Mutagenic Characteristics and Contribution of Polycyclic Aromatic Hydrocarbons to Mutagenicity of Concentrates from Municipal River Water by Blue Chitin Column

Akira Nagai,*[,a] Yoshinao Kano,[a] Ryozo Funasaka,[a] and Katsuhiko Nakamuro[b]

[a]Gifu Research Center for Public Health, 4–6 Akebono-cho, Gifu-shi, Gifu 500–8148, Japan and [b]Faculty of Pharmaceutical Sciences, Setsunan University, 45–1 Nagaotoge-cho, Hirakata-shi, Osaka 573–0101, Japan

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The mutagenic characteristics of concentrates from environmental water such as the effluent from various treatment plants and river waters using a modified Blue Chitin column method were determined by Ames assay with *Salmonella typhimurium* TA98, YG1021 and YG1024. This was done to estimate the quantitative contribution rate of mutagenicity estimated from analyzed polycyclic aromatic hydrocarbons (PAHs) content in the same concentrates to the mutagenicity of environmental water. Moreover, PAHs content and the mutagenicity of the river sediment-extract were measured to elucidate the fate of PAHs in the river. Many kinds of environmental waters and river sediment possessed principally indirect frame-shift type mutagenicity. Concentrates from many kinds of environmental waters possibly contained aminoarenes assayed with YG1021 and YG1024. In many kinds of environmental waters, the contribution rates of mutagenic magnitude estimated from seventeen analyzed PAH contents to the mutagenic magnitude of these water concentrates in TA98 with S9 mix were recognized to be from 0.10 to 1.15%. However, the contribution rate of mutagenic magnitude estimated from ten analyzed PAHs contents to the mutagenic magnitude of the Arata River sediment extract in TA98 with S9 mix was 64.2%. The high concentration of PAHs in the river sediment suggested that hydrophobic PAHs in the water might easily accumulate in the river sediment after adsorbing to the suspended solid.

Key words —— polycyclic aromatic hydrocarbons, mutagenic magnitude, contribution rate, environmental water, Blue Chitin

INTRODUCTION

The surface water of municipal river water has served as a source of supply water. This river water contains a large amount of trace toxic organic pollutants, because it receives a large quantity of waste water drained from the activities of human life, sewage treatment plants, industrial waste plants, agriculture and road runoff. Many studies have been reported in which municipal river water shows mutagenicity[1–14] and its character is derived from polycyclic aromatic hydrocarbons (PAHs) including nitro- and amino-derivatives.[1–6] Recently, WHO established the guidelines for fluoranthene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, benzo(*ghi*)peryrene and indeno(1,2,3-cd)perylene as possible carcinogenic pollution indicators of drinking-water.[15]

In mutagenicity investigations on environmental water, water samples need to be concentrated for Ames assay. One of the concentration methods is that using a solid support able to trap selectively molecular type mutagenic materials. XAD-2 resin traps a wide range of spectra of soluble organic substances in water. Blue Cotton, Blue Rayon and Blue Chitin which have ligand linked copper phthalocyanine trisulphonate are capable of adsorbing PAHs with three or more fused rings. These have been used as a preconcentration method in many mutagenicity investigations.[1–14,16–21]

Yamauchi *et al.*[2] and Sasaki *et al.*[13] used the Blue Cotton hanging method for concentration of organic substances in river water, measured the mutagenicity of concentrate and analyzed 10 kinds of PAHs, Trp-P-1 and Trp-P-2, respectively. Ohe and Nukaya reported the genotoxicity and the content

*To whom correspondence should be addressed: Gifu Research Center for Public Health, 4–6 Akebono-cho, Gifu-shi, Gifu 500–8148, Japan. Tel.: +81-58-247-1300; Fax: +81-58-248-0229; E-mail: nagai@koeiken.or.jp

of 1-nitropyrene in concentrates from river water using the XAD-2 resin column.[17] Mouri *et al*. reported the genotoxicity and the content of 6 kinds of heterocyclic amines in concentrates not only from river water, but also from the effluent from various treatment plants using the Blue Rayon column method.[19]

These reports described the contribution of PAHs or heterocyclic amines to the mutagenicity or the genotoxicity of river water, however, few target compounds contributing to the mutagenicity or the genotoxicity have been reported, and recovery of such compounds by preconcentration method was not quantitative.

