

Measurement of Polynuclear Aromatic Hydrocarbons in Canine Lung after Alkaline Decomposition

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ABSTRACT. An alkaline decomposition method employing a KOH/alcohol solution was studied, and polynuclear aromatic hydrocarbons (PAHs) contained in particles remaining in canine lung were measured. As a result, BaA, BkF, BaP, and BghiP were found. By this method, PAHs extracted from the lungs of 32 dogs were 13.0–166.0 ng (mean, 63.0 ng) for BaA, 6.6–90.2 ng (mean, 27.4 ng) for BkF, 9.8–167.4 ng (mean 47.2 ng) for BaP, and 10.8–206.0 ng (mean, 61.8 ng) for BghiP. The results showed no correlation between the age and the concentration of PAHs in the lung, but some correlation was found between the age and the lung weight ($p < 0.01$). There were significant correlations among the concentrations of the compounds in the lung ($p < 0.01$). These results suggest that dogs, like humans, are affected by automobile exhaust and other common generation sources of such substances.

KEY WORDS: alkaline decomposition, canine lung, PAH.

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Particles suspended in ordinary atmospheric air contain different kinds of trace carcinogenic substances [2–6, 9, 10, 12, 19, 20]. Of these, polynuclear aromatic hydrocarbons (hereinafter referred to as PAHs) are generated incidentally to incomplete combustion and, therefore, are considered major carcinogenic substances in the air centered on urban districts where there are many automobiles, and other combustion engines. It is suspected that atmospheric pollution level influences the occurrence of lung cancer, because the incidence rate of lung cancer in urban districts is higher than that in rural districts [15].

As a popular companion to humans, dogs are closely related with humans' lives and environments, particularly in urban districts as well. Dogs tend to be affected by environmental pollution. Takemoto [18] reported that dust is deposited in the lungs of dogs as well as humans. Abe [1] examined 1,000 lungs of dogs that lived in urban districts and found seven cases of lung cancer among 14 cases of neoplasm. Thus, the influence by worsening the environmental air pollution is a major concern not only for humans but also for dogs. There have been, however, few reports on the measurement of PAHs in canine lungs.

In this study, we examined pretreatment methods for measuring PAHs, which are major airborne carcinogenic substances. All the lungs used in this study were obtained from dogs sacrificed for other experimental purposes. The results are based on analysis of lungs from 32 dogs.

MATERIALS AND METHODS

Collection of canine lungs: The canine lungs examined for the present study were unused ones from dogs subjected to other experiments between 1995 and 1997. Each of the

collected canine lungs was chopped, minced and homogeneously mixed.

Collection of airborne particles: Airborne particle samples were collected on quartz fiber filters (Pallflex Products Corporation, Tokyo) with a high-volume air sampler (Kimoto Electric Corporation, Tokyo) at a flow rate of 1.18–1.25 m³/min. Reagents: A solution of 1M KOH/ethanol (hereinafter referred to as KOH/EtOH) was prepared from special-grade potassium hydroxide (Kokusan Chemical, Tokyo), ethanol of agricultural chemicals of residue-test grade (Wako Pure Chemical, Osaka) and a minimum quantity of deionized water. For liquid-liquid distribution extraction after decomposition, n-hexane of agricultural chemicals of residue-test grade (Wako Pure Chemicals, Tokyo) was used and, for its dehydration, special-grade anhydrous sodium sulfate (Kokusan Chemicals, Tokyo) was used.

Seven PAHs were tested: benzo(a)anthracene (BaA), benzo(a)pyrene (BaP) (Wako Pure Chemicals, Osaka); benzo(k)fluoranthene (BkF), benzo(b)chrysene (BbC), dibenzo (a, h) anthracene (dBaA) (Koch-Light Laboratories, UK); benzo(ghi)perylene (BghiP) (Aldrich Chemical Company, U.S.A.); and dibenzo(a,e)pyrene (dBaEP) (Chemical Procurement Laboratories, U.S.A.). Standard source liquids of these PAHs were prepared with dimethyl sulfoxide (DMSO) (Wako Pure Chemical, Osaka) of agricultural chemicals of residue-test grade and subsequently diluted with acetonitrile of high-performance liquid chromatography grade (Kokusan Chemicals, Tokyo) to produce about 1 ng/ml as standard solutions.

PAH analysis by HPLC: A pre-concentration HPLC/fluorescence spectrophotometry system was used for PAH analysis. This instrument consists of three Shimadzu HPLC

pumps (LC-6A), auto sample injector (SIL-6A), system controller (SCL-6A), column oven (CTO-2A), fluorescence spectrophotometer (RF-540), integrator (C-R3A), high-pressure six-way control valve (FCV-2AH), Hitachi mixer, Elma Optical degasser (ERC-3520) and waveform selecting NEC personal computer (PC-9801). The concentrating column used was a stainless steel column (ID 4.6 mm, 30 mm length) equipped with ODS-60-5 (Tokyo Chemicals, Tokyo), and the separation column was a stainless steel column (ID 4.6 mm, 250 mm length) equipped also with ODS-60-5.

