to previous results using EI-MS/MS, suggesting that PCB interference was not a primary factor. In fact, our results for P50 compared favorably with a previous study that also utilized GC-NCI/MS (Stern *et al*. 1996). Caution is warranted when reporting concentrations of P40 and P41, congeners that co-elute using common non-polar stationary phases (e.g. 5%-phenyl methylpolysiloxane), and for P44, a peak that is only partially resolved from other Cl-8 toxaphene homologs (Rogers *et al*. 2004). Additional laboratory intercomparison studies may be needed to resolve the differences observed for these congeners.

Individual congener and total toxaphene concentrations in white croaker and Alaskan halibut were two orders of magnitude lower in comparison with SRM 1946 (Table 4). The Alaskan halibut sample contained low background concentrations of PCBs and OCPs, including toxaphene (Tables 4, SI-2 and SI-3). Since the effect of PCB interference was low, total toxaphene concentrations with and without subtraction of PCBs were not substantially different (9.7 vs. 13 ng/g). In contrast, white croaker contained elevated levels of PCBs compared to toxaphene (Tables 4, SI-2 and SI-3), resulting in a substantial difference between PCB corrected and uncorrected estimates of total toxaphene (6.7 vs. 56 ng/g, respectively). A fourth possible scenario -- low PCB relative to toxaphene – was not explicitly addressed in the present study but would not be expected to be adversely affected by PCB interferences. It follows that the most important scenarios to consider PCB interferences are when PCB concentrations are comparable or higher than the level of toxaphene encountered.

Other Considerations

The quantitation of residues of toxaphene (or “weathered” toxaphene) vs. technical (i.e. unmodified) toxaphene are not fundamentally interchangeable processes. Components of technical toxaphene are selectively transformed once released in the environment, resulting in a toxaphene congener pattern dominated by recalcitrant homologs such as P26 and P50, and/or dead end transformation products such as Hx- and Hp-Sed (Stern *et al*. 1996, Maruya *et al*. 2000). Setting of appropriate retention time windows and knowledge of the homolog specific relative response are two important operational parameters to consider when targeting the appropriate measurement parameter (e.g. either technical or weathered toxaphene residues). This is especially pertinent for application of GC-NCI/MS protocols as the response of toxaphene homologs decreases rapidly with successive losses of chlorine atoms.

The selection and origin of technical toxaphene standards can also have a profound impact on quantitation of total toxaphene. Xia *et al*. (2009) reported the ratio of relative response for toxaphene calibration standards from NIST and Accustandard to be 1.21. This difference could explain the lower mean total toxaphene concentration (846 ±72 ng/g) estimated in their study. When correcting for this ratio, the adjusted toxaphene concentration in SRM 1946 from Xia *et al*. (2009) was 1015 ± 86 ng/g, which compares favorably to that estimated in the present study (Table 3). Moreover, Howdeshell and Hites (1996) compared three different toxaphene standards and observed batch by batch variability in homolog distributions for the commercial product. Therefore, a consistent set of standards is critical in standardizing GC-NCI/MS protocols for estimation of total toxaphene concentration.

A final consideration is the workup of environmental samples prior to instrumental analysis. It is not uncommon to employ a clean-up and/or fractionation step to reduce GC interferences after exhaustive extraction of "dirty" environmental matrices like fish tissue and sediment. However, one must be cognizant of imparting negative bias by losing target analytes when utilizing such steps. The pre-separation of PCB interferences using preparatory solid-liquid chromatography can reduce the complexity of GC-NCI/MS chromatograms, effectively reducing or eliminating altogether the need to monitor for PCB oxygen adducts (Maruya and Lee 1998, Kucklick and Helm 2006). Lastly, the selection of quantitation and recovery standards fortified into sample extracts should be made judiciously to avoid, if possible, undue PCB oxygen interferences. As observed in the present study, PCB 204, a commonly used internal standard in GC/MS analysis (Swackhamer *et al*. 1987), can generate significant positive bias for quantitation of total toxaphene. Alternative, non-PCB options such as polybrominated diphenyl ether (PBDE) congeners not commonly found in environmental samples (e.g. BDE172) merit consideration as such standards.

The formation of PCB-oxygen adduct ions can lead to overestimation of toxaphene concentrations when analyzing samples that also contain co-occurring PCBs by GC-NCI/MS. A three step process to identify and eliminate positive bias was instituted on fish tissue samples with different intrinsic levels of toxaphene and co-occurring PCBs. Corrected results using the approach were similar to those reported for techniques that are immune to this interference (e.g. GC-EI-MS/MS). Simultaneous monitoring of PCB congeners and oxygen adducts that interfere with characteristic ions for target toxaphene compounds and a rigorous, regularly scheduled maintenance program are recommended when analyzing samples suspected of containing co-occurring PCBs at concentrations that are comparable or higher than that expected for toxaphene.

Literature Cited

Alder, L. and B. Vieth. 1996. A congener-specific method for the quantification of camphechlor (toxaphene) residues in fish and other foodstuffs. *Fresenius Journal of Analytical Chemistry* 354:81-92.

Bordajandi, L.R., J.J. Ramos, J. Sanz, M.J. Gonzalez and L. Ramos. 2008. Comprehensive two-dimensional gas chromatography in the screening of persistent organohalogenated pollutants in environmental samples. *Journal of Chromatography A* 1186:312-324.

Chan, H.M. and F. Yeboah. 2000. Total toxaphene and specific congeners in fish from the Yukon, Canada. *Chemosphere* 41:507-515.

Chu, S.G. and C.S. Hong. 2004. Retention indexes for temperature-programmed gas chromatography of polychlorinated biphenyls. *Analytical Chemistry* 76:5486-5497.

