IDENTIFICATION OF HALOGENATED POLYCYCLIC AROMATIC COMPOUNDS IN BIOLOGICAL SAMPLES FROM ALBERTA OIL-SANDS REGION

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1 INTRODUCTION

Polycyclic aromatic compounds (PACs) are a complex class of compounds that are present in fossil material, such as petroleum oils. The most common PACs are the polycyclic aromatic hydrocarbons (PAHs) compounds of which 16 have been identified as priority compounds by United States Environmental Protection Agency (US EPA).[1] However, there are other important PACs that to date have received less attention. These include halogenated polycyclic aromatic compounds (HPACs), non-halogenated alkylated PAHs and heterocyclic aromatic compounds that contain S-, O- and N- atoms.[1,2] Like the PACs, HPACs are likely to be persistent in the environment.[2] In addition, the toxicity of some HPACs has been found to be similar to dibenzo-p-dioxins and dibenzofurans (PCDD/Fs).[3,7]

HPACs has been detected and quantified in environmental matrices and abiotic samples, such as waste incinerators, electronic wastes, atmosphere, and sediment.[6-9] To date, there are limited numbers of reports of HPACs in biological organism. This is due in part to the lack of analytical method for identification and quantification of these compounds and because of the lack of analytical standards for HPACs.[4]

Historically, the Alberta Oil-Sands (AOS) was covered by seawater millions year ago, which would have contained high concentrations of chloride and bromide ions.[5] Based on this, we hypothesize that are HPACs present in biotic samples from this region. To test this hypothesis, biological samples from the AOS were analyzed for HPACs using gas chromatography high resolution time of flight mass spectrometry (GC/HRTOF-MS) operating at a resolving power of ~25,000 (*see* Figure 1). Under these conditions, 3 HPACs were identified in biota.

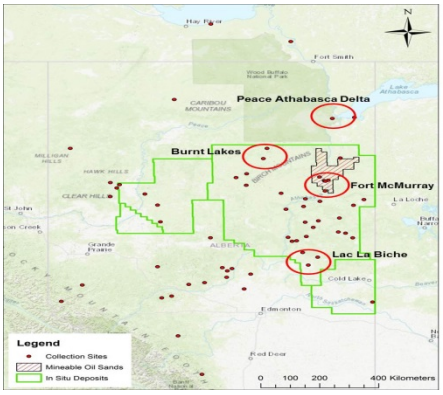

Fig 1. Sample collection sites

2 EXPERIMENTAL

2.1 Materials

All organic solvents used were of high-purity (Optima grade) and obtained from Fischer Chemical. Individual HPACs of 1-chloropyrene (1-Cl-Pyr), 7-bromobenz[a]anthracene (7-Br-BaA), 7-chlorobenz[a]anthracene (7-Cl-BaA), 9-chloroanthracene (9-Cl-Ant), 9-chlorophenanthrene (9-Cl-Phe), 7,12-dichlorobenz[a]anthracene (7,12-Cl2-

BaA) were from Cambridge Isotope Laboratories (Tewksbury, MA, USA). 1-bromopyrene (1-Br-Pyr), 1-chloroanthracene (1-Cl-Ant), 1,5-dichloroanthracene (1,5-Cl2-Ant), 2-bromofluorene (2-Br-Fle), 2,7-dibromofluorene (2,7-Br2-Fle), 3-bromophenanthrene (3-Br-Phe), 5-bromoacenaphthene (5-Br-Ana), 9-bromophenanthrene (9-Br-Phe), 9,10-dibromoanthracene (9,10-Br2-Ant), 9,10-dibromophenanthrene (9,10-Br2-Phe) were from Sigma Aldrich (St Louis, MO, USA).

