DETERMINATION OF GASEOUS ORGANIC COMPOUNDS IN HYOGO PREFECTURE, JAPAN

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Abstract. The aim of this study was to analyze gaseous organic chemicals (GOCs) of high traffic (Nishinomiya City: 979,987 vehicles/day) and low traffic areas (Miki City: 29,338 vehicles/day) by gas chromatography-mass spectrometry (GC-MS) and to evaluate general environment exposure by PAHs in GOCs. After air sampling using an OMNIPORE membrane filter (< 0.45 μm) and Porapak-QS, sorbents were extracted with solvent (dichloromethane: acetone (4:1 v/v), and analysis was carried out by GC-MS. Oxidative derivatives of diethylbenzene, such as diacethylbenzene and ethylacetophenone, were detected in GOCs. PAHs and phthalates in GOCs were measured. Pyrene, benz[a]anthracene, benzo[a]pyrene and benzo[ghi]perylene level were significantly higher in high traffic areas. The geometric mean of pyrene was 0.76 ng/m^3 for low traffic areas and 1.96 ng/m^3 for high traffic areas; benz[a]anthracene was found at 0.72 ng/m^3 and 1.80 ng/m^3 in low and high traffic areas, respectively; benzo[a]pyrene was found at 0.87 ng/m^3 and 3.60 ng/m^3 in low and high traffic areas, respectively and benzo[ghi]perylene was found at 0.57 ng/m^3 and 3.04 ng/m^3 in low and high traffic areas, respectively. The bis(2-ethylhexyl) phthalate (DEHP) level was the highest in the detected GOCs. The geometric mean of the DEHP levels in high traffic and low traffic areas were 484.85 and 387.26 ng/m^3, respectively. Adult and child DEHP exposure levels were 145.32 and 300.33 ng/kg/day, respectively, in high traffic areas. In low traffic areas, adult and child DEHP exposure levels were 116.18 and 240.10 ng/kg/day, respectively.

INTRODUCTION

Major air pollution incidents in the 1950s raised public awareness of the health hazards associated with deterioration in air quality. For Japan, the health hazards of sulfur oxides, nitrogen oxides and particulate matter (PM) also became a public concern. The sulfur oxide level has decreased following regulation of exhaust gas and the control of power plant fuel, except for nitrogen oxides. However, PM has not tended to decrease.

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Quinones present in PM can act as catalysts to produce reactive oxygen directly and may be key compounds in PM-based oxidative stress (Xia *et al*, 2004). Polycyclic aromatic hydrocarbons (PAHs) can induce oxidative stress indirectly, through biotransformation by cytochrome P450, expoxide hydrolase, and dihydrodiol dehydrogenase to generate redox active quinones (Penning *et al*, 1999). Therefore, many reports have considered the actions of the compounds contained in PM. It is well known that PM contains many PAHs and exposure to PAHs increases the risk of cancer in humans. The PAHs have received considerable attention as an important class of environmental organic pollutants. Epidemiological studies have suggested that there is a clear relationship between mortality and the

concentration of ambient PM with an aerodynamic diameter less than 10 μm (PM_{10}), and an increase in the PM_{10} level is associated not only with respiratory disorders but also with acute cardiovascular events (Dockery *et al*, 1993; Samet *et al*, 2000; De Leon *et al*, 2003). Recently, the focus has begun to shift to health effects arising from inhalation of fine particles. It has been reported that the association between $PM_{2.5}$ and cardiovascular disease mortality is strong and a 10 $\mu g/m^3$ increase in $PM_{2.5}$ from mobile sources accounts for a 3.4% increase in daily mortality (Laden *et al*, 2000; Pope *et al*, 2004). The air contains ultrafine particles, which are smaller than $PM_{2.5}$ and have unknown health effects. Further, the no bound-particle fraction of PAHs and other chemicals (gaseous organic compounds: GOCs) has the possibility to exist in ambient air. Numerous epidemiologic studies have demonstrated an association between elevated levels of ambient particles and morbidity or mortality. However, gas-phase ambient organic air contaminants have not been studied thoroughly. The aim of this study was to determine and compare GOCs in high and low traffic areas and evaluate PAHs exposure on the general population.

