Short communication

Mutagenicity in the Surface Waters from Rivers in the UK and Japan from 1997 to 2005

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Mutagenicity was monitored in the surface water from the river Thames in 1997, 2002, 2003 and 2005. All samples from the Thames taken at sites in London (a place close to Tower bridge and a site near Teddington lock) and in Windsor show significant mutagenicity in the Ames test. This suggests mutagenic pollution in the Thames was not improved from 1997 to 2005. Water samples from the Serpentine in Hyde Park, London and a sample from the river Dee in Chester also show mutagenic activities toward *S. typhimurium* YG1024. The mutagenicity (500–2500 revertants per liter) toward *S. typhimurium* YG1024 in the presence of metabolic activation found in the water from the Thames in London was comparable with that found in the water from the river Asahi in Okayama and the river Katsura in Kyoto, Japan in 2003 and 2005.

A comparison of the degree of mutagenicity was made between water samples from the Thames, Asahi and the Katsura rivers. Higher mutagenicity was observed with the *O*-acetyltransferase-overproducing strains, *S. typhimurium* YG1024 and YG1029, than with the parental strains *S. typhimurium* TA98 and TA100. This suggests the presence of amino- or nitro-groups in the structure of molecules polluting the water from all three rivers. Water samples taken from the Thames in 2002 and 2005 exerted mutagenicity in *S. typhimurium* YG1029, YG1024 and TA98 in the presence of metabolic activation but not in the absence of S9 mix, whereas samples from the Asahi and the Katsura showed mutagenicity in *S. typhimurium* YG1029, YG1024 and TA98 both in the presence and in the absence of S9 mix. This suggests that mutagenic pollution in the Thames and the Japanese rivers took place with different mutagenic contaminants.

Key words: mutagenicity, river water, blue rayon, Ames test, river Thames

Introduction

Surface water is required as a water source for drinking water or is used directly for agricultural or industrial purposes. Contamination of water by mutagens may have importance for humans by means of the safety of food and drink as well for the ecological

safety of organisms. Using different approaches, a large number of studies of surface waters have been verified to be contaminated by toxic substances, for example, polyaromatic hydrocarbons (PAHs), 2-phenylbenzotriazole (PBTA) compounds, heterocyclic amines and heavy metals, which could induce genetic damages (1–3). The methods for sample preparation described vary between blue-rayon, XAD resin, liquid-liquid extraction and others (2,4). Assay procedures for the genotoxicity studies employed also vary with the Salmonella/mutagenicity test, the comet assay, the micronucleus test and so on. The lack of unity of the methods gives rise to inconvenience for mutual comparison and diachronic monitoring of the genotoxicity of the water from the rivers.

Since the blue rayon method (5,6) is one of the most popular methods for sample preparation and much of the published water mutagenicity studies employed the Salmonella/Ames test (7), we prepared a ‘thorough protocol’ with the blue rayon method and the Ames test, which was used for long-term monitoring of surface water mutagenicity. Although many rivers have been evaluated for the presence of mutagenic activity (2), only a few studies have reported the mutagenicity of surface water from river in UK (8–10). This study presents an evaluation of the mutagenicity of the water from the Thames from 1997 to 2005 and the comparison of mutagenic specificities between rivers in the UK and Japan using the thorough protocol for monitoring.

Materials and Methods

Chemicals: Blue rayon was prepared from amorphous rayon staple fibers (Daiwabo, Hyogo, Japan) by dying with C. I. Reactive Blue 21 from Sumitomo Chemical (Osaka, Japan), as described previously (5). 2-

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Fig. 1. Site maps of water sampling from the river Thames in Greater London and Windsor, the river Dee in Chester and the Serpentine in London in UK.

Aminoanthracene, 6-aminochrysene, 2-aminofluorene, 9,10-dimethylbenzo[*a*]pyrene, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-nitrofluorene and 1-nitropyrene were reagent grade and obtained from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of analytical reagent grade.

Bacterial strains: *Salmonella typhimurium* strains TA98, and TA100 were a gift from Dr. B. N. Ames of the University of California, Berkeley. *S. typhimurium* strains YG1024/TA98(pYG219)/ and YG1029/TA100 (pYG219)/possessing elevated *O*-acetyltransferase levels (11), and YG1021/TA98(pYG216)/possessing elevated nitroreductase levels (12) were supplied by Dr. T. Nohmi of the National Institute of Health Sciences, Tokyo. Strains were characterized and maintained as previously recommended (7).

