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Polycyclic Aromatic Hydrocarbons in the Digestive Glands of the American Lobster, *Homarus americanus*, Captured in the Proximity of a Coal-Coking Plant

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For many years two coal-coking plants on the shoreline of Sydney Harbour, Nova Scotia, discharged their liquid effluents through a pond into the South Arm of the harbor (Uthe and Musial 1986). One plant was shut down in 1981 and the second in 1983. In 1982 the South Arm of Sydney Harbour was closed to commercial lobster fishing due to elevated polycyclic aromatic hydrocarbon (PAH) concentrations in the digestive glands (hepatopancreas) of American lobsters (*Homarus americanus*) (Uthe and Musial 1986). A study in 1984 (Uthe and Musial 1986) supported the continuation of the closure.

Sydney Harbour lobsters were reexamined in 1991. In addition to the commonly measured PAHs, a multitude of unknown peaks were present in the 1991 gas chromatograms in contrast to the earlier liquid chromatography chromatograms . Some PAHs are known carcinogens or are suspected of playing a role in the carcinogenic process (Lee et al 1976). Investigation of PAHs and sulfur-, nitrogen-, and oxygen-containing heterocyclic aromatic hydrocarbons (PASH, PANH, and PAOH, respectively) in lobster digestive glands is described in this study.

MATERIALS AND METHODS

Lobsters (generally 10) were captured using standard commercial traps and transported live to the laboratory where the intact digestive gland was removed, weighed, and frozen. Samples from a pool of the digestive glands from five market-sized (0.87 ± 0.12 kg) lobsters were extracted and extracts cleaned up according to Uthe and Musial (1986), substituting Biobeads SX-12 for SX-3 to improve separation. Pooled samples were used because of the large variation in PAH concentrations in individual lobster digestive glands (Uthe and Musial 1986). Samples were selected from three different pools and analyzed in triplicate for PAHs and heterocyclic aromatic hydrocarbons (HACs). Operational blanks, spike and recovery studies for common PAHs made up 20 % of every batch study. PAH and HAC standards (Table 1) were purchased from Supelco (Canada) or Ultra Scientific (Toronto, Canada).

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Extracts were analyzed by gas chromatography (Hewlett-Packard, 5890 Series II gas chromatograph) with mass spectrometry (Hewlett-Packard, 5971 Mass Selective Detector) (GC/MS) in both full scan and selective ion monitoring (SIM) modes. Information collected from the full scan (Figure 1) was used to develop a SIM method to: 1). focus on specific compounds; 2). increase peak response; and 3). filter out interfering data. PAHs and HACs were separated on a Supelco Spb^{thm-5} capillary column (30 m, 0.25 mm id, 0.25 μm film thickness). The gas chromatograph was equipped with a cool on-column, pressure and temperature programmable injection system. Cool on-column injection is a technique of introducing a sample as a liquid sample directly into a GC column. Due to the lack of prior vaporization the cool on-column injector eliminates mass discrimination problems. Analyses were carried out using the injection system in the oven tracking mode to maximize chromatographic resolution. The oven tracking feature maintains the inlet temperature three degrees higher than the oven temperature to optimize repeatibility. Spectra were acquired and processed with a Hewlett-Packard Vectra 386/25 data system. Identification was accomplished by using retention indices, mass spectral information, and authetic reference standards. Retention indices were compared with those of Vassilaros et al. (1982) and Wise et al. (1988). Mass spectra interpretation was based on interpretation by hand, comparison with published information (Later et al. 1981; Willey et al. 1981; Yu and Hites 1981; Bjorseth et al. 1983 and 1985), and comparison with standards.

RESULTS AND DISCUSSION

The earlier PAH studies measured only common, non-alkylated PAHs; however coal tar contains many PAHs and HACs (Wise et al. 1988). GC/MS scan (Figure 1) revealed a multitude of compounds in the extract from a pooled heptopancreas sample: 1. alkylated and non-alkylated PAHs, 2. PANH, 3. PAOH, and 4. PASH.