MATERIALS AND METHODS

Reagents

Dichloromethane and acetone were purchased from Wako Pure Chemical Industries, (Osaka, Japan). 1,2-Diacetylbenzene, 1,2-diethylbenzene, 1,3-diacetylbenzene, biphenyl, benzo[ghi]perylene, 1,4-diacetylbenzene and 1,4-diethylbenzene were purchased from Sigma-Aldrich (St Louis, USA). Benz[a] anthracene, acenaphthene, acenaphthyrene, benzo[a]pyrene and phenanthren were purchased from Tokyo Chemical Industry (Tokyo, Japan). Fluorene, pyrene, n-butyl benzyl phthalate and fluoranthene were purchased from Nakarai tesque (Kyoto, Japan). These compounds were used for mass spectra structure analysis. All reagents and solvents used were analytical grade.

Study sites

As shown in Fig 1, an area of high traffic and an area of low traffic in Hyogo Prefecture were selected. The distance between Nishinomiya City (representing the high traffic area) and Miki City (representing the low traffic area) is about 35 km (about 22 miles) in a straight line. Nishinomiya, with an approximate population of 446,500, has two highways: route 43 (67,138 vehicles/day) and Hanshin Expressway (912,849 vehicles/day) through the city, with a total of 979,987vehicles/day. Miki, with an approximate population of 75,000 people, has route 175 (29,338 vehicles /day).

GOC collection sites and number of samples

GOCs were collected from low traffic and high traffic areas in Hyogo Prefecture, Japan. The sampling period was October 2005 to December 2006. The total number of collected samples were 14 (low traffic area was 6 samples and high traffic area was 8 samples) and the sampling time was from two to four weeks (about 20 to 40 m^3). The sampling periods in the low traffic and high traffic areas overlapped.

Collection of GOCs

The GOC collecting system was composed of a MP-∑3 pump (Sibata, Tokyo, Japan), a 100 mm x 22 mm (length x diameter) Allihn type funnel with fused-in fritted glass disc (100-160 mesh), OMNIPORE membrane filter (0.45 μm Millipore, USA) and Porapak-QS polymer beads (50-80 mesh). Before use, the Porapak-QS polymer beads were washed with methanol followed by dichloromethane to remove organic contaminants. To prevent adsorption of impurities from the air, polymer beads were stored in a methanol solvent until use. The Allihn type funnel was packed with Porapak-QS (5 g) and the inlet was closed with an OMNIPORE membrane filter, which was

wound with Tephrone tape. The outlet of the Allihn type funnel was connected to the MP-∑3 pump. GOCs were collected on the Porapak-QS at a flow rate of 1 liter/minute. The Allihn type funnels were protected against light during and after collection by wrapping in aluminium foil.

Preparation of collected GOCs

The GOC was collected on Porapak-QS and then extracted with 250 ml of mixed solvent [dichloromethane:acetone (4:1 v/v)] in a soxhlet apparatus for 24 hours. The extraction solvent was evaporated to 1.0 ml in a KD-condenser.

GC-MS analysis and calibration procedure

GC-MS measurements were performed in a model QP-5000 (Shimadzu, Kyoto, Japan) connected to a CG-17A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a SLB-5ms fused silica capillary column (30 m x 0.25 mm i.d. with 0.25 μm film thickness) (SUPELCO, Bellefonte, PA) with helium as the carrier gas.

Injection was performed in split less mode (spilt opening after 4 minutes, column head pressure 150 kPa, injector 250ºC). The oven temperature program was as follows: 80ºC for 10 minutes, then increased at a rate of 10ºC/minute up to 350ºC, and holding at 350ºC for 8 minutes. The mass spectrum scan mode and the selected ion monitor (SIM) were operated in electron impact mode at 70 eV. At least five different concentrations across the working range were measured in duplicate. Calibration curves were calculated by the least-squares method.

