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Mutagenicity of Diesel-Exhaust Particulates

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Abstract

Organic extracts of diesel-exhaust particulates were analyzed for mutagenicity using Ames *Salmonella typhimurium* assay system.

An experimental diesel microbus used was drived on the chassis dynamometer according to CVS-75 mode. The samples were taken from the mixed gases in a dilution tunnel. With a high-volume air sampler equipped with double filter holders, particulate matters were collected on a teflon-coated glass fiber filter placed behind a activated carbon filter.

After ultrasonic extraction with benzene-ethanol and evaporation to dryness, the residue was dissolved in dimethylsulfoxide. Each sample was tested toward 2 strains, TA100 and TA98 by the pre-incubation method in the absence and presence of S-9mix.

Average concentration of diesel-exhaust particulates was about 116.6㎎/m^3, and 44.1 ~62.2% to the total weight of particulates, consisted of organic matters.

The mutagenicities of diesel-exhaust particulate organic matters were 4,512 and 2,205 revertants/m^3 toward TA100 without and with S-9mix, respectively. Those toward TA98 were 13,367 and 3,715 revertants/m^3, respectively.

1. INTRODUCTION

Of increasing concern is the possibility that chemicals released in the environment may be a significant cause of cancer in man(Doll, 1977; Tomatis et al., 1978). Thus, the search for, and identification of, carcinogenic compounds in ambient air is particularly important, as air pollutants have been implicated in the rising rate of incidence of human lung cancer in urban areas(Heath, 1978; Pitts et al., 1977). Because animal studies required to test various known or suspected carcinogens are complex, time-consuming and expensive, thus experiments directed to elucidating the potential carcinogenicity of air pollutants have been relatively limited.

However, a simpler and more sensitive test system for determining the mutagenicity of well-known carcinogens has been recently developed by Ames et al.(1975a) and it was clear that there is a close relationship between mutagenicity and carcinogenicity(McCann et al., 1975 ; Sugimura et al., 1976). The mutagenicity of many kinds of pure chemicals and complex mixture, such as urine (Durston et al., 1974 ; Yamasaki et al., 1977), cigarette smoke condensates(Kier et al., 1974), hair dyes(Ames et al., 1975b ; Yoo and Kuroda, 1988), foods or amino acid pyrolysates (Commonor et al., 1978 ; Matsumoto et al., 1977 ; Nagao et al., 1977 ; Sugimura et al., 1977 ; Uyeta et al., 1978 ; Yoo, 1985) and airborne particulate organic pollutants(Yoo and Kuroda, 1986 ; Yoo, 1987 ; Kuroda and Yoo, 1991 ; Yoo et al., 1991) was studied using Ames mutagenicity test method.

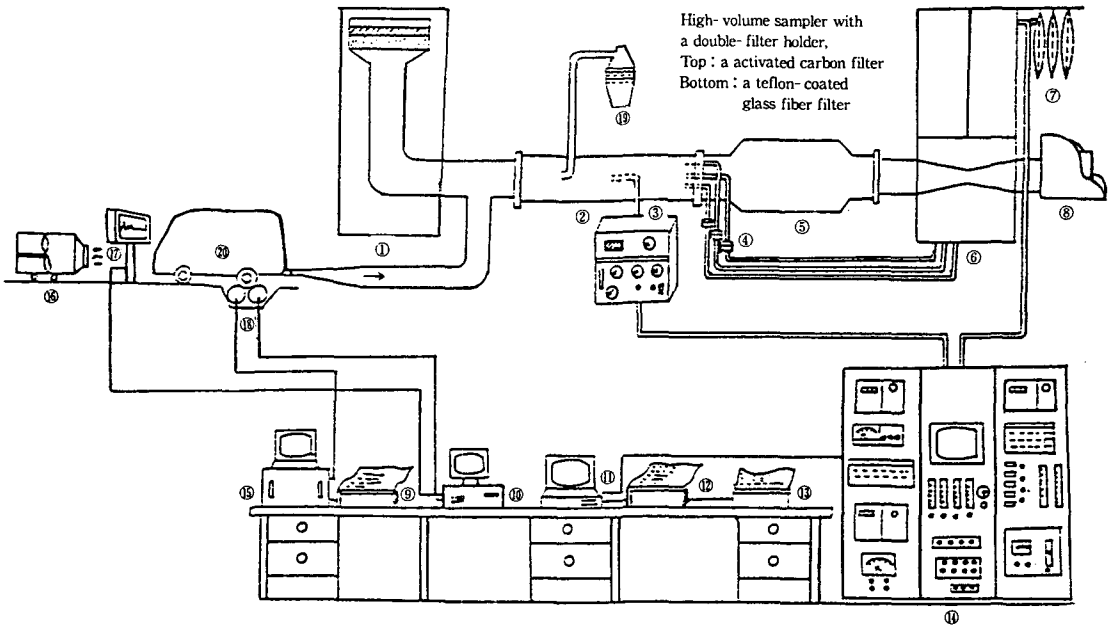
According to the previous report(Yoo et al., 1992), the mutagenicity of organic matters extracted from airborne particulates collected in the Seoul area was relatively strong. Although the exact

sources of mutagens in the urban area like Seoul were not known, it is possible to imagine some. Of the numerous sources, automobile-exhaust emission might be the greatest. The aim of the study presented here was to assess the mutagenicity in organic extracts of a diesel engine-emitted particulates.