S9 mix: S9 fraction was prepared from the livers of male Sprague-Dawley rats that had been induced by administering polychlorinated biphenyl (PCB54, with a chlorine content of 54%, Tokyo Kasei, Tokyo). Protein content of S9 fraction was 5.75 mg/mL determined by Coomassie Brilliant Blue staining. Activities of 7-ethoxoresorufin *O*-deethylase and 7-methoxyresorufin *O*-demethylase of S9 were 617 and 312 pmol resorufin/

min/mg protein, respectively. Experiments were carried out in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, Japanese Government Animal Protection and Management Law (No. 105) and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). S9 mix contained 10% (by volume) S9 fraction and the following sterile co-factors: 100 mM sodium phosphate buffer pH 7.4, 8 mM magnesium chloride, 33 mM potassium chloride, 5 mM glucose-6-phosphate, 5 mM nicotinamide adenine dinucleotide phosphate, 5 mM glucose-6-phosphate, 5 mM nicotinamide adenine dinucleotide and 5 mM adenosine triphosphate.

Protocol for water sampling and extraction of mutagens with blue rayon: The sampling locations were points at (A) north bank of the Thames close to Tower Bridge, London, UK, (B) south bank of the Thames close to Teddington, Teddington, UK, (C) north bank on the Serpentine in Hyde Park, London, UK, (D) south bank of the Thames, close to Windsor Bridge, Windsor, UK, (E) north bank of the River Dee close to Queen's Park Bridge, Chester, UK (Fig. 1), (F) east bank of the river Asahi located near the Ezaki area, Okayama, Japan, (G) east bank of the river Katsura,

Fig. 2. Site maps of water sampling from the river Asahi in Okayama and the river Katsura in Kyoto, Japan.

close to Kuga bridge, Kyoto, Japan (Fig. 2). Point A is located in central London, between the Tower pier and St. Katharine's pier. Point B is located upstream and 19 km west of central London. Point C is located in a park in central London. Point D is located in a town 32 km west of central London. Point E is located in a city. Point F is located in the city center inside the urban area but an upstream of industrial areas; at the another side of the riverbank small clams are occasionally gathered up. Point G is located within an urban area, including a large factory for kimono-textile and close to a sewage plant. Hereafter, these locations are referred to as Tower Bridge, Teddington, the Serpentine, Windsor, Chester, Okayama and Kyoto, respectively. The weather on the sampling dates was sunny or cloudy, but not rain. The tide was checked and the sampling was performed at low tide. Sample number, sampling location and date are summarized in Table 1.

River water was sampled into a plastic container and divided into a series of one-liter samples in plastic bottles (one liter bottle). Blue rayon (2 g/one-liter of sample) was put into the sample water, and the bottles were mechanically shaken for 60 min at room temperature. After the 60 min shaking, the blue rayon was removed, and washed twice with distilled water and squeezed to remove excess water. 10 mL of methanol

was then added to the blue rayon in a sealed tube and stored in the refrigerator at 4°C. The extraction of absorbed substances was performed as follows. Blue rayon (2 g of each) containing any adsorbed water sample contaminants was put into an Erlenmeyer flask (500 mL) with 200 mL of methanol and 4 mL of aqueous 25% ammonia solution. The flask was mechanically shaken for 30 min and the extract was collected and poured into a Kjeldahl flask. The remaining blue rayon in the Erlenmeyer flask was extracted twice with 100 mL of methanol-ammonia (50:1) each for 30 min, and the resulting extract was taken and added to the Kjeldahl flask. The combined extracts (200 mL, 100 mL and 100 mL) were evaporated to dryness under reduced pressure. The residue was taken up in 10 mL of methanol and the resulting solution was put into a test tube and evaporated to dryness. The residue was dissolved in 0.1 mL of dimethylsulfoxide and subjected to the Ames test. For the negative control, a series of one-liter samples of de-ionized water were placed into plastic bottles, blue rayon (2 g each) was put into the water, and the bottles were mechanically shaken at for 60 min at room temperature. The extraction and the Ames test were performed as described above.