Calculation of phthalate esters and PAH exposure

We estimated phthalate and PAH exposure with the following equation (On-line reference):

Inhalation exposure (ng/kg/day) = C_n x V_i ÷ Bw_i

C_n= mean concentration of phthalates or PAHs in GOCs (ng/m^3)

The value of this C_n is the mean concentration of phthalates and PAHs as shown in Table 2.

V_i = Respiration volume (adults: 15 m^3/day, children: 9.3 m^3/day)

Bw_i = body weight (adults: 50 kg, children: 15 kg).

Statistical analysis

The data were analyzed with the Mann-Whitney *U* test with a level of significance set at $p<0.05$.

RESULTS

The GC-MS total ion chromatograms (TIC) of GOCs for Nishinomiya and Miki City are shown in Fig 2. The TIC of Nishinomiya was different from Miki. Many peaks were observed after 25 minutes in the TIC for Nishinomiya (Fig 2A). These peaks may be diesel engine discharge compounds (> C_{22}).

The mass spectra of the congener compounds in the GOCs are shown in Fig 3. These mass spectra were observed for TIC of GOCs at retention times from 5 to 15 minutes. The mass spectrum in Fig 3A indicates 1,2-diethylbenzene (1,2-DEB). The mass number at m/z 134 appeared to be a molecular ion (M^+). The fragment ion m/z 105 appeared to be $(C_2H_5C_6H_4)^+$. The mass spectrum in Fig 3B indicates 2′-ethylacetophenone (2-EAP). The mass number at m/z 148 appeared to be M^+. The fragment ions m/z 133 and m/z 105 appeared to be $(M\text{-}CH_3)^+$ and $(C_2H_5C_6H_4)^+$, respectively. DEB has three structural isomers, and these hydroxyl derivatives of DEB had an asymmetric carbon. The mass spectrum in Fig 3C shows presumably one of 1,2-EPE or 1,2-EPEi. The mass number m/z 222 appeared to be M^+. The fragment ions m/z 207 and m/z 132 appeared to be $(M\text{-}CH_3)^+$ and $(C_2H_5C_6H_4CH{=}CH_2)^+$, respectively. The six