2. MATERIALS AND METHODS

2.1 Collection of diesel-exhaust particulates

An experimental diesel microbus(2522cc, 8872km -drived) used was drived on the chassis dynamometer(DC-80, 40HP) according to CVS-75 mode (Fig. 1). Exhaust gases were mixed with clean air in a dilution tunnel. With a high-volume air sampler equipped with double filter holders, particulate matters were collected on a teflon-coated glass fiber(Advantec, PG-60) placed behind a activated carbon filter(Toho, 350~400㎡/g) in order to avoid a formation of artifacts, new reaction products such as nitro-substituted aromatic hydrocarbons during the sampling process.



1. Filter trap
2. Dilution tunnel
3. H.C. analyzer
4. Particulates sampler
5. Heat exchanger
6. Diesel CVS
7. Gas sample bag
8. Blower
9. 10. 12. Printer
11. Analyzer computer
13. X-Y plotter
14. Analyzer for CO, CO_2, NO_x
15. System computer
16. Cooling fan
17. Driver's aid
18. Chassis dynamomter
19. High-volume air sampler
20. Experimental car

Fig. 1. Scheme of the sampling system

2.2 Extraction of organic matters from collected particulates

The organic components were extracted by ultrasonication (28kHz, 15min)(Goto, et al., 1982) with benzene-ethanol(4:1, v/v) (Goto, et al., 1981). The organic extracts were filtered and evaporated to dryness and then dissolved in dimethylsulfoxide. Each sample was stored at -20 ℃ until mutagenicity test.

2.3 Mutagenicity test

The mutagenicity test of organic extracts were carried out by the procedure of Ames et al.(1975a) and pre-incubation(37℃, 20min) method(Yahagi et al., 1977). Each sample was tested toward 2 strains, *Salmonella typhimurium* TA100 and

TA98 in the absence and presence of S-9mix. S-9 was prepared from rats treated with Aroclor 1254 (Maron et al., 1983). S-9 was used at 50 $\mu\ell$/plate. Values of the mutagenicity represented are the average of duplicate determinations.

3. RESULTS

3.1 Concentrations of gaseous materials in diesel-exhausts

The concentrations of CO_2, CO, NO_X and total hydrocarbons in diesel-exhaust gas were shown in Table 1. CO_2 and CO were measured by non-dispersive infrared analysis, and NO_X and hydrocarbons were measured by chemiluminescence and GC-FID, respectively. As shown in Table 1, samplings were carried out twice and samples were collected at the cold-start and hot-start condition. The concentrations of CO, NO_X and hydrocarbons had a range of 0.74~1.27, 0.69~0.80 and 0.17~0.29 g/m^3, respectively. These values do not exceed the vehicles-emission standard values in Korea.

Table 1. Concentrations of gaseous materials in diesel-exhausts

Sampling	Driving condition		CO_2 [1] (g/m^3)	CO [2] (g/m^3)	NO_x [3] (g/m^3)	Hydrocarbons [4] (g/m^3)
1st	Cold	Initial	231.54	1.13	0.73	0.19
		Stable	229.89	1.27	0.80	0.28
	Hot	Initial	230.95	0.74	0.69	0.17
2nd	Cold	Initial	233.34	0.92	0.78	0.18
		Stable	220.31	1.13	0.75	0.29
	Hot	Initial	233.36	1.00	0.77	0.22

Measuring method, [1] NDIR, [2] NDIR, [3] CLD, [4] GC-FID

3.2 Concentrations of particulate matters in diesel-exhausts

Table 2 showes the concentrations of particulate matters exhausted from a diesel engine. The concentration of particulate matters had a range of 108.20~124.93mg/m^3, and the value at cold-start condition was higher than that at hot-start condition. This fact suggests that the efficiency of internal combustion at cruised driving is higher than that at idling. Dilution rates were calculated from 13.4/(CO_2)+(Hydrocarbons)+(CO).

Table 2. Concentrations of particulates matters in diesel-exhausts

Sampling	Driving condition	Weight(mg) of particulates collected	Gas volume (m^3)	Dilution rate	Concentration of particulates(mg/m^3)
1st	Cold	176.0	24.64	17.49	124.93
	Hot	79.3	9.71	14.79	120.79
2nd	Cold	246.3	31.71	14.49	112.55
	Hot	140.2	12.75	9.84	108.20

3.3 Concentrations of organic extracts

As shown in Table 3, 44.1~62.2% to the total weight of particulates, consisted of extractable organic matters. This rate is about 3-fold higher than that of airborne particulates reported in the previous study(Yoo et al.,1991). The concentrations of organic matters extracted from diesel-exhaust particulates had a range of 49.65~75.11mg/m^3, estimated to unit per cubic meter · exhaust gas.