Mutation test: The blue rayon extracts of river water (0.5 or 1 liter) dissolved in 0.1 mL of dimethylsul-

Table 1. Monitoring of the mutagenicity of the river water

Samples River No. Location		Sampling date	Amount of water (L)	Average no. of His ⁺ revertants/plate*									
				YG1024		YG1029		<i>S. typhimurium</i> TA98		TA100		YG1021	
				+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9	− S9
River Thames													
1.	Tower Bridge	Oct. 31, 1997	1	<u>1384</u>	nd	nd	nd	nd	nd	nd	nd	nd	nd
			0.5	<u>300</u>	nd	nd	nd	nd	nd	nd	nd	nd	nd
2.	Tower Bridge	Aug. 8, 2002	1	<u>652</u>	nd	344 [†]	nd	nd	nd	nd	nd	nd	nd
3.	Tower Bridge	Sep. 26, 2003	1	<u>549</u>	nd	nd	nd	nd	nd	nd	nd	nd	nd
4.	Tower Bridge	July 22, 2005	1	<u>834</u>	nd	194	nd	nd	nd	nd	nd	nd	nd
5.	Teddington	Oct. 31, 1997	1	<u>973</u>	nd	nd	nd	nd	nd	nd	nd	nd	nd
			0.5	<u>450</u>	nd	nd	nd	nd	nd	nd	nd	nd	nd
6.	Teddington	August 23, 2002	1	<u>1005</u>	nd	<u>2384</u>	170	nd	nd	<u>270</u>	nd	nd	nd
7.	Teddington	July 25, 2005	1	<u>1274</u>	55	<u>371</u>	140	<u>105</u>	39	<u>73</u>	68	nd	nd
			0.5	<u>544</u>	nd	164	nd	nd	nd	nd	nd	nd	nd
8.	Windsor	July 28, 2005	1	<u>268</u>	nd	138	nd	nd	nd	nd	nd	nd	nd
			0.5	<u>247</u>	nd	104	nd	nd	nd	nd	nd	nd	nd
The Surpentine													
9.	Hyde Park	Sep. 21, 2002	1	<u>293</u>	nd	<u>277</u> [†]	nd	nd	nd	nd	nd	nd	nd
River Dee													
10.	Chester	Dec. 31, 2002	1	<u>741</u> [†]	nd	nd	nd	nd	nd	nd	nd	nd	nd
River Asahi													
11.	Okayama	May 20, 2003	1	<u>2101</u>	nd	148	nd	23	nd	nd	nd	nd	nd
12.	Okayama	July 15, 2003	1	<u>882</u>	<u>93</u>	218	82	51	nd	110	nd	nd	nd
13.	Okayama	December 15, 2005	1	<u>1073</u>	<u>2267</u>	<u>288</u>	<u>254</u>	78	<u>105</u>	204	131	<u>187</u>	<u>115</u>
			0.5	<u>544</u>	nd	<u>240</u>	nd	nd	nd	nd	nd	nd	nd
River Katsura													
14.	Kuga bridge	May 31, 2005	1	<u>1412</u>	<u>114</u>	<u>352</u>	163	<u>186</u>	24	118	98	nd	nd
			0.5	<u>776</u>	nd	nd	nd	nd	nd	nd	nd	nd	nd
Negative control [‡]													
DMSO		0.1 mL		83 ± 25	28 ± 9	108 ± 29	118 ± 27	37 ± 15	30 ± 7	131 ± 53	125 ± 30	33 ± 6	23 ± 6
Positive control (nmole/plate)													
2-Aminoanthracene			1	1456	nd	628	nd	143	nd	109	nd	nd	nd
			10	nd	nd	nd	nd	728	nd	1022	nd	280	nd
6-Aminochrysene			1	3463	nd	1200	nd	nd	nd	nd	nd	nd	nd
			10	nd	nd	nd	nd	2978	nd	224	nd	nd	nd
2-Aminofluorene			1	1929	nd	728	nd	41	nd	95	nd	nd	nd
			40	nd	nd	nd	nd	1242	nd	535	nd	nd	nd
9,10-Dimethylbenzo[<i>a</i>]pyrene													
			100	212	nd	2249	nd	365	nd	1183	nd	nd	nd
Trp-P-2			1	4493	nd	126	nd	2185	nd	94	nd	nd	nd
IO			0.01	4715	nd	341	nd	906	nd	115	nd	nd	nd
2-Nitrofluorene			1	nd	1120	nd	721	nd	71	nd	130	nd	515
			10	nd	nd	nd	nd	nd	742	nd	728	nd	5181
1-Nitropyrene			1	nd	21457	nd	1465	nd	924	nd	283	nd	nd
			4	nd	nd	nd	nd	nd	3964	nd	1825	nd	nd

nd: not determined. Underline: Water samples are judged to be mutagenic.

