Target, Suspect and Non-Target Screening of Dioxin-Like Compounds in Sediment and Fish Using A Sensitive High-Resolution Time-of-flight Mass Spectrometer

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Introduction

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and planar PCBs (pPCBs) are highly toxic and present at very low concentrations (ppt-ppq) in environmental samples. Highly sensitive and selective gas chromatography sector high-resolution MS (GC-HRMS) systems have therefore been the gold standard for measurement of these compounds. In the last decade, GC triplequadrupole MS systems have become sufficiently sensitive to offer an alternative to GC-HRMS. Recent developments in ion source, analyzer and detector design of high-resolution GC quadrupole time-of-flight MS (GC/Q-TOF) systems has resulted in improved sensitivity and resolution. Such instruments may now be feasible alternatives to GC-HRMS, offering high sensitivity, high selectivity and verification of identity through full-spectrum EI spectra. The performance of such a system was evaluated in the current study for the analysis of both biotic (fish) and abiotic (sediment) samples.

The utility of the GC/Q-TOF systems extends beyond trace-analysis of target compounds, such as PCDD/Fs and pPCBs. The entire full-spectrum data is written to disc and is available for various post-acquisition tasks, such as suspect and non-target screening. To illustrate this we have screened a sediment sample for dioxin-analogues such as polychlorinated naphthalenes (PCNs), polychlorinated dibenzothiophenes (PCDTs) and polychlorinated thianthrenes (PCTAs) and polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs). We also used non-target screening workflows to find additional planar halogenated compounds.

Materials and methods

Samples were spiked with ^{13}C-internal standards and extracted by Soxhlet (sediment) or column extraction (salmon) in Umeå. Extracts were cleaned up using sulfuric acid treatment and carbon column fractionation using ISO/IEC 17025 accredited methods. The planar halogenated aromatic compound fraction from the carbon column was shipped (coded) to Agilent, CA; data were acquired using a novel 7250 GC/Q-TOF system equipped with a novel ion source. The GC analysis was performed by split-less injections (1 μL) onto an Agilent DB-5MS column (60m long, 0.25 mm ID, 0.25 μm film thickness). The oven temperature program was 90 °C (2 min), raise at 15 °C/min to 190 °C, raise at 3 °C/min to 300 °C. Helium was used as carrier at a constant flow of 1.2 mL/min. Electron ionization was used at an electron energy of 70 eV. Centroid and profile data was collected over the m/z range 50 to 1200. The mass resolution was 25,000 or better.

To aid the data analysis, an accurate mass library containing spectra of PCDD/Fs and pPCBs (natural and 13C-labelled analogs) was created in a .cdb format. The data were processed using MassHunter Qualitative Analysis Find-by-Fragment screening workflow for quick detection of the target compounds, which were later quantified using MassHunter Quantitative Analysis software. The quantification was based on calibration curves and internal standard normalization.

Results and discussion

The accurate mass PCDL (Personal Compound Database and Library) that was created using spectra of 46 native and labeled PCDD/Fs and pPCBs proved highly useful in both qualitative screening and quantitative workflows. The new Find-by-Fragment screening workflow was effective in extracting target compound features from the complex data and provided a comprehensive overview of the results.

The sensitivity was good with the lowest point of the calibration graph generally below 100 fg (even lower for tetra- and penta-congeners). Two sets of comparison data were available: one small set from GC-HRMS analyses of the sample extracts prior to shipment to CA, and one large set from previous analyses of the samples (in-house reference materials, RMs). There was good agreement between the GC-HRMS data and the average RM values (Table 1). The GC/Q-TOF data for the toxicologically most relevant congeners (PCB126, 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, 1,2,3,7,8- and 2,3,4,7,8-PeCDF) were within ± 25 % of the GC-HRMS and RM values. Similarly, the dioxin toxic equivalency values (TEQs) were within ± 19 % of the comparison data. Larger deviations between the GC/Q-TOF and the comparison data were observed for hexa- and hepta-CDD/Fs and octa-CDD/F that were present at levels close to the limit-of-quantification. All congeners with good signal quality, i.e. a signal-to-noise ratio equal to or better than 10, were within +/– 40 % of the RM values.

Table 1: Comparison of GC-QTOF-HRMS and GC-Magnetic Sector-HRMS results.

