Determination of Endocrine Disruptors in Automobile Exhaust Particulate Matter

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Thirty-one endocrine disruptors, including alkylphenols, phthalates and styrene oligomers, in diesel and gasoline exhaust particulate matter were determined simultaneously by gas chromatography/mass spectrometry (GC/MS). When particulate matter was subjected to Soxhlet extraction and Kuderna-Danish concentration, four phthalates, an adipate and five styrene trimers were found to exist as contaminants from the ambient air. Regarding the other 21 compounds, only benzo[*a*]pyrene and *n*-pentylphenol were detected in the range of 0.5–11 and 0.1–1.1 μg/km driving distance, respectively, from both diesel and gasoline exhaust particulate matter. When particulate matter was subjected to only ultrasonic extraction, the above phthalates, adipate and styrene trimers were not detected as contaminants from the ambient air. Using this treatment method, the above phthalates, adipate and styrene trimers were not detected in either of the two types of exhaust particulate matter. The above results revealed that the presence of such endocrine disruptors as benzo[*a*]pyrene and *n*-pentylphenol in diesel and gasoline exhaust particulate matter must be investigated in greater detail. Furthermore, it becomes clear that prevention of contamination during sample pretreatment is necessary.

Key words —— endocrine disruptor, diesel exhaust, gasoline exhaust, gas chromatography/mass spectrometry

INTRODUCTION

Hormones influence many aspects of the body, including metabolism regulation and the body's sexual characteristics. Estrogens are the hormones that influence the development and maintenance of female sex characteristics and the maturation and function of the sex organs, and testosterone serves a similar function in males. It is now becoming clear that a wide variety of both man-made and natural chemicals are capable of mimicking these hormones. These so-called endocrine disruptors have caused great concern all over the world.[1)] The Japan Environment Agency has listed 67 chemicals that were suspected to be endocrine disruptors in 1998. This list included 47 pesticides and related chemicals, 17 industrial chemicals and 3 by-products of combustion.[2)]

In recent years, there has been an increase in the contribution to air pollution from diesel exhaust in urban areas. Diesel exhaust has been reported to have both anti-estrogenic[3,4)] and anti-androgenic[5)] activities *in vitro*, and to influence the reproductive-system *in vivo*.[6–8)] However, endocrine disruptors such as phthalates and styrene oligomers in diesel and gasoline exhaust have yet to be measured.

In order to determine the amount of endocrine disruptors in diesel and gasoline exhausts, we have previously developed a gas chromatography/mass spectrometry (GC/MS) method for the simultaneous determination of 31 endocrine disruptors in diesel and gasoline exhaust particulate matter.[9)] In this study, we determined the 31 endocrine disruptors in diesel and gasoline exhaust particulate matter.

MATERIALS AND METHODS

Chemicals —— Thirty endocrine disruptors, *p*-*n*-pentylphenol (Ph5), *p*-*n*-hexylphenol (Ph6), *p*-*n*-heptylphenol (Ph7), *p*-*n*-octylphenol (Ph8), *p*-*n*-nonylphenol (Ph9), bisphenol A (BPA), 2,4-dichlorophenol (DCP), diethyl phthalate (DEP), di-*n*-propyl phthalate (DPrP), di-*n*-butyl phthalate (DBP), di-*n*-pentyl phthalate (DPeP), di-*n*-hexyl phthalate (DHP), benzyl *n*-butyl phthalate (BBP),

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Table 1. Automobiles Tested in This Study

Vehicle	Type	Fuel	Particulate (μg/test)
D1	sedan	diesel	0.8
D2	truck	diesel	4.1
D3	truck	diesel	4.5
D4	truck	diesel	6.7
G1	wagon	gasoline	0.8
G2	truck	gasoline	0.6
G3	truck	gasoline	0.3
G4	sedan	gasoline	0.2