Cochran, J.W. and G.M. Frame. 1999. Recent developments in the high-resolution gas chromatography of polychlorinated biphenyls. *Journal of Chromatography A* 843:323-368.

de Geus, H.J., R. Baycan-Keller, M. Oehme, J. de Boer and U.A. Brinkman. 1998. Determination of enantiomer ratios of bornane congeners in biological samples using heart-cut multidimensional gas chromatography. *Journal of High Resolution Chromatography* 21:39-46.

United States Environmental Protection Agency (USEPA). 2010. Method 8276 Toxaphene and Toxaphene Congeners by Gas Chromatography/ Negative Ion Mass Spectrometry (GC/NIMS): Test Methods for Evaluating Solid Waste, Physical/ Chemical Methods (SW-846). USEPA. Washington, DC.

Fowler, B. 2000. The determination of toxaphene in environmental samples by negative ion electron capture high resolution mass spectrometry. *Chemosphere* 41:487-492.

Frame, G.M. 1997. A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns .2. Semi-quantitative Aroclor congener distributions. *Fresenius Journal of Analytical Chemistry* 357:714-722.

Frame, G.M. 1999. Improved procedure for single DB-XLB column GC-MS-SIM quantitation of PCB congener distributions and characterization of two different preparations sold as "Aroclor 1254". *Journal of High Resolution Chromatography* 22:533-540.

Glassmeyer, S.T., K.E. Shanks and R.A. Hites. 1999. Automated toxaphene quantitation by GC/MS. *Analytical Chemistry* 71:1448-1453.

Gouteux, B., M. Lebeuf, S. Trottier and J.P. Gagne. 2002. Analysis of six relevant toxaphene congeners in biological samples using ion trap MS/MS. *Chemosphere* 49:183-191.

Howdeshell, M.J. and R.A. Hites. 1996. Historical input and degradation of toxaphene in Lake Ontario sediment. *Environmental Science & Technology* 30:220-224.

Korytar, P., L.L.P. van Stee, P.E.G. Leonards, J. de Boer and U.A.T. Brinkman. 2003. Attempt to unravel the composition of toxaphene by comprehensive two-dimensional gas chromatography with selective detection. *Journal of Chromatography A* 994:179-189.

Krock, B., W. Vetter and B. Luckas. 1997. PCB/toxaphene group separation on silica prior to congener specific determination of toxaphene residues in fish and other samples by GC/ECD. *Chemosphere* 35:1519-1530.

Kucklick, J.R. and P.A. Helm. 2006. Advances in the environmental analysis of polychlorinated naphthalenes and toxaphene. *Analytical and Bioanalytical Chemistry* 386:819-836.

Kucklick, J.R., K.J.S. Tuerk, S.S. Vander Pol, M.M. Schantz and S.A. Wise. 2004. Polybrominated diphenyl ether congeners and toxaphene in selected marine standard reference materials. *Analytical and Bioanalytical Chemistry* 378:1147-1151.

Maruya, K.A. and R.F. Lee. 1998. Aroclor 1268 and toxaphene in fish from a southeastern US Estuary. *Environmental Science & Technology* 32:1069-1075.

Maruya, K.A., S.G. Wakeham, W. Vetter and L. Francendese. 2000. Prominent chlorobornane residues in estuarine sediments contaminated with toxaphene. *Environmental Toxicology and Chemistry* 19:2198-2203.

Maruya, K.A., T.L. Walters and R.O. Manning. 2001. Residues of toxaphene in finfish and shellfish from Terry and Dupree Creeks, Georgia, USA. *Estuaries* 24:585-596.

Rogers, E., M. Petreas, J.-S. Park, G. Zhao and M.J. Charles. 2004. Evaluation of four capillary columns for the analysis of organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers in human serum for epidemiologic studies. *Journal of Chromatography B* 813:269-285.

Santos, F.J., M.T. Galceran, J. Caixach, J. Rivera and X. Huguet. 1997. Characterization of toxaphene by high resolution gas chromatography combined with high resolution mass spectrometry and tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* 11:341-348.

Skopp, S., M. Oehme, F.L. Chu, F. Yeboah and H.M. Chan. 2002. Analysis of "total toxaphene" and selected single congeners in biota by ion trap HRGC-EI-MS/MS using congener-optimized parent ion dissociations. *Environmental Science & Technology* 36:2729-2735.

Stern, G.A., M.D. Loewen, B.M. Miskimmin, D.C.G. Muir and J.B. Westmore. 1996. Characterization of two major toxaphene components in treated lake sediment. *Environmental Science & Technology* 30:2251-2258.

Swackhamer, D.L., M.J. Charles and R.A. Hites. 1987. Quantitation of toxaphene in environmental-samples using negative-ion chemical ionization mass-spectrometry. *Analytical Chemistry* 59:913-917.

Veyrand, B., A. Venisseau, P. Marchand, J.P. Antignac and B. Le Bizec. 2008. Determination of toxaphene specific congeners in fish liver oil and feedingstuff using gas chromatography coupled to high resolution mass spectrometry. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 865:121-126.

Xia, X.Y., B.S. Crimmins, P.K. Hopke, J.J. Pagano, M.S. Milligan and T.M. Holsen. 2009. Toxaphene analysis in Great Lakes fish: a comparison of GC-EI/MS/MS and GC-ECNI-MS, individual congener standard and technical mixture for quantification of toxaphene. *Analytical and Bioanalytical Chemistry* 395:457-463.

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Supplemental Information

Supplemental Information is available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_05SI.pdf