Alkaline decomposition: Three-gram samples were taken from each of the canine lungs and, in reference to the method of Seto *et al.* [14], subjected to alkaline decomposition as follows. 50 ml of KOH/EtOH solution (1M) was added to the sample, after which the entire solution was heated with a mantle heater and subjected to 2-hr of decomposition. The solution was left to cool to about 50°C, mixed with 15 ml of n-hexane, then allowed to cool to room temperature and mixed with 50 ml of deionized water. The solution was transferred to a separating funnel and vigorously shaken for one minute to extract organic components. The lower layer (water layer) was transferred to another separating funnel, then mixed with 15 ml of n-hexane and vigorously shaken. This cycle of steps was repeated again. The upper layer (organic layer) was collected, mixed with 50 ml of deionized water and then vigorously shaken for washing. The extract obtained was dehydrated with anhydrous sodium sulfate and then concentrated by rotary evaporator; only the solvent was removed by distillation under nitrogen flow. Thereafter, it was dissolved in 1 ml of acetonitrile for HPLC analysis.

The recovery rate of the standard solutions of PAHs by alkaline decomposition and reproducibility: To verify the

effectiveness of the alkaline decomposition method for extraction of PAHs from dog lungs, the recovery rate of the PAH standard solutions and the reproducibility of the results were examined. Table 1 shows the recovery rate of the standard solutions alone, Table 2 that of the standard solutions in the presence of the canine lungs, and Table 3 that of the standard solutions after alkaline decomposition in the presence of the canine lungs and airborne particles. Figure 1 shows the HPLC chromatograms of individual compounds. Since, BaA, BkF, BaP and BghiP provided satisfactory results among the compounds tested, these four PAHs were subjected to further measurements in this study.

RESULTS

Measurement of PAHs on lung specimens of 32 dogs: Alkaline decomposition was performed with the lungs of 32 dogs, and the solutions obtained were subjected to HPLC for measurement of BaA, BkF, BaP and BghiP. Table 4 sum-

Table 1. Recovery in percentage after standard PAH addition to canine lung

Compound	Standard (%)	Lung+Standard (%)
BaA	117.8	106.8
BkF	100.7	99.4
BaP	99.1	96.8
dBahA	102.0	105.6
BbC	104.3	99.5
BghiP	112.4	96.7
dBaeP	104.0	105.8
Average	105.8	101.5

Table 2. Results of extraction efficiency of PAHs in suspended particulate canine lung

Compound	Particle (ng/mg)	Lung (ng/g)	RR (%)
BaA	3.85	0.12	94.0
BkF	4.63	0.05	100.2
BaP	4.98	0.12	85.5
BghiP	10.40	0.10	103.0

RR: recovery ratio.

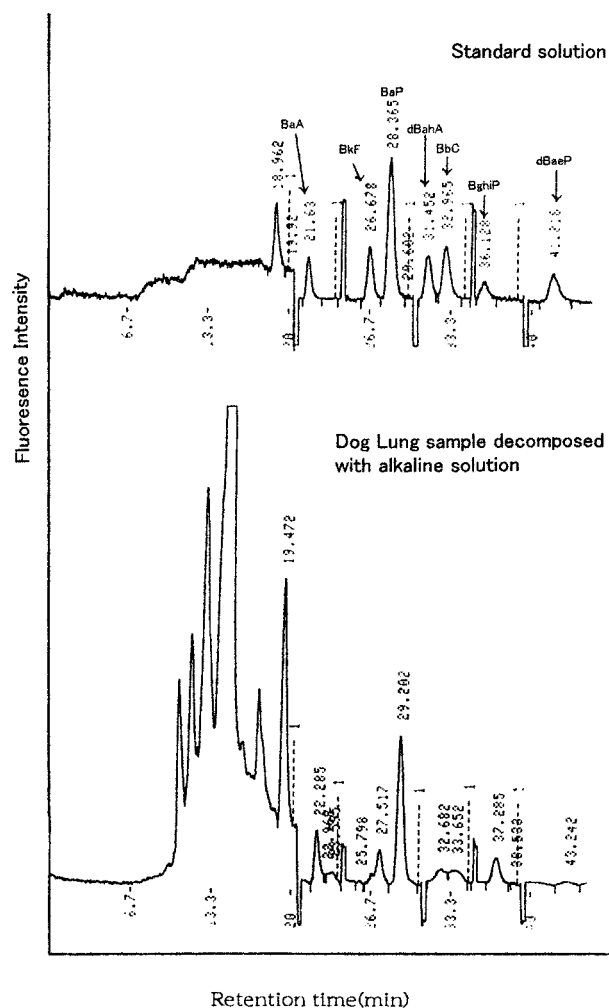


Fig. 1. Excitation and emission wavelengths.