3.4 Mutagenicity of organic extracts

The mutagenicities of organic matters extracted from diesel-exhaust particulates were 4,512(4,280~4,930) and 2,205(1,990~2,450) revertants/m^3 · exhaust gas toward TA100 strain in the absence

and presence of S-9mix, respectively(Table 4). Those toward TA98 strain in the absence and presence of S-9mix were 13,367(11,800~15,640) and 3,715(3,250~4,270) revertants/m^3 · exhaust gas, respectively.

Table 3. Concentrations of organic matters extracted from diesel-exhaust particulates

Sampling	Driving condition	Weight(mg) of organic extracts	Concentrations of organic extracts	
			per exhaust particulates(%)	per exhaust gas(mg/m^3)
1st	Cold	78.57	44.6	55.77
	Hot	49.31	62.2	75.11
2nd	Cold	108.65	44.1	49.65
	Hot	72.71	51.9	56.12

Table 4. Mutagenicity of organic extracts in *S. typhimurium* TA100 and TA98

Sampling	Driving condition	Strain	Revertants/m^3 · exhaust gas*	
			without S-9mix	with S-9mix
1st	Cold	TA100	4,280	2,060
		TA98	12,820	3,480
	Hot	TA100	4,930	2,320
		TA98	13,210	4,270
2nd	Cold	TA100	4,560	1,990
		TA98	15,640	3,250
	Hot	TA100	4,280	2,450
		TA98	11,800	3,860

*: The numbers of spontaneous revertants per plate, 180(TA100 without S-9mix), 160(TA100 with S-9mix), 20(TA98 without S-9mix), 25(TA98 with S-9mix) were subtracted and then estimated to per m^3 · exhaust gas.

4. DISCUSSION

In the Ames *Salmonella* reverse-mutation assay system, TA100 and TA98 strains are respectively available for testing mutagens of base-pair substitution and frameshift type(Maron and Ames, 1983).

Promutagens, indirect-acting mutagens, such as benzo(a)pyrene as a typical carcinogenic air pollutant, are metabolized by adding S-9mix and then show the mutagenic activity. However, nitro-substituted aromatic hydrocarbons such as nitropyrenes, recently recognized air pollutants (Yoo, 1989), show directly strong mutagenic/carcinogenic activity without metabolisms (Campbell et al., 1981 ; Rosenkranz and Mermelstein, 1983 ; Tokiwa et al., 1984 ; Hirose et al., 1984).

In this study, the mutagenicity toward TA98 without S-9mix was the highest among those in the case of other test systems. This fact suggests that mutagens of frameshift and/or direct-acting types are major contributors to mutagenicities of diesel-exhaust particulates.

The nitro-substituted aromatic hydrocarbons and derivatives might be produced to a large extent by nitration during the combustion process and in the exhaust stream. Wang et al.(1978) reported that the source of direct-acting mutagens in particulate matters in city air was automobile-exhausts. Pitts et al.(1978) have suggested a possible formation of directly mutagenic nitro-derivatives by the exposure of non-carcinogenic as well as carcinogenic polycyclic aromatic hygrocarbons to nitrogen dioxide. Actually they have been recently found in airborne particles(Gibson, 1983) and diesel-exhaust particles(Schurtzle, 1983 ; Xu et al., 1982 ; Bechtold et al., 1986). Hisamatsu et al. (1991) reported that 2-, 4-nitropyrenes and 2-

nitrofluoranthene were formed by the photochemical reaction of pyrene and fluoranthene with nitrogen dioxide in atmosphere.

The samples of diesel-exhausts used in this experiments were collected on a teflon-coated glass fiber filter placed behind a activated carbon filter in order to avoid a formation of artifacts, new reaction products such as nitro-substituted arenes in the sampling process.

Although some kinds of air pollutants emitted from automobiles, such as CO, NO_x and hydrocarbons, are subject to control in Korea, the mutagenicity of automobile-exhausts that might produce the air pollution, was not yet surveyed. The emissions of small engines used for the generation of electric power or farm machinery are not regulated at all. Small diesel engines emitted exhausts of very high mutagenicity(Ohnishi et al., 1980). To reduce pollution levels, car engines must have improved emission-cleaning attachments or be adapted to work at high air/fuel ratios. The problems are compounded by the continued use of many high-polluting jalopies, including old diesel-powered vehicles.

5. CONCLUSION

The mutagenicity of organic extracts of diesel-exhaust particulates was measured with Ames *Salmonella typhimurium* strain TA100 and TA98 in the presence and absence of S-9mix.

Average concentration of diesel-exhaust particulates was about 116.6mg/m^3 and 44.1~62.2% to the total weight of particulates, consisted of organic matters.

The mutagenicities of diesel-exhaust particulate organic matters were 4,512 and 2,205 revertants/m^3 toward TA100 without and with S-9mix, respectively. Those toward TA98 were 13,367 and 3,715 revertants/m^3, respectively.

The mutagenicities toward TA98 and in the absence of S-9mix, has higher value than those toward TA100 and in the presence of S-9mix, which suggests mutagenic materials of frameshift and/or direct-acting type such as nitro-substituted aromatic hydrocarbons(nitro-PAHs) are major contributors to mutagenicities of diesel emission particulates.

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