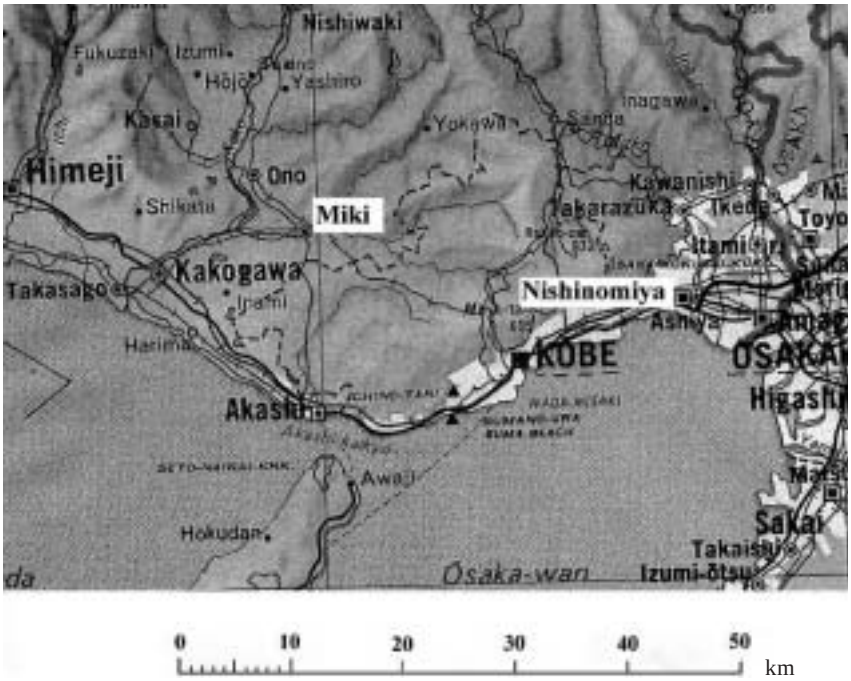

Fig 1–The geographical surroundings of the sampling points. Nishinomiya (high traffic area 979,987 traffic/day) has route 43 and the Hanshine expressway in the southern of the city and is located of Amagasaki City. Miki (low traffic area 29,338 traffic/day) is surrounded by mountains and is located north of Akashi City. The distance between Nishinomiya and Miki is about 35 km (about 22 miles) in a straight line.

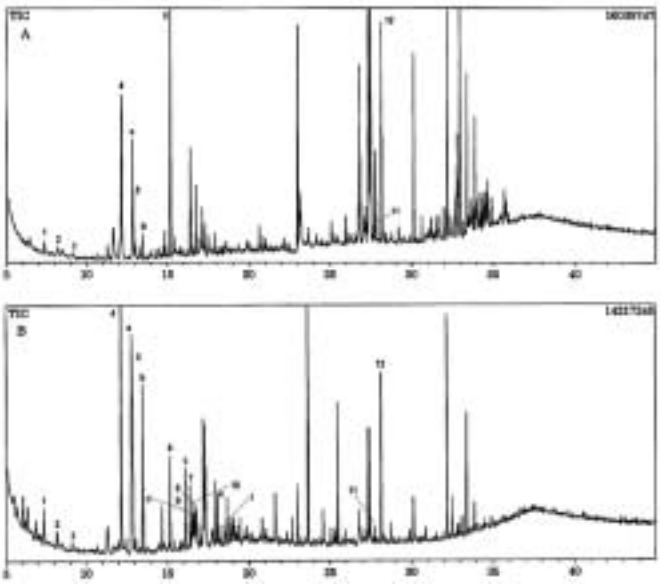

Fig 2–Total ion chromatogram (TIC) pattern of PM in Nishinomiya, and GOCs in Nishinomiya and Miki City. The peak labels correspond to those appeared in Table 1. A: TIC pattern of GOCs in Nishinomiya City. B: TIC pattern of GOCs in Miki City. The oven temperature program was as follows: 80ºC for 10 minutes; increase at a rate of 10ºC/minute up to 350ºC, and holding at 350ºC for 8 minutes.