In the previous paper[20] we reported a quantitative preconcentration method for 26 kinds of PAHs in water, and suggested that a modified Blue Chitin column method was useful for 14 kinds of PAHs with 4 and 5 rings. Moreover, we indicated the occurrence of PAHs surveyed by GC-MS in the effluent from various treatment plants and river water.

In this paper we measured 17 kinds of PAHs including 6 kinds of PAHs established as WHO's guideline in the environmental water and investigated the mutagenic characteristics of concentrates obtained from the effluent from various treatment plants and river water using this same method. We evaluated in detail quantitative contribution of mutagenic magnitude estimated from analyzed PAH contents in the same concentrates to the mutagenic magnitude of river water concentrates as well as the river sediment.

MATERIALS AND METHODS

Chemicals ——
Chemical sources were as follows: acenaphthylene (Anl), acenaphthene (An), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (FA), pyrene (PY), benz[*a*]anthracene (B*a*A), chrysene (Chr), benzo[*a*]pyrene (B*a*P) and dibenz[*a*,*h*]anthracene (diB*ah*A) were from Wako Pure Chemical Industries, Ltd.; benzo[*ghi*]perylene (B*ghi*P), 2-aminoanthracene (2-AAnt), 3-aminofluoranthene (3-AFA) and 1-aminopyrene (1-APY) were from Aldrich Chemical Co. Inc.; benzo[*b*]fluoranthene (B*b*FA) and benzo[*k*]fluoranthene (B*k*FA) were from RK Chemical Co.; methanol (CH_3OH), ammonia (NH_4OH), dichloromethane (CH_2Cl_2) and dimethyl sulfoxide (DMSO) were also from Wako Pure Chemical Industries, Ltd., Japan.

The column (14 mm i.d. × 60 mm) was packed with 200 mg of Blue Chitin (Funakoshi, Ltd., Japan) which was in a powdered form and contained 40 μmol of copper phthalocyanine trisulfonate-chitin.

Sample —— The effluents from a night soil treatment plant, sewage treatment plant and septic tanks combined with aerobic treatment were sampled in Gifu city on September 1998.

The surface waters of the Katsura River, the Uji River and the Kizu River were sampled on September 1997. The surface waters of the Nagara River, the Sakai River, the Arata River and the Kuwabara River were sampled on September 1998.

These water samples were filtered through a glass-fiber filter as quickly as possible after collection.

The Arata River sediment was sampled on May 1999. The sample was dried in a dark place at room temperature, and was screened through a 2 mm mesh sieve.

Preconcentration Method by Modified Blue Chitin Column Method[20] —— Five liters of effluents from various treatment plants and river waters was passed through a Blue Chitin column, which was washed[11] at a flow rate of 20 ml/min with a peristaltic pump, and then the column was washed with 20 ml of water. The organics adsorbed to Blue Chitin were consecutively eluted with 100 ml of $CH_3OH \cdot NH_4OH$ (50 : 1) and 20 ml of CH_2Cl_2 at a flow rate of 5 ml/min. Fractions of both $CH_3OH \cdot NH_4OH$ (50 : 1) and CH_2Cl_2 were combined and evaporated to dryness with a rotary evaporator in a water bath at about 40°C under vacuum. The residue was dissolved in 2 ml of DMSO for Ames assay. The concentrates from upstream and downstream of the Nagara River were dissolved in 1 ml of DMSO, respectively.

Fifty grams of the river sediment was extracted with 200 ml of $CH_2Cl_2 \cdot C_2H_5OH$ (4 : 1) twice under ultrasonic vibration (28 Hz) for 15 min and then centrifuged at 3000 rpm for 10 min. The extract was concentrated to about 100 ml with a rotary evaporator in a water bath at about 40°C under vacuum, washed with 300 ml of 5% NaCl solution, and evaporated to dryness with a rotary evaporator in a water bath at about 40°C under vacuum.[21] The residue was dissolved in 10 ml of CH_3OH and then 90 ml of H_2O added. This solution was passed through a Blue Chitin column and the same condition as for water sample was followed. Half amount of concentrate was dissolved in 2 ml of DMSO for Ames assay and