The digestive glands extracts were re-analyzed by GC/MS in the SIM mode. Table 1 lists alkylated and unalkylated PAHs and HACs shown in Figure 2, their retention indices, their major ion, and their estimated concentrations in the original tissue pool. Compounds in Table 1 joined by a "/" indicate possible identities, within our capabilities. Most reported concentrations are estimates, based on external standardization and using the response factor of the nearest eluting PAH or HAC present in the external standard. Variation is estimated to be 30-40 % by this technique. The common PAH results (Table 1) are based on internal standardization. Recovery studies were carried out only for a few common PAHs, as noted in Table 1.

Response factors for lighter and heavier compounds (Figure 2) were comparable. Chromatography process starts at relatively low temperatures, therefore thermally liable components may not be exposed to thermal stress. Little maintenance was required with the injector system, because the sample passes directly into the

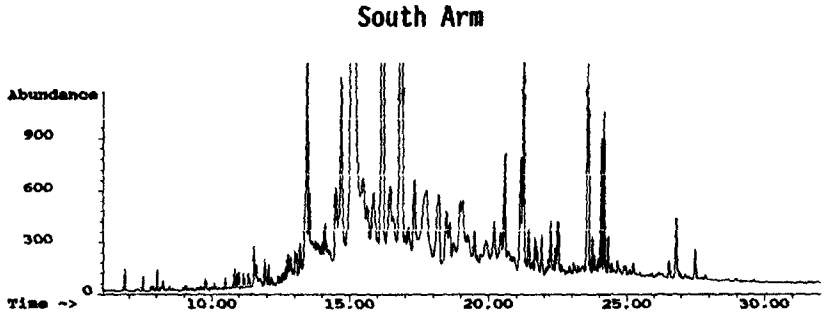

Figure 1. GC/MS, full scan mode to analyze digestive glands extract.

column.

Benzo[*b*]fluoranthene/benzo[*j*]fluoranthene and chrysene/ triphenylene could not be distinguished by GC/MS (Figure 3). Benzo[*k*]fluoranthene and 7,12-dimethylbenz[*a*]anthracene also co-eluted, but were separated based on their mass spectra (Figure 3).

Fortification studies showed 50-100% PANH loss by our extraction procedure. Specific PANH, e.g. carbazoles, benzocarbazoles, dibenzocarbazoles, and acridines, present in coal tar (Lee et al. 1982), were not found when fortified at 10 to 2000 ng·g^{-1} levels and those present in the sample and quantified in Table 1 are biased low due to such losses. Heavier molecular weight PAHs, such as dibenzopyrenes, were also lost. Few PAOH and PASH were identified in digestive glands extracts. Many unidentified peaks are still present at low concentrations (~5 to ~20 ng·g^{-1} wet wt.). Taking recovery losses into consideration,

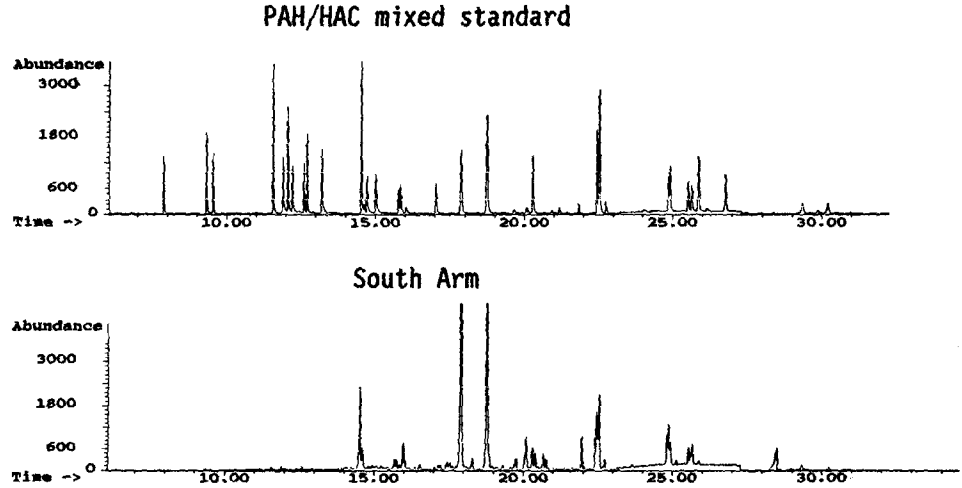

Figure 2. Comparing PAH/HAC mixed standard to South Arm sample (SIM).