dicyclohexyl phthalate (DCHP), di-2-ethylhexyl phthalate (DEHP), di-2- ethylhexyl adipate (DEHA), 1,3-diphenylpropane (DPP), 2,4-diphenyl-1-butene (DPB), *cis*-1,2-diphenylcyclobutane (DPCB1), *trans*-1,2-diphenylcyclobutane (DPCB2), 2,4,6-triphenyl-1-hexene (TPH), 1e-phenyl-4e-(1-phenylethyl)tetraline (PPET1), 1a-phenyl-4e-(1-phenylethyl)tetraline (PPET2), 1a-phenyl-4a-(1-phenylethyl)tetraline (PPET3), 1e-phenyl-4a-(1-phenylethyl)tetraline (PPET4), 1a,3e,5e-triphenylcyclohexane (TPCH1), 1e,3e,5e-triphenylcyclohexane (TPCH2), *n*-butyl benzene (BB), benzophenone (BP) and *p*-nitrotoluene (NT) were purchased from Wako Pure Chemical Industries (Osaka, Japan). The benzo[*a*]pyrene (BaP) standard solution was from Supelco (Bellefonte, PA, U.S.A.). Pesticide analysis-grade dichloromethane was from Wako Pure Chemical Industries.

Sampling and Pretreatment —— Diesel and gasoline exhaust particulate matter were collected on Pallflex Products (Putnam, CT, U.S.A.) Tx filers. Four diesel engine vehicles (D1–4) and four gasoline engine vehicles (G1–4) were used in this study (Table 1), and the vehicles were driven at 80 km/hr for 20 min.

In cases D1–3 and G1–3, the filters were weighed and the soluble organic fraction (SOF) was extracted with 100 ml of dichloromethane using a Soxhlet extractor. The extract was concentrated by a Kuderna-Danish (KD) evaporative concentrator. The residue was dissolved in 0.6 ml of dichloromethane and 1 μl of the solution was subjected to the GC/MS system.

In cases D4 and G4, the filters were washed twice ultrasonically with dichloromethane prior to the sampling of the particulate matter. Particulate matter was collected on a Tx filter, and SOF was immediately extracted ultrasonically for 10 min twice with 10 ml of dichloromethane. One micro liter of extract was subjected to the GC/MS system. It took less than 2 hr from the washing of the filter to the GC/MS injection.

GC/MS Analysis —— The GC/MS determination was performed in a Hewlett-Packard (Wilmington, DE, U.S.A.) 6890 gas chromatograph coupled to a Hewlett-Packard 5973 mass spectrometer. A Hewlett-Packard HP-5MS capillary column (30 m $\times$ 0.25 mm i.d. coated with 0.25 μm film thickness) was used for the separation of the 31 endocrine disruptors. The oven temperature was programmed at 40°C for 1 min, then to 10°C/min until 300°C, holding the final temperature for 30 min.

MS detection was performed in the selected ion monitoring (SIM) mode. Monitoring ions (*m*/*z*) were 164 for Ph5, 178 for Ph6, 192 for Ph7, 206 for Ph8, 220 for Ph9, 213 for BPA, 162 for DCP, 149 for the all phthalates, 129 for DEHA, 196 for DPP, 104 for DPB and DPCB2, 91 for DPCB1, TPH and BB, 207 for the PPET isomers, 313 for the TPCH isomers, 182 for BP, 137 for NT, 252 for BaP. The temperatures of the transfer line and the ion source were set at 250°C and 230°C, respectively.

RESULTS AND DISCUSSION

Prior to the analysis of the exhaust particulate matter, we determined the 31 endocrine disruptors in the extract from blank filters subjected to Soxhlet extraction and KD concentration. As listed in Table 2, 10 out of the 31 endocrine disruptors were significantly detected in the extracts from blank filters. These chemicals were four phthalates (DEP, DBP, BBP and DEHP), an adipate (DEHA) and five styrene trimers (TPH and PPET1–4). We considered these chemicals were contaminants from the ambient air, because they constitute the main components of plastics. Since the other 21 chemicals were not detected in the blank filters, it was concluded that the 21 compounds in the extracts were from exhaust particulate matte

Table 2 also lists the concentrations of the 21 endocrine disruptors in the extracts from diesel and gasoline exhaust particulate matter (D1–3, G1–3) subjected to Soxhlet extraction and KD concentration. Only BaP and Ph5 were detected in the range of 0.5–17 and 0.1–0.8 μg/ml, respectively, in the extracts from both diesel and gasoline exhaust particulate matter. Their values were calculated to be 0.5–11 and 0.1–1.1 μg/km driving distance, respectively.