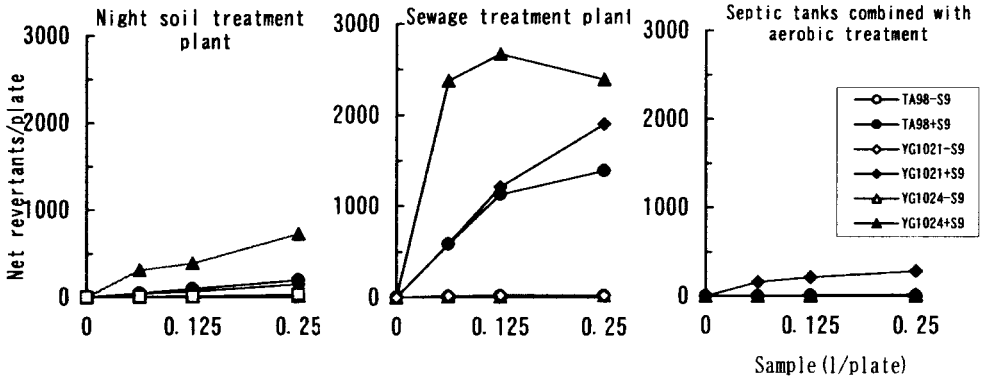

Fig. 1. Mutagenicity of Concentrates from Effluent from Various Treatment Plant in TA98, YG1021 and YG1024

The numbers of spontaneous revertants per plate 25 ± 6.0 (TA98-S9), 33 ± 3.9 (TA98+S9), 20 ± 3.3 (YG1021-S9), 32 ± 5.7 (YG1021+S9), 23 ± 5.0 (YG1024-S9) and 60 ± 12 (YG1024+S9) were subtracted.

the other amount was dissolved in 1 ml of CH_2Cl_2 for GC-MS analysis.

GC-MS Analysis —— Seventeen kinds of PAHs were analyzed by GC-MS using a Thermoquest GCQ. The conditions of GC-MS were described previously.[20]

Ames Salmonella/Microsome Assay[22] —— The bacterial *Salmonella typhimurium* strains used in the Ames Salmonella/microsome assay were TA98 and derivatives of TA98 capable of detecting mutagenic nitroarenes or aminoarenes: YG1021 and YG1024, respectively.[23,24] YG1021 is a nitroreductase-overproducing strain and is highly sensitive to the mutagenic action of typical nitroarenes. YG1024 is an *O*-acetyltransferase-overproducing strain and is also extremely sensitive to the mutagenic action of nitroarenes and aminoarenes.

Ames assay was carried out by the liquid preincubation procedure. The concentrates were applied at a volume of 25, 50 and 100 μl per plate. Effect of microsomal metabolism on mutagenic activity was determined by addition of cofactors and liver S9: S9 mix (50 μl) from rats pretreated with phenobarbital and 5,6-benzoflavon (Oriental Kobo, Ltd., Japan). Mutagenicity was judged from the dose-response curve showing at least a two-fold increase over the spontaneous revertants. Mutagenic activity was estimated by extrapolation of the dose-response curve.

The numbers of spontaneous revertants per plate were 25 ± 6.0 (TA98-S9), 33 ± 3.9 (TA98+S9), 20 ± 3.3 (YG1021-S9), 32 ± 5.7 (YG1021+S9), 23 ± 5.0 (YG1024-S9) and 60 ± 12 (YG1024+S9), respectively.

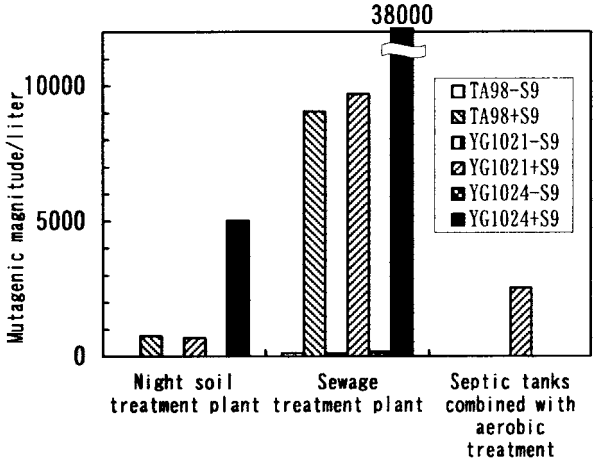

Fig. 2. Mutagenic Magnitude of Concentrates from Effluent from Various Treatment Plants in TA98, YG1021 and YG1024

RESULTS

Mutagenicity of Concentrates from Effluent from Various Treatment Plants

Figure 1 shows the results of the mutagenicity of concentrates from the effluent from various treatment plants in TA98, YG1021 and YG1024 with and without S9 mix. Their mutagenic magnitude estimated from Fig. 1 is shown in Fig. 2.