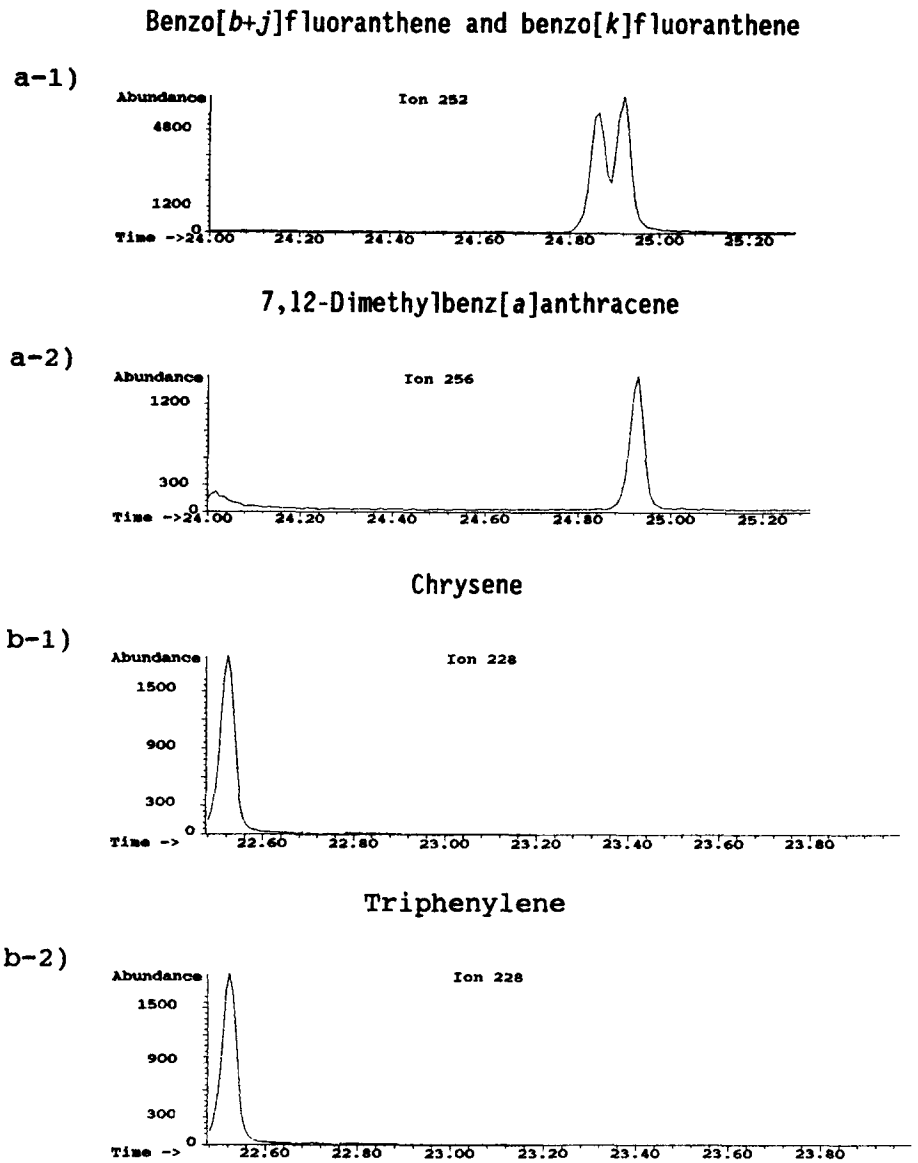

Figure 3 Co-elution problems: a-1)benzo[*b*+*j*]fluoranthene; benzo[*k*] fluoranthene a-2) 7,12-dimethylbenz[*a*]anthracene; b-1) chrysene and b-2) triphenylene

concentrations of some of the unknown peaks may be high enough to warrant investigation. Common PAH concentrations were comparable with those determined by reversed-phase liquid chromatography/fluorescence used previously (Uthe and Musial 1986). Our study confirmed the continuing elevated PAH concentrations in digestive glands of lobsters from the South Arm of Sydney Harbour. PAH concentrations led us to investigate

Table 1. PAH and HAC concentrations (ng·g^{-1} wet wt.) in digestive glands of lobsters. SIM data.