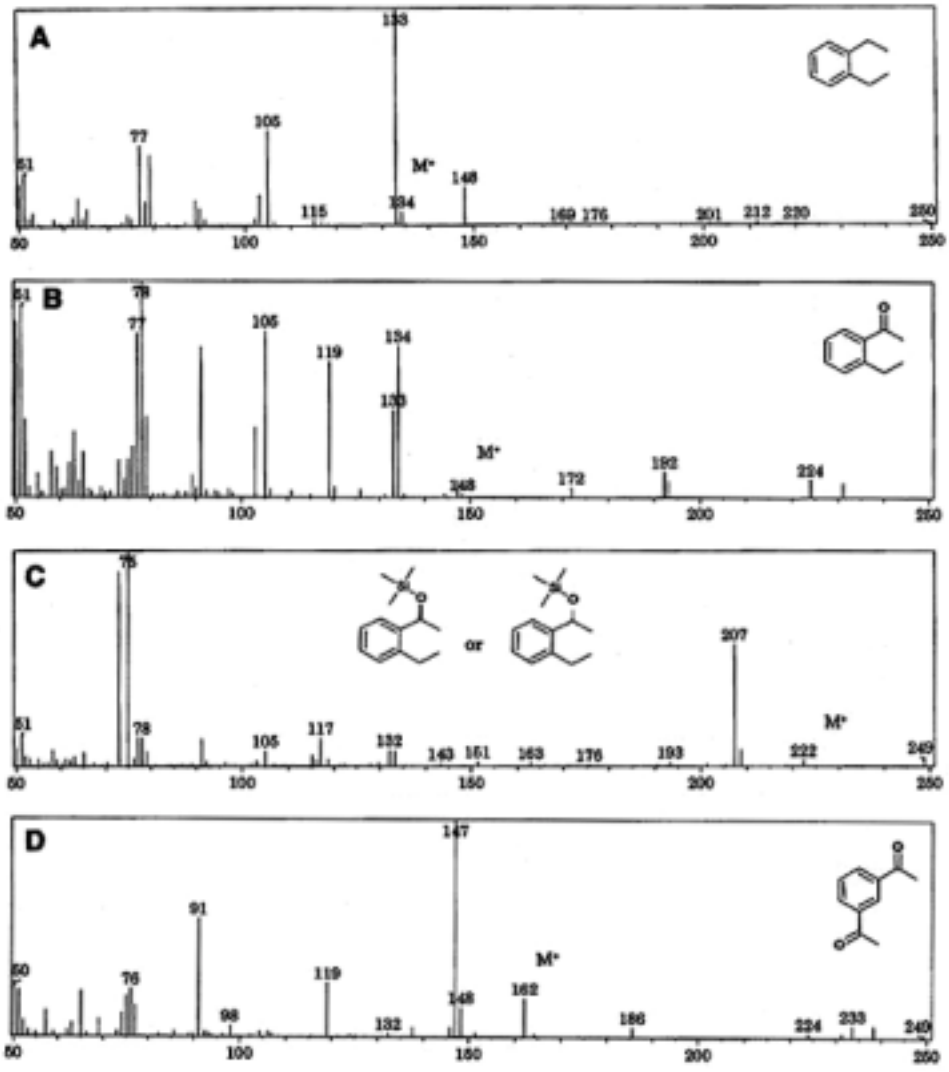

Fig 3–Mass spectra of compounds in gaseous organic chemicals.

A 1,2-Diethylbenzene; B 2′-Ethylacetophenone; C 1-(2′-ethylphenyl)ethanol or its symmetrical carbon containing isomer; D 1,3-Diacetylbenzen

peaks of the mass number m/z 207 were observed in GOCs. The fragment ion m/z 117 appeared to be $(CH_2C_6H_4CH{=}CH_2)^+$. The mass spectrum in Fig 3D is 1,3-Diacetylbenzene (1,3-DAB). The mass number m/z 162 appeared to be M^+. The fragment ion m/z 147 and m/z 119 appeared to be $(M\text{-}CH_3)^+$ and $(C_6H_4COCH_3)^+$, respectively.

The detected GOC compounds are shown in Table 1. All detected compounds had mass spectra and retention times of authentic compounds, except for six EPEs. Many detected compounds were related to DEB, except for BIP, DBBQ, BBP and DEHP. The levels of phthalate esters and PAHs in the GOCs are shown in Table 2. Pyrene, benz[a]anthracene, benzo[a]

Table 1
Compounds found in the air samples.