The concentrate from the effluent of septic tanks combined with aerobic treatment did not show both direct and indirect mutagenicity in TA98, however, that from the effluent from a night soil treatment plant showed indirect mutagenicity in TA98 with 761 net revertants/l; the concentrate from effluent from a sewage treatment plant showed direct and indirect mutagenicity in TA98 with 124 net revertants/l and 9050 net revertants/l, respectively. The

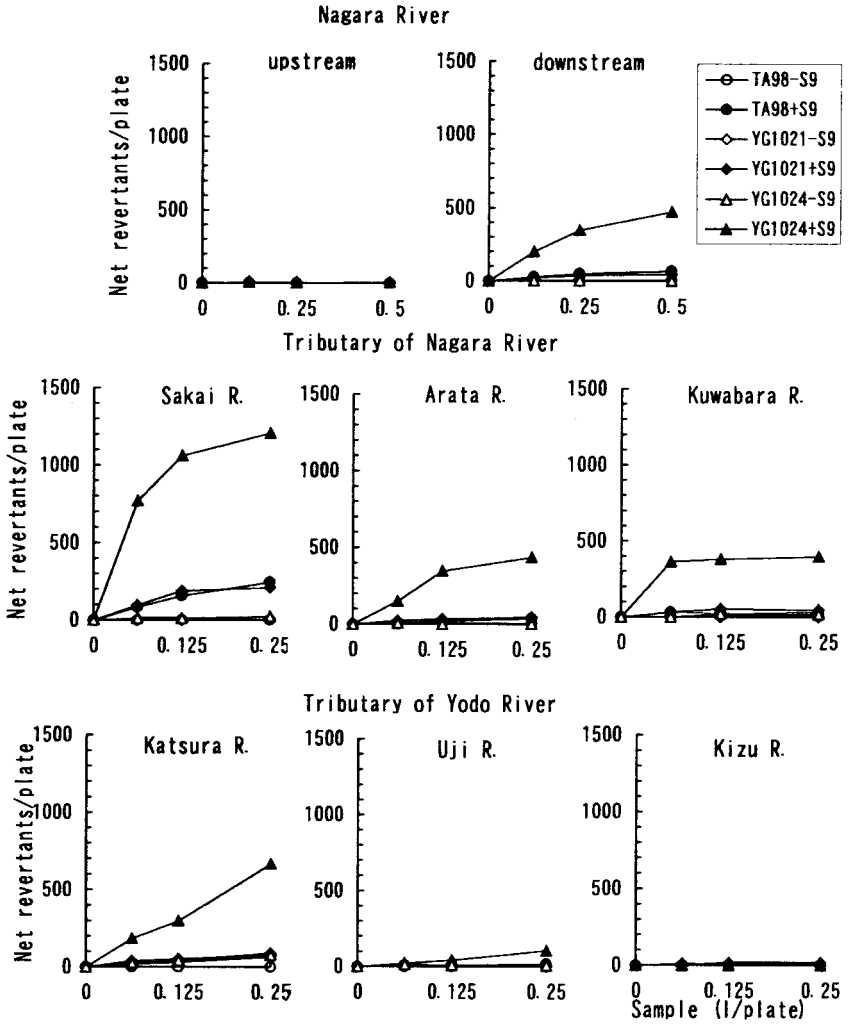

Fig. 3. Mutagenicity of Concentrates from Water of the Nagara River and the Yodo River in TA98, YG1021 and YG1024

The numbers of spontaneous revertants per plate 25 ± 6.0 (TA98-S9), 33 ± 3.9 (TA98+S9), 20 ± 3.3 (YG1021-S9), 32 ± 5.7 (YG1021+S9), 23 ± 5.0 (YG1024-S9) and 60 ± 12 (YG1024+S9) were subtracted.

mutagenic activity was increased by the addition of S9 mix. From these results, the effluent from a night soil treatment plant and a sewage treatment plant appeared to possess frame-shift type mutagenicity.

To confirm characteristics of the frame-shift type mutagens in detail, strains YG1021 and YG1024 which are sensitive to nitroarenes and/or aminoarenes were used for Ames assay. The results were as follows: the mutagenic magnitude of concentrates from effluent from a night soil treatment plant and a sewage treatment plant was 5020 and 38000 net revertants/l in YG1024 with S9 mix, respectively. Although the direct mutagenic activities against YG1021 were not enhanced comparing with TA98, the indirect mutagenic activities against YG1024 were enhanced comparing with TA98. Accordingly, these results suggested that the concentrates from effluent from the two plants possibly contained aminoarenes.