2.2 Sample preparation

Liver samples of otters (*n=6*), northern pike (*n=4*), lake whitefish (*n=4*) and snails (*n=3*) were collected in 2014 and 2015 from the sites shown in Figure 1 (circled in red). Approximately 1.5 grams of each sample (wet weight) was weighed and mixed with diatomaceous earth and transferred to an accelerated solvent extraction cell size and spiked with the RIS (100 ng) prior to extraction. Details of the ASE extraction and clean-up steps can be found in Idowu *et al*. [10]

2.3 GCxGC-HRTOFMS condition

Analysis of the HPAC extracts and standard solutions were performed using a 7890A GC (Agilent Technologies, Wilmington, DE, USA) fitted with a split/splitless injector, coupled with high-resolution time-of-fight mass spectrophotometer (TOF-MS) Pegasus 4D HRT (LECO, St Joseph, MI, USA) operated in positive electron ionization mode and calibrated with perfluorotributylamine, PFTBA. The stationary phase was Rxi -5Sil MS (60 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA) followed by a Rxi-17Sil MS (2 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA). Standards and samples (2 μL) were injected at 250 ° C in splitless mode. The sample was analyzed in 1D mode. The parameters of GC and MS were reported in Idowu et al.[10] In 1D mode, the oven was programmed from 80 ºC (held for 1 min), heated to 210 ºC at 35 ºC/min, further to 260 ºC at 3 ºC/min and finally to 315 ºC at 10 ºC/min (held for 5 min). The MS transfer line temperature was at 300 ºC, ion source temperature at 250 ºC and Helium as carrier gas supplied at 1.4 mL/min in both modes. The HRTOF-MS was operated at a mass range of m/z 50 – 500 with an acquisition rate of 13 spectra/second (1D) and approximately 200 spectra/ second (2D) at 70 eV. The MS system was calibrated daily using PFTBA as the mass calibration gas. The resolving power (full width half height) of the system was typically greater than 25 000 based the peak width of *m/z* 218.9851 of PFTBA.

3.0 RESULTS AND DISCUSSION

Instrument Verification. As a check of the performance of the GC/HRTOF-MS, the mean experimental Δm was first measured on the 16 HPACs. Based on our knowledge of the EI fragmentation behavior of the HPACs, the theoretical exact *m/z* values for the abundant ions characteristic to each HPAC were compared with the experimentally measured mean *m/z* values (5 replicate injections of the standard solution) determined by our system at a RP of 25,000. The repeatability of replicate measurements of the analytical standards ranged from 0.1 to 0.6 mmu and in all cases the mean mass accuracy was lower than ±3.5 ppm. Taken together, these results imply that the GC/HRTOF-MS is well-suited for identifying these compounds in environmental samples.

Compound Identification Workflow. The next step was to use the verified method to determine if any HPACs could be identified in any of the 4 biota samples. To do so, total ion chromatograms (TICs) were first deconvoluted by constructing exact mass extracted ion chromatograms (XICs) for the 2 prominent ions characteristic of each of the 16 HPACs. The next step in the identification workflow was to compare the experimentally measured mass for the 2 prominent ions in the samples to the theoretically expected *m/z* value. Measured Δm's of ±5ppm were considered reliable for analyte identification. Another consideration in the identification of HPACs in our samples was to insure that r_t of identified compounds eluted within a reasonable r_t relative to the analytical standard of similar halogenated content and PAC backbone. Concentrations of HPACs in the samples were then estimated by

comparing the electronically integrated area of peaks in the exact mass XIC in the samples to the area of HPAC standard of similar halogenated type and content and with a similar PAC backbone. With the limited number of isomers currently available it is not possible to discern the substitution positions of the halogen atoms on the HPACs identified in our samples.

