POTENTIAL INTERFERENCES FROM PENTA-BDES IN THE DETERMINATION OF BROMO CHLORO BIPHENYLS (PXBS) IN FISH FROM THE CANADIAN GREAT LAKES

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Introduction:

Polybrominated chlorinated biphenyls (PXBs) are a group of environmental contaminants that have one or more bromine atoms introduced into the polychlorinatedbiphenyl backbone. The introduction of a second type of halogen into the polychlorinated moiety increases the number of possible congeners from 209 to 9180 (Ohta et al. 2008). Currently, there are only a limited number of PXB standards commercially available. These compounds are thought to be undesired by-products generated during incineration processes in which consumer products containing brominated flame retardants are present and are anticipated to concentrate into various environmental compartments.

Our preliminary assessment of fish form the Laurentian Great Lakes, as presented at Dioxin 2008, focused on five custom synthesized PXBs that were currently available at the time (PXB 126, 118, 105, 169 and 126A) – all were based on a co-planar PCB substitution (Alaee et. al 2008). Preliminary results showed that 4'-MoBr-3, 3',4,4'5,5'-PeCB (PXB 169) was present in all samples, 4'-MoBr-2,3,3',4-TeCB(PXB 105) were not detectable in two channel catfish and brown trout, and 4'-MoBr-2, 3',4,5-TeCB (PXB 118) was detected in 9 out of 22 samples.

Of particular interest was the observation of two large unidentified peaks that were present in the 403.7913/405.7886 monobromopentachloro mass channels that met the minimum criteria for PXB confirmation in terms of isotopic ratios. Ion structure elucidation (ISE) was attempted unsuccessfully to identify the mass apex of these unknown compounds so that structural formulae could be calculated. GC TOF mass spectrometry was ultimately used to identify these unknown peaks as being PBDE 99 and PBDE 100.

Analytical Methods:

Approximately 5 g of sample was spiked with 15 13C12-PCDD/Fs and 12 13C12 Co-PCB surrogates prior to extraction. Recoveries were based on 13C12 PCBs (13C12 CB-156, 157 and 189) that had been added at the beginning of the extraction process. 13C12 CB-138 was used as an instrument performance standard. Each sample was acid digested overnight using concentrated HCl and extracted with hexane the following day. Fractionation and cleanup of the samples were accomplished using sequential columns based on sulfuric acid/silica, alumina and 5% PX21-Amoco Carbon Silica. Extracts were analysed initially by GC HRMS using a split/splitless injector and a 60m DB5MS column. The chromatographic conditions were as follows: initial temperature 120^{o}C, initial held 1.5minutes, raised to 220^{o}C at 20^{o}C/min, raised to 320^{o}C @2.5^{o}C/min, held for 15 min. The injection temperature and transfer line temperature were held at 280^{o}C and 260^{o}C respectively, and the ion source was held at 280^{o}C

The GC methods were transferred to a GC TOF and the extracts were re-injected using a PTV injector. The GC TOF was operated in EI mode with the source temperature at 200^{o}C, the GC re-entrant was at 260^{o}C and the heptacosa reference reservoir was at 80^{o}C. Detector voltage was at 2900,

the electron energy was at 70eV and the TOF MS scan m/z was 100-950amu. The scan time was 0.9sec and the interscan delay was 0.1sec.

Results and Discussion:

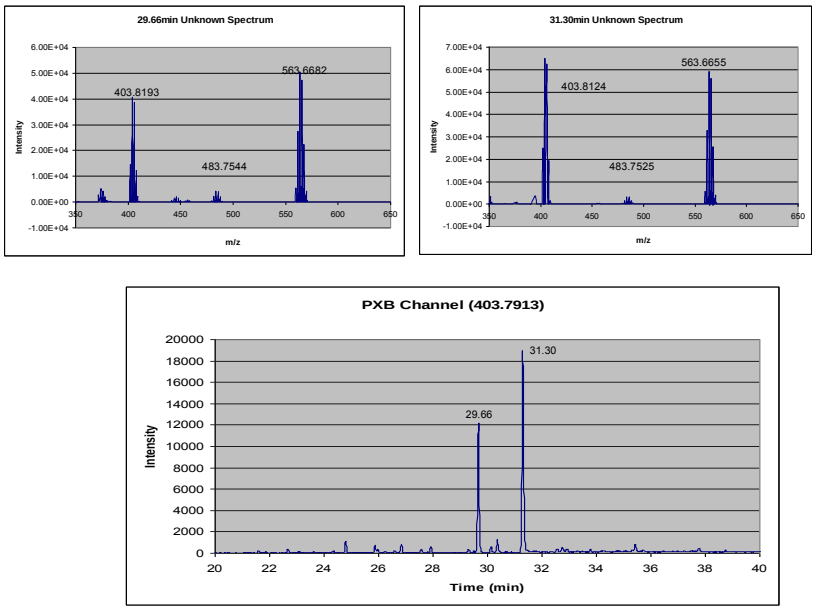
The use of accurate mass GC/TOF increased the sensitivity of the unknown compounds in full scan mode and thus permitted subsequent structural identification. The unknowns were identified as penta-BDEs from the NIST mass spectral libraries included with the instrument. Further confirmation by GC HRMS showed that these two compounds were indeed PBDE 100 and 99. We had been observing the M-Br_2 ion (403.7870/405.7850) of these two compounds in this PXB channel (403.7913/405.7866). An instrument resolution $m/\Delta m \geq 90\,000$ would be necessary to separate the M-Br_2 penta-BDE ions from the PXBs. Extracts from the non ortho/mono ortho fractions as identified by MOE method 3418 were not expected to contain any PBDEs and as such we can only surmise that the concentration of PBDE 100 and 99 in the original samples were so large that fractionation after extraction was compromised and subsequent breakthrough of these compounds into the dioxin like PCB fraction is what we had been observing. MOE Method 3418 was developed prior to PBDE determinations and the presence of these compounds did not affect the Co-PCB measurements. This method has since been modified to ensure that any breakthrough has been minimized. Monitoring M^+ for penta-BDEs to identify and correct for any potential breakthrough problems that could occur is suggested.

Table 1: PXB Molecular Ions and Penta-BDE Molecular Ions and M-Br_2 Ions

PXB	77	126A	126B	126C	118	105	157	169
ION 1	379.8191	369.8305	457.7296	413.78	369.8305	369.8305	403.7913	403.7913
ION 2	381.8167	371.8276	459.7274	415.7776	371.8276	371.8276	405.7866	405.7866
Penta-BDE							Molecular Ion	M-Br_2 Ion
ION 1							563.6215	403.787
ION 2							565.6195	405.785

We initially thought that the peaks at masses 403.7913/405.7886 indicated the presence of isomers of the monobrompentachlorobiphenyl homolog series (ie the same mass channel as PXB 169). In instances where there is a lack of commercial standards available it is not uncommon to assume that compounds in the same mass channels share common structural characteristics and can be assumed to be isomers of each other as long as isotopic ratio criteria are met. The use of response factors compared to an identified compound within the mass window can then be used to calculate a concentration of that unknown compound and that concentration can be included to create a total homolog concentration calculation. This is typically the case in the analysis of hydroxy PBDEs and hydroxy PCBs where several compounds are not commercially available and many unidentified peaks that meet minimum isotopic ratio criteria are present in the resulting chromatogram.

Figure 1: Ion Spectra of the two observed unknowns



Conclusions:

QA/QC criteria are important in compound confirmation. Selected ion monitoring and the determination of peak area ratios $(M)^+/(M+2)^+$ with acceptance criteria set at +/-15% is crucial. Other potential isobaric interferences in this mass range may possibly include tribromodibenzofuran. (403.7870/405.7850) which when compared to PBDE100 and 99 and other pentaPBDEs would require a mass spectral resolution of greater than 90,000 for separation. The importance of generating or having access to the full scan spectra of routine compounds could be of great benefit when dealing with the analysis of non-routine low level compounds. Focusing on the molecular ion may lead to misleading conclusions. As a result, caution should be used when considering unknowns that happen to fall into the same mass range as Penta-BDE homologues as they may not be structural isomers as has been presumed historically. One must also be aware of carry over that may occur with high level samples which may have ramifications not only in further clean-up procedures but subsequent analysis methods. Even at $m/\Delta m \geq 10{,}000$ the potential for interferences from other compounds and/or other homolog groups exists.

Refereneces:

Ohta, S., H. Tokusawa, T. Nakao, O. Aozasa, H. Miyata and M. Alaee. 2008. Global Contamination of Coplanar Polybrominated/Chlorinated Biphenyls (Co-PXBs) in the Market Fishes from Japan. Chemosphere 73:S31-38.

Alaee, M, Pacepavicius, G, Reiner, E, MacPherson, K, Fayez, L, Nakao, T, Ohta, S (2008), Determination of Co-Planar Polybrominated-chlorinated Biphenyls in Fish from Great Lakes: Preliminary Results and Analytical Challenges. Organohalogen Compounds, Volume 70, 768- 771