Mutagenicity of Concentrate from River Water

Figure 3 shows the results of the mutagenicity of concentrates from river waters in strains TA98, YG1021 and YG1024 with and without S9 mix, and Fig. 4 shows the mutagenic magnitude estimated from Fig. 3.

The concentrates from river waters of downstream of the Nagara River, and its tributaries the Sakai River and the Arata River were indirectly mutagenic in TA98 with 200, 1570 and 156 net revertants/l, respectively. The concentrate from river water of the Katsura River, a tributary of the Yodo River was also indirectly mutagenic in TA98, showing 272 net revertants/l.

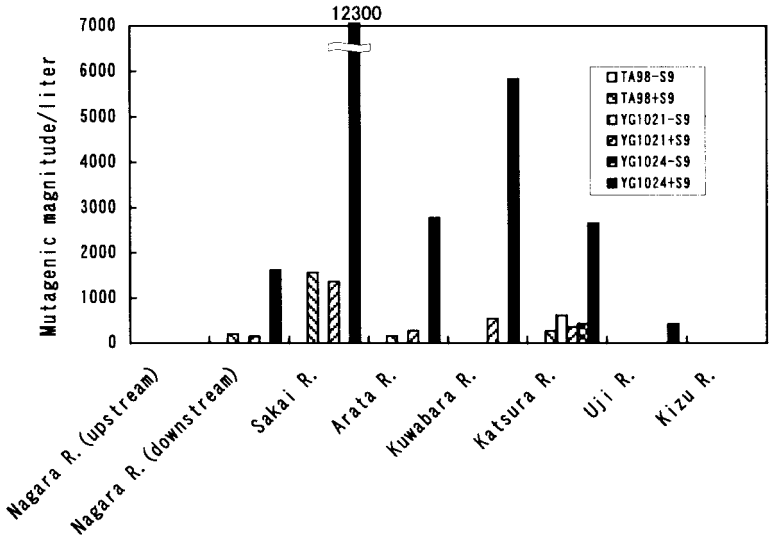

Fig. 4. Mutagenic Magnitude of Concentrates from Water of the Nagara River and the Yodo River in TA98, YG1021 and YG1024

The results using strains YG1021 and YG1024 suggested that the concentrates from river waters of downstream of the Nagara, the Sakai, the Arata and the Katsura rivers possibly contained aminoarenes, because indirect mutagenic magnitude against YG1024 was enhanced from 8 to 18-fold comparing with TA98.

Mutagenicity of Extract from River Sediment and Occurrence of PAHs in the Same Extract

The extract from the Arata River-sediment showed indirect mutagenicity in TA98 as shown in Fig. 5. The extract possessed the same frame-shift type mutagenicity as the effluent from various treatment plants and river waters. However, the mutagenic activities against YG1021 and YG1024 with and without S9 mix were not enhanced comparing with TA98. This fact suggested that the extract from the Arata River-sediment possibly did not contain nitroarenes or aminoarenes.

Ten kinds of PAHs were detected in the extract from the Arata River-sediment by GC-MS, and these detectable levels were in a range from 4 ng/g-dry of dibenz(*a*,*h*)anthracene to 480 ng/g-dry of fluoranthene.

Contribution of PAHs to the Mutagenicity of Various Water Concentrates and Sediment Extract

Mutagenicity of PAH compounds were measured by Ames assay[22] to learn the quantitative contribution of mutagenic magnitude estimated from their analyzed content to mutagenic magnitude of water concentrates and sediment extract. The mutagenic magnitude against TA98 with S9 mix of PAH compounds is shown in Table 1.

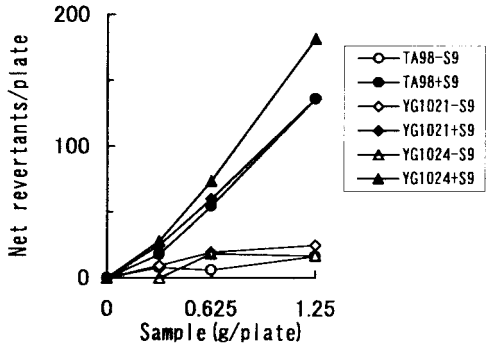

Fig. 5. Mutagenicity of Extract from Arata River-Sediment in TA98, YG1021 and YG1024

The numbers of spontaneous revertants per plate 25 ± 6.0 (TA98-S9), 33 ± 3.9 (TA98+S9), 20 ± 3.3 (YG1021-S9), 32 ± 5.7 (YG1021+S9), 23 ± 5.0 (YG1024-S9) and 60 ± 12 (YG1024+S9) were subtracted.