Compound	Retention Index[A]	Major Ion	ng·g^{-1}
Naphthalene	200.00[E]	128	50[B]
2-Methylnaphthalene	220.50[E]	142	~30[C]
1-Methylnaphthalene	223.39[E]	142	~20[C]
2,7-Dimethylnaphthalene/ 2,6-Dimethylnaphthalene	240.38	156	~50[C]
1,7-Dimethylnaphthalene	242.84	156	~80[C]
1,6-Dimethylnaphthalene/ 1,3-Dimethylnaphthalene	243.43	156	~50[C]
1,4-Dimethylnaphthalene/ 2,3-Dimethylnaphthalene	246.21	156	~30[C]
Acenaphthylene	248.01[E]	152	48[B]
Acenaphthene	253.71[E]	154	69[B]
Dibenzofuran	259.17[E]	168	~90[C]
Trimethylnaphthalene	262.70	170	~50[C]
Trimethylnaphthalene	264.68	170	~20[C]
2,3,6-Trimethylnaphthalene	265.13	170	~80[C]
2,3,5-Trimethylnaphthalene	267.19	170	~70[C]
Fluorene	270.04	165	150[B]
Trimethylnaphthalene	270.99	170	~30[C]
9-Methylfluorene	274.18	180	~50[C]
2-Methylfluorene	288.34	180	~120[C]
1-Methylfluorene	289.66	180	~170[C]
Methylfluorene	291.25	180	~110[C]
9-Fluorenone	294.79	181	~100
Phenanthrene	300.00[E]	178	1140[B]
Anthracene	301.37[E]	178	230[B]
Benzoquinoline[D]	307.27	179	~150[C]
Phenanthridine[D]	308.81	179	~150[C]
Benzo[*f*]quinoline[D]	309.09	179	~130[C]
Benzoquinoline[D]	310.68	179	~70[C]
Benzoquinoline[D]	311.75	179	~180[C]
Benzoquinoline[D]	312.46	179	~60[C]
Methyldibenzothiophene	313.83	198	~150[C]
3-Methylphenanthrene	319.26[E]	192	~150[C]
4H-Cyclopenta[*def*]phenanthrene[D]	321.88	190	~1000[C]
1-Methylphenanthrene/ 4-Methylphenanthrene/ 9-Methylphenanthre	323.66	192	~250[C]
Methylbenzoquinoline[D]	336.22	193	~40[C]
3,6-Dimethylphenanthrene	337.86[E]	206	~90[C]

Table 1. continued.