Detected compound	Abbreviation	MW	Formula	Peak label
1,2-Diethylbenzene	1,2-DEB	134	$C_{10}H_{14}$	1
1,3-Diethylbenzene	1,3-DEB	134	$C_{10}H_{14}$	2
1,4-Diethylbenzene	1,4-DEB	134	$C_{10}H_{14}$	3
2'-Ethylacetophenone	2-EAP	148	$C_{10}H_{12}O$	4
4'-Ethylacetophenone	4-EAP	148	$C_{10}H_{12}O$	5
Biphenyl	BIP	154	$C_{12}H_{10}$	6
D-1-(2′-ethylphenyl)ethanol	D-1,2-EPE	148	$C_{10}H_{12}O$	a
L-1-(2′-ethylphenyl)ethanol	L-1,2-EPE	148	$C_{10}H_{12}O$	b
D-1-(3′-ethylphenyl)ethanol	D-1,3-EPE	148	$C_{10}H_{12}O$	c
L-1-(3′-ethylphenyl)ethanol	L-1,3-EPE	148	$C_{10}H_{12}O$	d
D-1-(4′-ethylphenyl)ethanol	D-1,4-EPE	148	$C_{10}H_{12}O$	e
L-1-(4′-ethylphenyl)ethanol	L-1,4-EPE	148	$C_{10}H_{12}O$	f
1,2-Diacetylbenzene	1,2-DAB	162	$C_{10}H_{10}O_2$	7
1,3-Diacetylbenzene	1,3-DAB	162	$C_{10}H_{10}O_2$	8
1,4-Diacetylbenzene	1,4-DAB	162	$C_{10}H_{10}O_2$	9
Di-tert-butyl-1,4-benzoquinone	DBBQ	220	$C_{14}H_{20}O_2$	10
n-Butyl benzyl phthalate	BBP	312	$C_{19}H_{20}O_4$	11
Bis(2-ethylhexyl) phthalate	DEHP	390	$C_{24}H_{38}O_4$	12

MW=molecular weight

Table 2
Concentration of phthalates and PAHs in urban and rural GOCs.

Phthalate and PAHs	SIM	Gaseous organic chemicals (< 0.45 μm)				Mann -Whitney *U* test p
		Nishinomiya (*N*=8)		Miki (*N*=6)		
		GM ng/m^3	Range ng/m^3	GM ng/m^3	Range ng/m^3	
n-Butyl benzyl phthalate	m/z 149	15.86	2.15 - 59.64	16.69	4.47 - 89.49	0.897
Bis(2-ethylhexyl)phthalate	m/z 149	484.58	183.72 - 1,002.86	387.26	138.5 - 1,024.40	0.606
Acenaphthylene	m/z 154	85.25	4.61 - 1,010.99	53.06	5.19 - 609.96	0.606
Acenaphthene	m/z 153	9.84	3.93 - 24.44	7.71	1.18 - 163.23	0.606
Biphenyl	m/z 154	243.21	69.35 - 1,998.34	175.58	49.93 - 868.71	0.439
Fluorene	m/z 165	4.40	0.17 - 26.12	6.12	1.19 - 27.39	0.699
Phenanthrene	m/z 178	11.07	2.56 - 31.48	12.64	4.35 - 40.77	0.846
Fluoranthene	m/z 202	3.23	0.62 - 28.02	1.45	0.23 - 5.12	0.245
Pyrene	m/z 202	1.96[a]	0.74 - 4.97	0.76	0.39 - 1.62	0.020
Benz[a]anthracene	m/z 228	1.80[a]	0.41 - 3.50	0.72	0.17 - 2.00	0.039
Benzo[a]pyrene	m/z 252	3.60[a]	1.10 - 9.39	0.87	0.04 - 4.13	0.024
Benzo[ghi]perylene	m/z 276	3.04[a]	0.09 - 18.07	0.57	0.16 - 1.33	0.020

[a]$p<0.05$

Table 3
Estimated average inhalation exposure (ng/kg/day) of phthalate esters, biphenyl and PAHs.

