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Polycyclic Aromatic Hydrocarbons in Human Fat and Liver

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Some polycyclic aromatic hydrocarbons (PAHs) are regarded as carcinogens. Many investigators have determined PAHs in various samples, for example, in water, air, cigarette smoke and food (OGAN 1979, DAISEY 1979, SNOOK 1977, FRETHEIM 1976, HORI 1973). Thus, one question arises. How much of these carcinogenic compounds has been accumulated in human tissues? TOMINGAS et al. (1976) showed that ppb levels of benzo(a)pyrene were detected in human bronchial carcinoma tissues.

Recently many investigators have applied HPLC to analyze for PAHs. In our previous study, PAHs in marine samples were determined with HPLC equipped with a reversed-phase column and a fluorescence detector (OBANA et al. 1981). Good separation of the isomers benzo(a)pyrene and benzo(e)pyrene was obtained with high sensitivities; for instance, the detection limit of benzo(a)pyrene was 10 pg. In this report, we determined nine PAHs in human fat and liver without complex pretreatment prior to analysis.

METHODS

Human fat and liver from six people and fat from four people were obtained from autopsies. These tissues were free from cancer. Smoking habit, occupation and residence of each person were not clear. The amount of liver was 40 to 120 g and that of fat was 40 to 90 g. Each tissue was minced. For liver samples, 300 mL of KOH-EtOH was added and saponified. Alkaline solution was extracted three times with 100 mL of n-hexane. Fat samples were initially divided into 25 g portions for each sample and then saponified in 300 mL of KOH-EtOH. Each alkaline solution was extracted three times with 300 mL of n-hexane. Each n-hexane layer was combined and evaporated to about 300 mL. n-Hexane layer was extracted three times with 100 mL of dimethylsulfoxide and the dimethyl sulfoxide layer was poured into 300 mL of 15% NaCl solution and then reextracted three times with 100 mL of n-hexane. The n-hexane layer was washed three times with 100 mL of water and dried with Na_2SO_4 and concentrated to about 5 mL. The n-hexane extract was eluted on silica gel (upper: Merck art 7734, 130 °C 4 h act, 5 g) and alumina (lower: Merck 1097, nonactivation, 6 g) column chromatography. The first fraction was eluted with 70 mL of n-hexane. The second fraction was eluted with 80 mL of 15% Et_2O + 85% n-hexane and concentrated to 2 mL. PAH extract was injected into a liquid chromatography equipped with a varible wavelength fluorescence detector and a 20 cm x 4.6 mm i.d. column

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Table 1. PAHs in human liver (ppt: on a wet basis)

	1 F,54	2 F,27	3 F,65	4 M,65	5 M,51	6 M,41
anthracene	200	240	170	180	140	110
pyrene	450	460	340	470	310	270
benz(a)anthracene	ND	ND	ND	ND	ND	ND
benzo(e)pyrene	ND	ND	ND	ND	ND	ND
benzo(b)fluoranthene	88	81	87	68	53	33
benzo(k)fluoranthene	15	23	10	17	8	6
benzo(a)pyrene	13	32	19	22	10	11
benzo(g,h,i)perylene	59	48	36	45	21	17
dibenz(a,h)anthracene	ND	ND	ND	ND	ND	ND

F = Female; M = Male, age ND< 5 ppt

packed with Lichrosorb RP-18 (Merck, reversed-phase C_{18}) of 5 μm particle size. The mobile phase was 70% acetonitrile + 30% water and the flow rate was 2.0 mL/min.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of PAHs in human fat. Peaks of seven PAHs were detected but two compounds such as benz(a)-anthracene and dibenz(a,h)anthracene were not detected.

PAH concentrations in liver and fat are listed in Table 1 and 2, respectively. Though PAHs are detected in human liver and fat, the concentrations are quite low (ppt levels). They averaged 1100 ppt in the fat and 380 ppt in the liver. The next highest level was that of anthracene and it averaged 260 ppt in the fat and 170 ppt in the liver. The known carcinogens, such as benz(a)-anthracene and dibenz(a,h)anthracene are not detected in either tissues, but benzo(a)pyrene is present at a rather low level, 20 ppt in both tissues. It seems that the differences of sex and age do not affect the PAH contents in the tissues.

PAHs in human fat and liver were rather different from PAHs in marine samples in both concentration and composition. In our previous report, PAH concentrations in marine samples are higher than in human tissues. Benzo(a)pyrene was 0.3-2.6 ppb in oyster and 0.6-9 ppb in wakame seaweed. Pyrene was 7-52 ppb in oyster and 12-41 ppb in wakame seaweed. Although pyrene was the most abundant PAH in all cases, the next most abundant compound was anthracene in human samples, but benzo(e)pyrene and benzo(b)-fluoranthene in the marine samples.

