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

Wenjian Lao, David Tsukada, Keith A. Maruya*

Southern California Coastal Water Research Project, Costa Mesa, CA 92626, USA

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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 745%, 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.

1. 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 [1,2]. 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 [1,3]. Isolation of co-eluting GC peaks using heart-cutting technology and two-dimensional separations [4,5], MS/MS [6–9], GC × GC-time-of-flight/MS [10], and high resolution mass spectrometry [11–13] 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 [14]. 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

* Corresponding author. Tel.: +1 714 755 3214; fax: +1 714 755 3299.
E-mail address: keithm@sccwrp.org (K.A. Maruya).

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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 Georgia, USA [1].

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 [15]. The characteristic masses of $[M-Cl+O]^{-1}$ and $[M-Cl_2+O]^{-1}$ 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.

2. Experimental

2.1. 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 °C and 250 °C respectively, deactivated with deionized water (3% by weight) and stored in hexane. Sodium sulfate (Mallinckrodt, Phillipsburg, NJ, USA) was baked at 500 °C for 4 h 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, USA). 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 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 (*Hippoglossus stenolepis*) ("Alaskan Halibut") was purchased from a local supermarket.

2.2. 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 PCB 209 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 s) 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 ~1–5 mL for gravimetric lipid content determination in triplicate (3 μL 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 $L \times 2.5$ cm i.d. glass column. Toxaphene and co-occurring organochlorines (including PCBs) were eluted with 1:1 DCM/hexane (v/v) in the 100–220 mL fraction. After concentration and exchange to hexane, the GPC extract was fractionated on a 30 cm $L \times 10$ 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.

2.3. 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/min. One μL of extract was injected splitless at 280 °C. The GC temperature program was as follows: 90 °C (1 min hold); ramp to 150 °C at 5 °C/min, ramp to 260 °C at 3 °C/min, ramp to 320 °C at 20 °C/min (5 min hold). A 10 min 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 $[M-Cl-1]^-$, $[M-Cl]^-$, and $[M-Cl+1]^-$ ions for toxaphene homologs and $[M]^-$, $[M-2]^-$ and $[M+2]^-$ 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 $[M-Cl]^-$ quantitation ions (i.e., *m*/*z* 309