Table 3. Results of reproducibility for alkaline decomposition of canine lung

Sample	PAHs concentration (ng/g •Lung)			
	BaA	BkF	BaP	BghiP
A (n=4)	0.18 ± 0.042 [23.3]	0.06 ± 0.003 [5.0]	0.12 ± 0.020 [16.7]	0.15 ± 0.022 [14.7]
B (n=4)	0.18 ± 0.007 [3.9]	0.1 ± 0.007 [7.0]	0.18 ± 0.013 [7.2]	0.23 ± 0.018 [7.8]
C (n=4)	0.14 ± 0.032 [22.8]	0.05 ± 0.008 [16.0]	0.1 ± 0.005 [5.0]	0.12 ± 0.012 [10.0]
D (n=4)	0.13 ± 0.013 [22.9]	0.05 ± 0.007 [14.0]	0.07 ± 0.007 [10.0]	0.11 ± 0.021 [19.1]

Average ± SD. []: C.V (%).

Table 4. PAH concentrations in canine lungs

	Age	Weight (kg)	Lung (g)	PAHs (ng/g•Lung)				PAHs (ng/Lung)			
				BaA	BkF	BaP	BghiP	BaA	BkF	BaP	BghiP
n	32	32	32	32	32	32	32	32	32	32	32
Ave.	5.06	17.08	350.18	0.19	0.08	0.13	0.17	62.90	27.35	47.25	58.75
S.D.	2.27	6.33	150.40	0.08	0.03	0.06	0.08	35.70	16.94	31.21	36.74
MAX	12.00	33.00	558.40	0.47	0.16	0.26	0.26	166.00	90.20	167.40	206.00
MIN	1.00	5.00	115.20	0.07	0.03	0.05	0.05	13.00	6.60	9.80	10.80

S.D.: Standard deviation.

marizes the mean concentrations of PAHs determined in the 32 dogs, the standard deviation, and the range. The age, body weight, and lung weight of the dogs are also shown. As shown in this table, the amount per dog was 13.0–166.0 ng (mean, 63.0 ng) for BaA, 6.6–90.2 ng (mean, 27.4 ng) for BkF, 9.8–167.4 ng (mean, 47.2 ng) for BaP, and 10.8–206.0 ng (mean, 61.8 ng) for BghiP. The difference between the maximum and minimum values for individual PAHs was about 13-fold (BaA), 14-fold (BkF), 17-fold (BaP), and 19-fold (BghiP). Table 5 shows the relationship between the amounts of different PAHs in terms of correlation coefficients, which was over 0.8 for all PAHs, indicating a significance

DISCUSSION

This is the first study measuring the PAH concentrations in canine lungs. It is also the first one in demonstrating the usefulness of the alkaline decomposition method in eliminating fat, protein, and of some other substances obtained in the canine lung tissue, which hinder sensitivity and accuracy of extraction or measurement. In this study, there were as large as 13 to 19 times differences in PAHs in the individual lungs of the 32 dogs observed upon pretreatment by alkaline decomposition. To identify the factors, correlations between age and PAHs were investigated. The results were $r = -0.09$ for BaA, -0.07 for BkF, -0.08 for BaP, and -0.12 for BghiP, showing no correlation between age and PAH concentration in the lung. We further investigated to find whether the concentration of PAHs was correlated with the body weight or lung weight. The correlation coefficient

Table 5. The correlation between PAHs in the canine lungs

	BaA	BkF	BaP	BghiP
BaA	1			
BkF	0.87**	1		
BaP	0.89**	0.97**	1	
BghiP	0.83**	0.87**	0.88**	1

**: $P < 0.01$.

was 0.2–0.4 for the body weight and 0.58–0.68 for the lung weight, indicating that the correlation was higher between the lung weight and the concentrations of PAHs.

The mean concentration of BkF was 0.41 ± 0.25 ng/g of canine lung, that of BaP 0.54 ± 0.34 ng/g, and that of BghiP 0.86 ± 0.57 ng/g. Although these levels were 1/4–1/5 of those reported by Seto *et al.* [13, 14] for human subjects, there was significant correlation among individual PAHs ($r = 0.83$ – 0.97), indicating that dogs are, like humans, most probably affected by car exhaust and other sources of these compounds [7, 8, 11, 16, 17], and that it is necessary to improve the environment to achieve reduction of PAHs in the lung. We will further investigate by accumulating data on the relationship between PAHs and sexes or living environments, individual differences, and absorption in to the lung.

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