TOMINGAS et al. (1976) reported that benzo(a)pyrene was detected at ppb levels in human bronchial carcinoma tissues. Among 24 carcinoma tissues, values were between 0.3 to 15,000 ppb

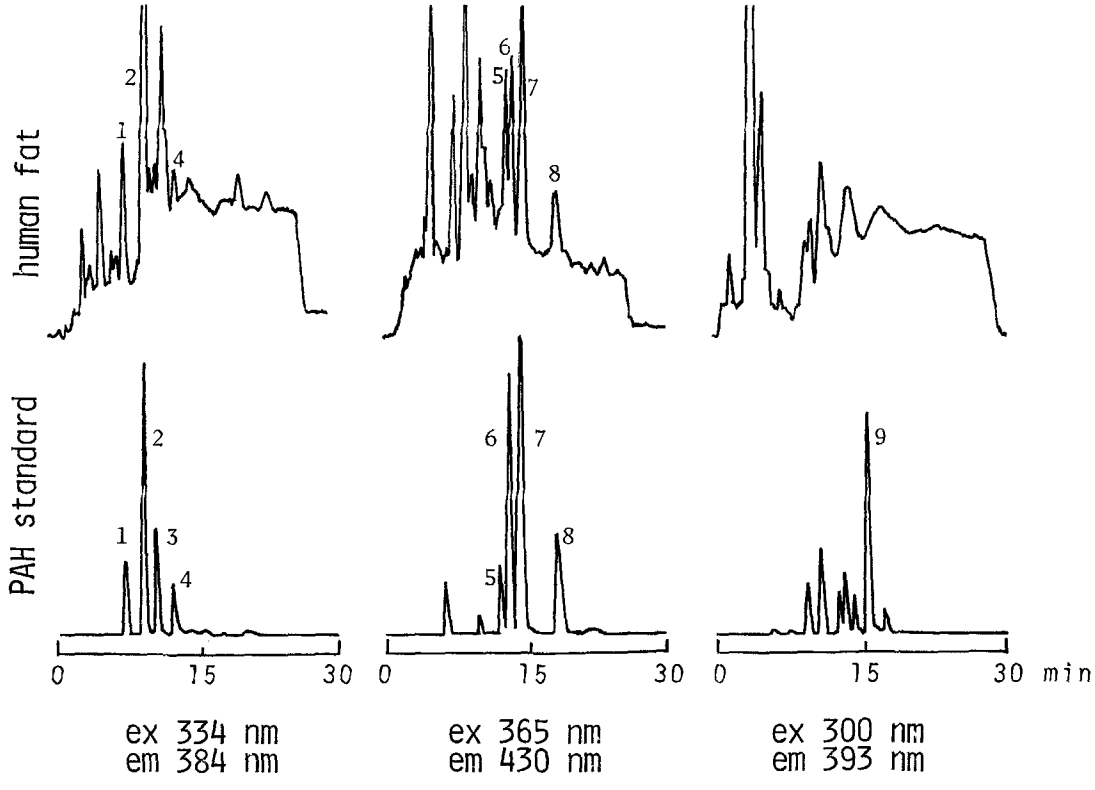

Fig. 1 HPLC chromatograms of PAHs from human fat and a mixture of nine PAH standards.
Peaks: 1 anthracene, 2 pyrene, 3 benz(a)anthracene, 4 benzo(e)pyrene,
5 benzo(b)fluoranthene, 6 benzo(k)fluoranthene, 7 benzo(a)pyrene,
8 benzo(g,h,i)perylene, 9 dibenz(a,h)anthracene.

Table 2. PAHs in human fat (ppt: on a wet basis)

	1 F,54	2 F,27	3 F,66	4 M,65	5 M,51	6 M,41	7 F,35	8 M,52	9 M,35	10 M,66
anthracene	575	440	260	190	420	140	ND	25	140	390
pyrene	780	920	890	650	1500	590	49	2000	1300	2700
benz(a)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
benzo(e)pyrene	71	110	64	57	140	83	49	30	41	150
benzo(b)fluoranthene	260	190	240	77	250	120	56	95	110	160
benzo(k)fluoranthene	28	40	38	17	48	27	ND	11	11	42
benzo(a)pyrene	31	25	24	18	59	18	ND	12	16	19
benzo(g,h,i)perylene	110	62	61	54	69	42	13	23	19	32
dibenz(a,h)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

F = Female; M = Male, age ND < 5 ppt

and 16 samples were less than ten ppb. However, the difference of benzo(a)pyrene concentration between the fat or liver and the lung might be due to the accumulation of inhaled particles in the lung. Other toxic chemicals, i.e., PCBs in human fat and liver were determined by WATANABE (1980). PCBs in human fat are 1.5 ppm and those in liver are 0.09 ppm. PCB levels are much higher than the PAHs in human fat and liver, though both are environmental contaminants. Although the levels of PAHs in human fat and liver are quite low, it is apparent that these compounds are accumulated to some degree in human tissues. It seems that HPLC with a fluorescence detector is a good apparatus to determine low levels of PAHs in human samples and further research is necessary to know if there is any relation between cancer and carcinogenic PAH accumulation.

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