Phthalate and PAHs	Nishinomiya		Miki	
	Adults	Children	Adults	Children
n-Butyl benzyl phthalate	4.76	9.83	5.01	10.35
Bis(2-ethylhexyl)phthalate	145.32	300.33	116.18	240.10
Acenaphthylene	25.58	52.86	15.92	32.90
Acenaphthene	2.95	6.10	2.31	4.78
Biphenyl	72.96	150.79	52.67	108.86
Fluorene	1.32	2.73	1.84	3.79
Phenanthrene	3.32	6.86	3.79	7.84
Fluoranthene	0.97	2.00	0.44	0.90
Pyrene	0.59	1.22	0.23	0.47
Benz[a]anthracene	0.54	1.12	0.22	0.45
Benzo[a]pyrene	1.08	2.23	0.26	0.54
Benzo[ghi]perylene	0.91	1.88	0.17	0.35

Adults: Respiration volume 15 m^3/day, Body weight 50 kg
Children: Respiration volume 9.3 m^3/day, Body weight 15 kg
(The respiration volume per day and the weight were the Japanese mean when the exposure of dioxin was estimated by the Japan Ministry of the Environment in 2001).

pyrene and benzo[ghi]perylene levels for Nishinomiya were significantly higher than that for Miki. As for other compounds Nishinomiya had higher levels than Miki except for fluorene.

Based on our measured phthalate and PAH levels, the inhalation exposure was calculated using the respiration volume per day and the weight (the respiration volume per day and the weight were the Japanese mean, when the Japan Ministry of the Environment estimated the exposure of dioxin in 2001). The estimated inhalation average exposure of phthalate esters and PAHs are shown in Table 3. The bis(2-ethylhexyl)phthalate (DEHP) level was the highest in the detected GOCs; adult and child DEHP exposures were 145.32 and 300.33 ng/kg/day, respectively, in Nishinomiya. In Miki, the adult and child DEHP exposures were 116.18 and 240.10 ng/kg/day, respectively. Phthalate and PAH exposures for children were higher than for adults. Pyrene, benz[a]anthracene, benzo[a]pyrene and benzo[ghi]perylene were significantly greater in adults and children in high traffic areas (Nishinomiya).

DISCUSSION

Many collection devices and sorbents have been employed to evaluate air pollution (Pellizzari *et al*, 1975). It has been reported that a good recovery pattern has been obtained for PAH on quartz micro-fiber, polyurethane foam (PUF), and XAD-2 (Iavicoli *et al*, 2006). Their results showed that pyrene, and benz[a]anthracene are adsorbed on quartz filter (at 0.3 and 2.3 ng/m^3, respectively), and naphthalene, and biphenyl are adsorbed on PUF (at 1,820 and 1,562 ng/m^3, respectively). In general, most PAHs are adsorbed or condensed onto airborne particles at high concentrations and in elevated temperatures present in smoke or exhaust pipes leading from the combustion processes in which these agents are generated. It has been reported

that the distribution of PAHs were determined for six sizes of airborne particles in Spain (Gutiérrez-Dabán *et al*, 2005). Their paper showed PAHs accumulated at higher percentages in the finest size range of submicron particles (smaller than 0.6 μm). It suggested that the existence of PAHs might relate to the number of benzene rings. For example, pyrene composed of four benzene rings, it easily adsorbed on the particle. Biphenyl, composed two benzene rings, exists as a gas. This present study did not use a quartz filter; the samples were collected through a membrane (<0.45 μm), and were adsorbed on Porapak-QS because the collected samples might contain particles <0.45 μm and gaseous substances. In this study pyrene was found in significantly higher levels in high traffic areas, Four PAHs (< 0.45 μm) were adsorbed and the other one may be a GOC. Some compounds were found on GC/MS analysis.

The detected compounds of GOC were DEB and DEB derivatives (Table 1). It has been reported that 1,2-DAB was metabolite of 1,2-DEB which behaves as a neurotoxin and a blue chromogen was induced (Gagnaire *et al*, 1990, 1991; Kim *et al*, 2002; Spencer *et al*, 2002; Tshala-Katumbay *et al*, 2005). 1,2-DEB is a moderately volatile, colorless liquid found in gasoline, kerosene, and fuel oil. A metabolic pathway of 1,2-DEB has been proposed that metabolizes to 1,2-EPE by ω-1 oxydation and forms 1,2-EPE and 1,2-DAB in rats (Payan *et al*, 1999).

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