Table 2. Concentrations (μg/ml) of the Endocrine Disruptors in Extracts from Diesel and Gasoline Exhaust Particulate Matter

Chemicals	Soxhlet-KD							Ultrasonic		
	Blank	D1	D2	D3	G1	G2	G3	Blank	D4	G4
Ph5	ND	0.4	0.7	0.4	0.8	0.3	0.1			
		(0.4)	(0.4)	(1.1)	(0.3)	(0.1)	(0.1)			
Ph6	ND	0.1	ND	ND	ND	ND	ND			
Ph7	ND	ND	ND	ND	ND	ND	ND			
Ph8	ND	ND	ND	ND	ND	ND	ND			
Ph9	ND	ND	ND	ND	ND	ND	ND			
BPA	ND	ND	ND	ND	ND	ND	ND			
DCP	ND	ND	ND	ND	ND	ND	ND			
DEP	16.3							ND	ND	ND
DPrP	ND	ND	ND	ND	ND	ND	ND			
DBP	7.9							ND	ND	ND
DPeP	ND	ND	ND	ND	ND	ND	ND			
DHP	ND	ND	ND	ND	ND	ND	ND			
BBP	2.1							ND	ND	ND
DCHP	ND	ND	ND	ND	ND	ND	ND			
DEHP	25.7							ND	ND	ND
DEHA	6.3							ND	ND	ND
DPP	ND	ND	ND	ND	ND	ND	ND			
DPB	ND	ND	ND	ND	ND	ND	ND			
DPCB1	ND	ND	ND	ND	ND	ND	ND			
DPCB2	ND	ND	ND	ND	ND	ND	ND			
TPH	0.8							ND	ND	ND
PPET1	0.2							ND	ND	ND
PPET2	1.3							ND	ND	ND
PPET3	0.3							ND	ND	ND
PPET4	0.6							ND	ND	ND
TPCH1	ND	ND	ND	ND	ND	ND	ND			
TPCH2	ND	ND	ND	ND	ND	ND	ND			
BB	ND	ND	ND	ND	ND	ND	ND			
BP	ND	ND	ND	ND	ND	ND	ND			
NT	ND	ND	ND	ND	ND	ND	ND			
BaP	ND	ND	17.0	3.8	3.2	1.4	0.5			
			(9.7)	(10.8)	(1.1)	(0.7)	(0.5)			

Values in the parentheses represent calculated values in μg/km driving distance. ND, not detected (below 0.1 μg/ml).

The other 19 chemicals were not detected.

To avoid contamination of the above phthalates, adipate and styrene trimers from the ambient air, the filters from D4 and G4 were extracted ultrasonically and the extracts were injected into the GC/MS system. The analytical results are also listed in Table 2. The above four phthalates (DEP, DBP, BBP and DEHP), adipate (DEHA) and styrene trimers (TPH and PPET1–4) were not detected in the extract, neither from the blank filter nor from D4 and G4. This result suggests that the above phthalates, adipate and styrene trimers were not present in significant concentrations in the extracts from diesel and gasoline exhaust particulate matter.

In conclusion, only BaP and Ph5 were detected from diesel and gasoline exhaust particulate matter in the range of 0.5–11 and 0.1–1.1 μg/km driving distance, respectively. medial effective concentration (EC_{50})-value of BaP in induction of cytochrome P4501A1 using rat hepatocyte culture has been reported to be 8.74×10^{-7} M.[10] In contrast, 10% of effective concentration (EC_{10})-value of Ph5 in estrogenic activity using yeast two-hybrid assay has been reported to be 3×10^{-6} M.[11] Although, endpoints of the two chemicals are different, it seems that BaP is more important than Ph5 by the data from emission amount and activity. These results revealed that the presence of such endocrine disruptors as BaP

and Ph5 in diesel and gasoline exhaust particulate matter must be investigated in greater detail. Furthermore, it becomes clear that prevention of contamination during sample pretreatment is necessary.

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