Congener	Sediment			Salmon		
	QTOF	Sector	RM mean	QTOF	Sector	RM mean
PCB-77	32	37	36	1125	900	910
PCB-81	3.5	1.8	1.8	23	23	26
PCB-126	8.8	8	7.3	464	410	430
PCB-169	3.9	1.8	1.6	53	53	58
2,3,7,8 -TCDD	**1.8**	**1.5**	**1.5**	**2.3**	**2.6**	**2.7**
12378 -PeCDD	**3.2**	**3.2**	**2.8**	**4.7**	**4.4**	**4.6**
123478 -HxCDD	6.4	1.8	2.1	0.4	0.3	0.3
123678 -HxCDD	14	11	11	3	1.7	1.9
123789 -HxCDD	8.2	7	6.8	0.2	0.1	0.2
HpCDD	40	34	36	0.7	0.3	0.3
OCDD	137	100	113	1.9	0.9	1.2
2378 -TCDF	**13**	**14**	**17**	**26**	**23**	**23**
12378 -PeCDF	**4.7**	**4.1**	**4.2**	**4.2**	**5.4**	**5**
23478 -PeCDF	**11**	**10**	**9.4**	**29**	**26**	**25**
123478 -HxCDF	7	7.1	9.1	1.3	0.9	0.9
123678 -HxCDF	10	5.1	4.5	2.7	1.2	1.2
234678 -HxCDF	8.5	6.1	6.1	1.5	1	0.9
123789 -HxCDF	3.7	1.9	2.3	ND	0.2	0.2
1234678 -HpCDF	95	76	77	0.2	0.3	0.3
1234789 -HpCDF	8	3.1	3.4	ND	0.1	0.1
OCDF	130	94	105	1.6	0.2	0.3
TEQ	**18**	**15**	**15**	**67**	**60**	**63**

Suspect screening revealed PCNs, PCDTs, and PBDDs in the sediment samples, with PCNs as the most abundant class of dioxin-like compounds. The PCN levels were slightly higher than those of PCDFs. Non-target screening showed that Polycyclic Aromatic Compounds (PACs), incl. PAHs, dominated the dioxin fraction, and also revealed many halogenated PAHs (halo-PAHs) (**Figure 1**). The abundances (area units) of halo-PAHs were about 100-fold lower than those of the parent PAHs, and were declining with increasing degree of halogenation.
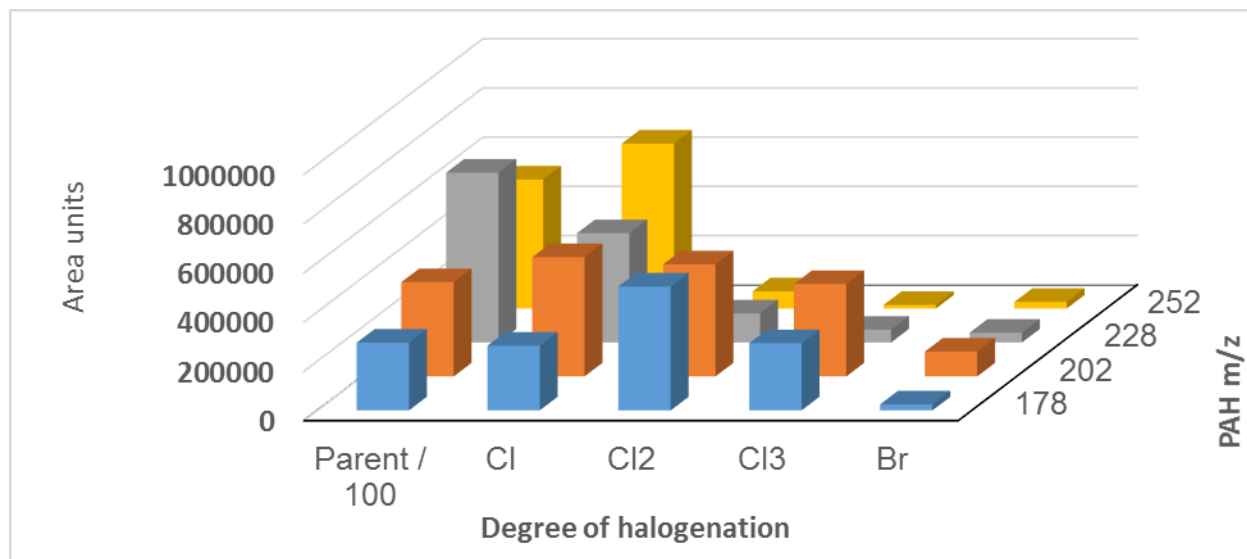
Figure 1. Abundances (peak heights) of parent PAHs and their halogenated derivatives in Baltic Sea sediment.