Identification of HPACs. For lake whitefish, a dichloro-anthracene/phenanthrene [$C_{14}H_8Cl_2$, (Cl_2-Ant/Phe)] was identified in all 4 samples (*see* Table 1*)*. The r_t time of this compound was ~18.0 mins which was in good agreement with the elution of the 1,5-Cl_2-Ant standard. The mean experimental measured masses (*n=4*) of the 2 characteristic ions in the EI mass spectra of this compound were 245.9995±0.0007 and 176.0619±0.0003 amu. Compared with the theoretically expected mass, this corresponds to respective Δm's of -1.0976±1.5037 and -0.9372±0.8399 ppm. Using the response factor of 1,5-Cl_2-Ant, mean concentrations of Cl_2-Ant were estimated to be 16.3 ± 11.4 ng/g, lipid weight (lw). Dichloro-anthracene/phenanthrene was also identified and detected in one river other sample. The peak in the chromatogram that we ascribe to be Cl_2-Ant/Phe had an experimental measured mass of 245.9993 and 176.0625 with Δm's of -1.7480 and 2.7263, respectively. The concentration of this compound in the river otter sample was ~3× smaller than those measured in lake whitefish.

Two brominated compounds were also tentatively identified in our samples. A monobrominated anthracene/phenanthrene [$C_{14}H_9Br$, (Br-Ant/Phe)] and a dibrominated fluorene [$C_{13}H_8Br_2$, (Br_2-Fle)] were detected in northern pike and in a snail sample (*see* Table 1*)*. The r_t of the Br-Ant/Phe was the same as that of the 9-Br-Phe and with a similar r_t it is reasonable to suggest that the compound in our sample is 9-Br-Phe. The experimentally measured masses of the 2 characteristic ions of Br-Ant/Phe in the northern pike samples were 255.9876/176.0621 which correspond to a Δm of -2.3829/0.5111 ppm, respectively. Similarly for the snail sample, Δm values of 0.8203 and 1.9879 ppm were obtained for the 2 prominent ions characteristic to this compound. The lipid-corrected concentrations of Br-Ant/Phe were estimated to be 12.5 and 170.5 ng/g in northern pike and snail, respectively, and were based on the response factor of 9-Br-Phe.

The experimentally measured *m/z*-values for the 2 prominent ions of Br_2-Fle in the northern pike and snail samples were 323.8971/163.0567 and 323.8959/163.05412, respectively. The r_t of this compound in our samples agreed well with that of 2,7-Br_2-Fle (19.8 mins). Measured Δm's were all within ± 3ppm of the expected *m/z* value. Based on the response factor of 2,7-Br_2-Fle, the concentration of Br_2-Fle in northern pike and snail were estimated to be 26.7 and 111.4 ng/g, lipid weight, respectively.

To date non-halogenated PACs have been the focus of monitoring activities in the AOSR. The addition of a halogen atom onto the PAC backbone, however, will make these compounds less water soluble than the non-halogenated parent PACs and increase their fugacity in water. Therefore, we can expect partitioning of HPACs from the aquatic environment into biological organisms to be greater than their non-halogenated analogs. Because the addition of a halogen atom, in particular a Cl-atom, will render PACs more environmentally persistent, we postulate that these compounds could in fact be more bioaccumulative than non-halogenated PACs. Clearly, the lack of authentic standards limited our ability to identify more of these compounds.

Table 1. Concentrations (ng/g, lw) of 3 HPACs in biota samples from AOS region using GC-HRTOF-MS.

Species	HPAC Detected	Measured Mass	Mass Accuracy (Δm, ppm)	Concentration (ng/g, lw)[a]
Lake Whitefish	Cl_2-Ant/Phe	245.9995	-1.0976	16.3 ± 11.4[b]

		176.0619	-0.9372	
Snails	Br-Ant/Phe	255.9884	0.8203	170.5
		176.0624	1.9879	
	Br_2-Fle	323.8959	-2.4390	111.4
		163.0541	-0.6746	
River Otter	Cl_2-Ant/Phe	245.9993	-1.7480	5.5
		176.0625	2.7263	
Northern Pike	Br-Ant/Phe	255.9876	-2.3829	12.5
		176.0621	0.5111	
	Br_2-Fle	323.8971	1.1114	26.7
		163.0547	2.7598	

[a]lw= lipid weight; [b]mean ± SD measured in 4 samples

4.0 ACKNOWLEDGEMENTS

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