Table 2 shows the concentrations of PAHs and their estimated mutagenic magnitude against TA98 with S9 mix in the effluent from various treatment plants, river waters and sediment. Individual magnitude was obtained by multiplying the concentration of PAH detected chromatographically and the mutagenic magnitude of the corresponding PAH compounds shown in Table 1. The sum of the estimated magnitude was made under the assumption that the synergistic or repressive interruption between PAH and the other chemicals was not caused

Table 1. Mutagenic Magnitude of PAH Compounds in TA98+S9 mix

Compound	Mutagenic magnitude revertants/μg
Acenaphthylene	—[a)]
Acenaphthene	—
Fluorene	—
Phenanthrene	—
Anthracene	—
Fluoranthene	64
Pyrene	—
Benz[*a*]anthracene	56
Chrysene	—
Benzo[*b*]fluoranthene	61
Benzo[*k*]fluoranthene	59
Benzo[*a*]pyrene	162
Dibenz[*a*,*h*]anthracene	39
Benzo[*ghi*]perylene	—
2-Aminoanthracene	955
3-Aminofluoranthene	1000
1-Aminopyrene	500

a) negative

on the mutagenicity in the concentrate.

Table 3 shows the contribution rates of mutagenic magnitude estimated from total analyzed PAH contents to mutagenic magnitude of the various environmental water concentrates and the sediment extract in TA98 with S9 mix.

In the effluent from a night soil treatment plant 12 kinds of PAHs were detected, the mutagenic magnitudes calculated from their concentrations and mutagenic magnitude of the corresponding PAH were from 0 to 2.50 net revertants/l, and the sum of these magnitudes was 8.74 net revertants/l. As the mutagenic magnitude obtained from Ames assay was 761 net revertants/l, the contribution rate of mutagenic magnitude estimated from total analyzed PAH content to the mutagenic magnitude of concentrate was 1.15%.

The effluent from the sewage treatment plant possessed high mutagenicity and 8 kinds of PAHs were detected; the sum of the mutagenic magnitude calculated from the concentrations of these PAHs was 21.4 net revertants/l. The contribution rate of mutagenic magnitude estimated from total analyzed PAH content to the mutagenic magnitude of the concentrate was very low at 0.23%.

Among river waters, that of Sakai River had high mutagenicity: the sum of the mutagenic magnitude calculated from concentrations of PAHs detected was 3.94 net revertants/l. The contribution rate of mutagenic magnitude estimated from total analyzed PAH contents to the mutagenic magnitude of concentrate was 0.25%. In the other river waters, the mutagenic contribution rates from the analyzed PAHs this time were low with 0.10 to 0.17%.

In the extract from the Arata River-sediment 10 kinds of PAHs were detected, and the sum of the mutagenic magnitude calculated from concentrations of these PAHs was 56.5 net revertants/g. The contribution rate of mutagenic magnitude estimated from total analyzed PAH contents to the mutagenic magnitude of sediment-extract was 64.2%. It was therefore suggested that 5 kinds of PAHs accounted for the majority of the mutagenic material of this sediment.

DISCUSSION

There have been reports on the relationship between the mutagenicity and the distribution of PAHs in river water. In this paper we evaluated the contribution of 17 kinds of PAHs including 6 kinds of PAHs established as WHO's guideline for drinking-water from the view point of the mutagenicity in the environmental water.

In this investigation, we evaluated the quantitative contribution rate of mutagenic magnitude estimated from analyzed PAH content to the mutagenic magnitude of environmental water by the following process. We applied the Blue Chitin column method which was clarified the characteristics of adsorption and elution of 17 kinds of PAHs. The same concentrates obtained by this method were applied for Ames assay and GC-MS analysis, and we measured the mutagenic magnitude of each PAHs compound in order to estimate the mutagenic magnitude from PAH concentration determined by GC-MS analysis.