2,7-Dimethylphenanthrene	339.47[E]	206	~100[C]
Fluoranthene	344.85[E]	202	4660[B]
Acephenanthrylene	347.76	202	~230[C]
Phenanthro[4,5-*bcd*]thiophene	349.34	208	~100[C]
Pyrene	351.85[E]	202	3310[B]
Benzo[*a*]fluorene	366.80[E]	216	~40[C]
Benzo[*b*]fluorene/Methylpyrene	369.03[E]	216	~300[C]
2-Methylpyrene	370.38	216	~280[C]
Methylfluoranthene	374.24	216	~250[C]
1-Ethylpyrene	385.32	230	~90[C]
Benzo[*b*]naphtho[2,1-*d*]thiophene	390.15	234	~190[C]
Benzo[*c*]phenanthrene[D]	391.54	226	~600[C]
Benz[*c*]acridine[D]	392.80	229	~70[C]
Phenanthro[*b*]naphtho[4,3-*b*] thiophene	395.32	234	~40[C]
Benzo[*b*]naphtho[2,3-*d*]thiophene	395.74	234	~50[C]
4H-Cyclopenta[*cd*]pyrene[C]	397.69	226	~60[C]
Benz[*a*]anthracene[D]	398.23[E]	228	940[B]
Chrysene[D]/Triphenylene	400.00[E]	228	1260[B]
Naphthacene	408.04[E]	228	~150[C]
Aza-chrysene[D]	411.26	229	~10[C]
8-Methylbenz[*a*]anthracene/ 6-Methylbenz[*a*]anthracene	417.53	242	~170[C]
2-Methylchrysene/ 5-methylchrysene[D]	419.04	242	~40[C]
4-Methylchrysene/ 6-Methylchrysene[D]	420.85	240	~90[C]
1-Methylchrysene	422.12	240	~130[C]
7-Methylbenz[*a*]anthracene[D]	422.97	240	~100[C]
1,3-Dimethyltriphenylene	431.47	254	~40[C]
Benzo[*b*+*j*]fluoranthene[D]	443.41[E]	252	1080[B]
Benzo[*k*]fluoranthene[D]	444.17[E]	252	571[B]
Benzo[*a*]fluoranthene[D]	446.86	252	~57[C]
Benzo[*e*]pyrene	452.40[E]	252	520[B]
Benzo[*a*]pyrene[D]	454.19[E]	252	720[B]
Perylene	457.10[E]	252	~100[C]
Dibenz[*a*,*h*]acridine[D]	488.26	279	~10[C]
Indeno[1,2,3-*cd*]pyrene[D]	493.70[E]	276	33[B]
Dibenz[*a*,*h*]anthracene[D]	496.30[E]	278	51[B]
Dibenz[*a*,*c*]anthracene[D]	497.14[E]	278	~50[C]
Benzo[*b*]chrysene[D]	499.15[E]	278	~60[C]
Picene	500.00[E]	278	~200[C]
Benzo[*ghi*]perylene[D]	501.21[E]	278	200[B]

Table 1. continued.

[A] Relative standard deviations for retention index (n=4) $\leq$ 0.088%.

[B] The common PAH concentrations were based on internal (deuterated (D-10 phenanthrene and D-12 chrysene) standardization. Recoveries ranging from 80 to 120 % for surrogates (D-10 anthracene; D-12 benz[*a*]anthracene; D-12 benzo[*b*]fluoranthene and D-12 benzo[*a*]pyrene) recoveries ranging from 80 to 120 %.

[C] Other PAH and HAC concentrations were determined using response factor of nearest eluting PAH or HAC component in external standard.

[D] Compound identified in literature as being involved in the carcinogenic process.

[E] Reference standard available for compound.

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other PAHs and HACs in digestive glands of lobsters. An estimated 80 compounds were identified in this study, most of them alkylated PAH. Our results summed PAH concentration was higher than that previously due to the increased number of compounds measured. Some of the new compounds identified are suspected carcinogens, namely benzo[*c*]phenanthrene, 7-methylbenz[*a*]anthracene, 5-methyl chrysene, benzo[*a*]fluoranthene, benzo[*b*]chrysene, and the benzacridines, dibenzacridines, benzoquinolines, and dibenzanthracenes (Lee et al. 1976; Dong et al. 1978; Thakker et al. 1978; Yu and Hites 1981). The concentration of benzo[*c*] phenanthrene is comparable to that of benzo[*a*]pyrene and should be analyzed routinely along with the common PAH. The benzoquinolines were also present at high levels and many of these warrant further investigation. Benz and dibenzacridines were present at low levels; an improved extraction procedure for this class of compounds (PANH) is needed. Alkylated phenanthrenes, alkylated fluorenes, and fluorenones are suspected mutagens (Yu and Hites 1981). Alkylated phenanthrenes and alkylated fluorenes were present at notable levels and many of these compounds also warrant further study.

Our results from the South Arm of Sydney Harbour confirm the accumulation of high PAH and HAC levels in lobsters. It is probable that the South Arm of Sydney Harbour will remain closed to commercial fisheries for a long time, at least until the action to clean up the PAH contamination is completed.

Lobster digestive gland is viewed as a delicacy by many consumers. It would be interesting therefore to compare PAH and HAC concentrations in Sydney Harbour lobsters with PAH and HAC concentrations found in lobsters captured in other industrial and municipal harbors, or areas contaminated by creosote, coal-tar pitch, or other sources of PAHs.

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