No PCTAs were found in the sediment, but an interfering signal was noted during octa-CTA screening. Its spectrum displayed a tetrabromo isotope cluster at (monoisotope) m/z 492.730, and another tetrabromo cluster at [M-15], likely corresponding to a methyl loss (**Figure 2**). Molecular formula generation returned C13 H7 N Br4 as the most likely formula and a ChemSpider search returned only one candidate, 1,3,6,8-tetrabromo-9-methyl-carbazole (TB-Me-CZ). A likely origin may be *in-situ* methylation of an halogenated natural product 1,3,6,8-tetrabromocarbazole (TB-CZ), which has been reported in Great Lakes sediment [1]. TB-CZ was also present in the investigated sediment at about twice the abundance of TB-Me-CZ (as expected at a slightly shorter retention time, 51.21 min).
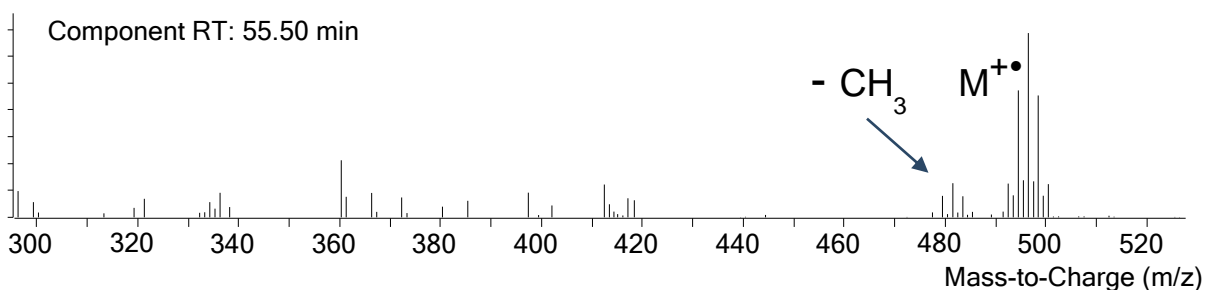
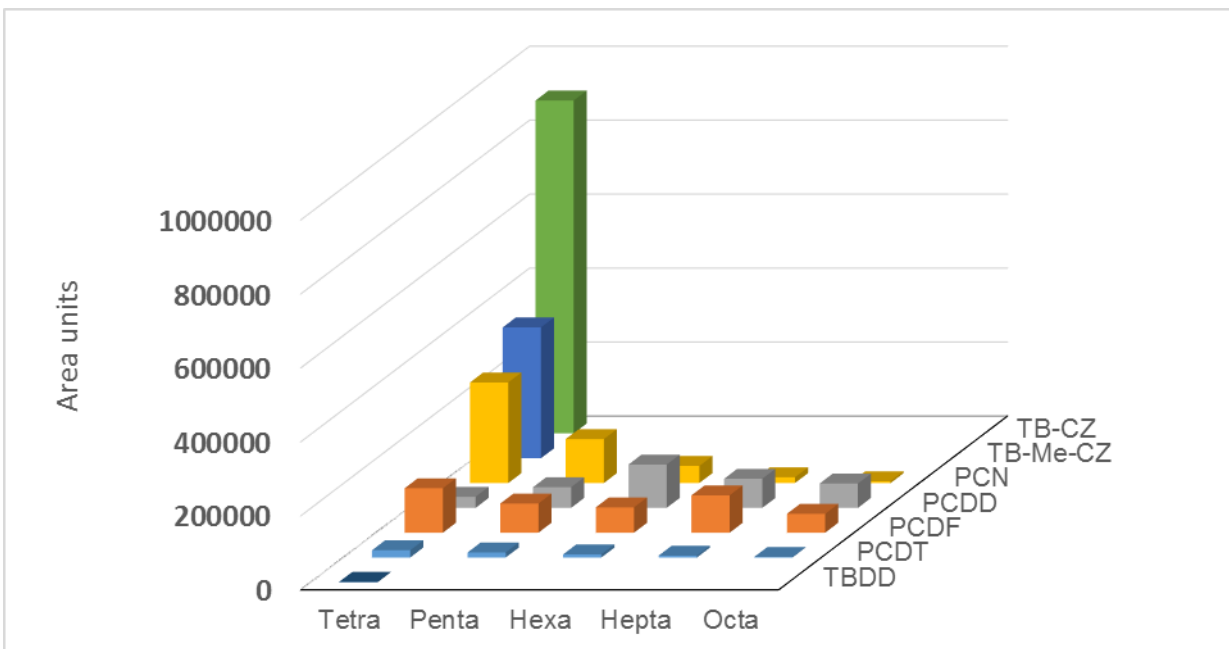**Figure 2.** EI-MS spectrum of C13 H7 N Br4 unknown.

Figure 3. Abundances of bi- and tri-cyclic dioxin like compounds detected in the Baltic Sea sediment sample.

The relative abundances of the bi- and tri-cyclic planar halogenated compounds that were detected in sediment are summarized and compared in **Figure 3.** The halogenated natural products (TB-CZ and TB-Me-CZ) was the most abundant group of dioxin-like compounds. They are N-analogues of PCDFs, and their total abundance was similar to the combined abundances of the anthropogenic compound groups PCNs, PCDDs, and PCDFs. The S-analogues of PCDFs, PCDTs, was much less abundant than the TB-CZs and the PCDFs. Least abundant was PBDD/Fs of which only two early eluting tetrabromo dioxin congeners (TBDDs) were found, likely corresponding to 1,3,6,8- and 1,3,7,9- TBDD. These are considered to be of natural origin, just like TB-CZ. Further work is needed to positively identify the TB-Me-CZ and investigate if it is of natural or anthropogenic origin.

Acknowledgements

References

1. Zhu L and Hites RA (2005) Identification of Brominated Carbazoles in Sediment Cores from Lake Michigan, *Environmental Science and Technology,* **39**, 9446-9451.