This study suggested that the municipal river waters and the effluent from a night soil treatment plant and a sewage treatment plant possess principally indirect frame-shift type mutagenicity. Further, their concentrates possibly contained aminoarenes from the results assayed with highly sensitive strains; YG1024. Similar suggestions were reported on mutagenicity of municipal river water by Sayato *et al.*[7] Moreover, it was apparent that the mutagenic characteristics of river water derive from waste water drained from human life activities, because the river waters and the effluent from the various treatment plants discharged into the river have the same frame-shift type mutagenicity.

Table 2. Concentrations and Mutagenic Magnitude of PAHs in Various Environmental Waters and Sediment

Sample		compound						
		Anl	An	Fl	Phe	Ant	FA	PY
Effluent of treatment plants								
Night soil treatment plant	conc.[a)]	< 10	< 2	3	4	3	18	8
	M.M.[b)]	0	0	0	0	0	1.15	0
Sewage treatment plant	conc.	< 10	< 2	< 2	3	2	17	10
	M.M.	0	0	0	0	0	1.09	0
Septic tanks combined	conc.	< 10	< 2	< 2	5	5	16	4
with aerobic treatment	M.M.	0	0	0	0	0	1.02	0
River water								
Nagara River (downstream)	conc.	< 10	< 2	< 2	< 2	< 2	3	3
	M.M.	0	0	0	0	0	0.19	0
Sakai River	conc.	< 10	< 2	< 2	3	2	17	10
	M.M.	0	0	0	0	0	1.09	0
Arata River	conc.	< 10	< 2	< 2	< 2	< 2	4	7
	M.M.	0	0	0	0	0	0.26	0
Kuwabara River	conc.	< 10	< 2	< 2	< 2	< 2	5	4
	M.M.	0	0	0	0	0	0.26	0
Katsura River	conc.	< 10	< 2	< 2	3	< 2	4	3
	M.M.	0	0	0	0	0	0.26	0
Uji River	conc.	< 10	< 2	< 2	< 2	< 2	3	< 2
	M.M.	0	0	0	0	0	0.19	0
Kizu River	conc.	< 10	< 2	< 2	< 2	< 2	3	< 2
	M.M.	0	0	0	0	0	0.19	0
Sediment								
Arata River	conc.	< 1	< 0.2	< 0.2	56	70	480	460
	M.M.	0	0	0	0	0	30.72	0

a) conc.: concentration; Data of effluents from treatment plants and river waters taken from Ref. 20 effluents from treatment plants and river water (ng/l), sediment (ng/g). *b*) M.M.: mutagenic magnitude; obtained by multiplying analytical PAH content and the mutagenic magnitude in TA98+S9 mix.

The contribution rate of aminoarenes in environmental water has been reported. Mouri *et al*. found that the contribution rate of Trp-P-2 to the genotoxicity of concentrates from the effluent from a sewage treatment plant and river water was more than 10% and several percent, respectively.[19)] Sasaki *et al*. reported that the contribution rate of Trp-P-1 and Trp-P-2 was several percent to the mutagenicity of river water concentrate.[13)] Yamauchi *et al*. reported that, although the rate of contribution of hydrophobic PAHs (8 kinds of PAHs) to the mutagenicity of river water concentrate was not calculated, that of hydrophilic PAHs was estimated to be 5%.[2)]

In this paper, the contribution rates of mutagenicity estimated from analyzed PAH content to the mutagenic magnitude of these water concentrates in TA98 with S9 mix were recognized to be 1.15 and 0.23% in the effluent from a night soil treatment plant and a sewage treatment plant, respectively, and from 0.10% to 0.25% in the river water. The contribution rates of these PAHs were very low, corroborating the report by Yamauchi *et al*.

The reason for the low mutagenic contribution rates of PAHs in water's mutagenicity might be due to the existence of other PAHs that had functional group in water. Moreover, it was speculated that PAHs might be hard to be dissolved and exist by adsorbing to the particles in river water depending on their solubility in water.

CH_3-PAHs (*e.g*., methylphenanthrene, methylpyrene, methylbenz(*a*)anthracene) or NO_2-PAHs (*e.g*., dinitropyrene) have been detected other than PAHs in the atmosphere,[25–27)] and Nakamuro *et al*. reported that these PAH derivatives might enter to environmental water via rainwater and road runoff.[28)]

Nitrated PAHs possessed mainly strong mutagenicity, however the contribution rate of 1-nitropyrene that was detected in the river water by Ohe was only 1%. It was assumed that the contribution of PAHs to the mutagenicity in water was low.

Table 2. Continued

compound										total
BaA	Chr	BbFA	BkFA	BaP	diBahA	B*ghi*P	2-AAnt	3-AFA	1-APY	
10	11	11	13	15	17	< 10	< 5	< 10	5	118
0.56	0	0.67	0.77	2.43	0.66	0	0	0	2.50	8.74
< 10	< 4	< 4	8	< 4	< 10	< 10	5	10	10	65
0	0	0	0.47	0	0	0	4.78	10.0	5.00	21.4
< 10	< 4	< 4	< 4	< 4	< 10	< 10	< 5	< 10	< 5	30
0	0	0	0	0	0	0	0	0	0	1.02
< 10	< 4	< 4	< 4	< 4	< 10	< 10	< 5	< 10	< 5	6
0	0	0	0	0	0	0	0	0	0	0.19
< 10	< 4	< 4	6	< 4	< 10	< 10	< 5	< 10	5	48
0	0	0	0.35	0	0	0	0	0	2.50	3.90
< 10	< 4	< 4	< 4	< 4	< 10	< 10	< 5	< 10	< 5	11
0	0	0	0	0	0	0	0	0	0	0.26
< 10	< 4	< 4	7	< 4	< 10	< 10	< 5	< 10	< 5	14
0	0	0	0.41	0	0	0	0	0	0	0.67
< 10	< 4	< 4	< 4	< 4	< 10	< 10	< 5	< 10	< 5	10
0	0	0	0	0	0	0	0	0	0	0.26
< 10	< 4	< 4	< 4	< 4	< 10	< 10	< 5	< 10	< 5	3
0	0	0	0	0	0	0	0	0	0	0.19
< 10	< 4	< 4	< 4	< 4	< 10	< 10	< 5	< 10	< 5	3
0	0	0	0	0	0	0	0	0	0	0.19
130	210	< 0.4	100	77	4	8	< 0.5	< 1	< 0.5	1595
7.28	0	0	5.90	12.47	0.16	0	0	0	0	56.53

Table 3. Mutagenic Magnitude in TA98+S9 mix and Mutagenic Contribution Rates of PAHs Detected at Various Environmental Water and Sediment

Sample	Mutagenic magnitude [a)]		Contribution rate (%)
	Ames assay [b)]	Analysis [c)]	
Effluent from treatment plants			
Night soil treatment plant	761	8.74	1.15
Sewage treatment plant	9050	21.4	0.23
Septic tanks combined with aerobic treatment	—[d)]	1.02	0.00
River water			
Nagara River (upstream)	—	0	0.00
Nagara River (downstream)	200	0.19	0.10
Sakai River	1570	3.94	0.25
Arata River	156	0.26	0.17
Kuwabara River	—	0.67	0.00
Katsura River	272	0.26	0.10
Uji River	—	0.19	0.00
Kizu River	—	0.19	0.00
Sediment			
Arata River	88	56.5	64.2

a) effluent from treatment plants and river water (revertants/L), sediment (revertants/g). *b*) Mutagenic magnitude was obtained by dose-response curve in Ames assay. *c*) Mutagenic magnitude was obtained by multiplying concentration of PAH detected and the mutagenic magnitude of corresponding PAH compound. *d*) negative.

In this paper, the relationship between mutagenicity and the distribution of 17 kinds of PAHs in effluent from various treatment plants, river waters and river sediment has been discussed. It was recognized that the contribution of 17 kinds of PAHs to mutagenicity in the effluent from various treatment plants and municipal river waters was low. As PAHs may exist with various forms in water, a complicated clean-up in the preconcentration and high technical analysis will be necessary to identify the many unknown PAHs in further study. From the point of view of analytical techniques, Ames assay was considered useful as a method to screen the mutagenic compounds in water, since this assay was able to comprehensively evaluate PAHs with mutagenicity in water.

Moreover, it was assumed that hydrophobic PAHs in water might easily accumulate in river sediment after adsorbing to a suspended solid, because the amount of hydrophobic PAHs of assay symmetry this time in the river sediment was more than that in environmental water. Accordingly, it is important for the fate of PAHs to investigate the occurrence of PAHs in the river sediment than in water. Therefore, it was considered that the elucidation of the distribution and the fate of PAHs in the river sediment was important for safety assessment of PAHs in environmental water.

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