*Averages of two or three plates except three samples marked †.

†Results of single plate.

‡Averages ± standard deviation of more than 10 plates.

foxide were assayed with *S. typhimurium* TA98, TA100, YG1021, YG1024 and YG1029 using preincubation techniques (13) of the Ames test (7) with or without metabolic activation. Briefly, the preincubation mixture was prepared by mixing the components in the following order: 0.1 mL of a sample in DMSO solution, 0.5 mL of 0.1 M sodium phosphate buffer at pH 7.4 or 0.5 mL of S9 mix, and 0.1 mL of an overnight culture of

bacteria. After incubation for 20 min at 37°C, molten agar was added and the mixture was poured onto a minimal agar plate. Plates were incubated for 72 hr at 37°C and revertant colonies were scored manually. When the number per plate exceeded 3000, colonies in a certain square area were counted, and from the average counts in 5 such areas the total number on the plate was estimated. Experiments were performed in duplicate or

in triplicate and average values of results were determined. Since aminoaromatics include PBTA, PAHs, nitro-PAHs and heterocyclic amines were found as major mutagens in the surface waters (2), amino-PAHs (2-aminoanthracene, 6-aminochrysene and 2-aminofluorene), heterocyclic amines (Trp-P-2 and IQ), PAH (9,10-dimethylbenzo[*a*]pyrene) and nitro-PAHs (2-nitrofluorene and 1-nitropyrene) were used as positive controls. These compounds were chosen because aminoaromatics like PBTA compounds (14–17), poly aromatic hydrocarbons (18–20), heterocyclic amines (21,22) and 1-nitropyrene (23) were reported as suspected mutagens in surface waters (2). Average numbers of His⁺ revertants/plate of positive controls were obtained from dose-response relationships with four or six dosages. All experiments were carried out in accordance with the Safety Guidelines of Okayama University and Japanese Government Management Law for the toxic chemicals (No. 303).

Results and Discussion

All samples from the Thames, Dee, Asahi, Katsura and the Serpentine were mutagenic towards *S. typhimurium* YG1024 with metabolic activation (Table 1). Water samples were judged to be mutagenic when average numbers of His⁺ revertant obtained were more than twice those of the negative control (underline in Table 1). Samples obtained from the Thames at Tower bridge in 2002 (sample No. 2), at Teddington in 2002 (sample No. 6) and in 2005 (sample No. 7), at Hyde Park (sample No. 9) and from the Katsura in Kyoto in 2005 (sample No. 14) were also mutagenic towards *S. typhimurium* YG1029 with metabolic activation (Table 1). No mutagenic activity was observed with the control sample extracted with blue rayon from de-ionized water (data not shown). Greater mutagenicity was observed with extracts from one-liter of water than that from 0.5 liter of water with sample No. 1 toward *S. typhimurium* YG1024 with S9 mix, No. 5 toward YG1024 with S9 mix and No. 7 toward YG1024 and YG1029 with S9 mix, No. 13 toward YG1024 and YG1029 with S9 mix and No. 14 toward YG1024 with S9 mix (Table 1). The mutagenicity of the samples from the Thames at Tower Bridge and Teddington were comparable from 1997 to 2005. This suggests mutagenic pollution in the Thames did not improve from 1997 to 2005. Although Teddington is located upstream of the center of London, the mutagenicity of the water samples obtained at Teddington in 2002 and 2005 was not lower than the samples at Tower Bridge. The water in the Thames was still mutagenically polluted at Windsor, although the activity was decreased to a quarter of the levels obtained at Teddington. Since water samples from the Serpentine and from the Dee were also mutagenic in the Ames test,

evaluation of the mutagenicity in water from rivers in the UK as well as the Thames should be performed for environmental preservation. Water samples (Nos. 6 and 7), taken from the Thames in 2002 and 2005, exerted mutagenicity in *S. typhimurium* YG1029, YG1024, and TA98 in the presence of metabolic activation but not in the absence of S9 mix (Table 1). Mutagens having three or more fused aromatic rings in their molecules can be adsorbed to the blue-rayon (5). Several amino-aromatics are reported as mutagens found in the river water, for example, heterocyclic amines in the river Danube (21), azo-dyes in the river Cristais (24) or PBTA-type mutagens in the Japanese rivers (25). Because of the necessity of metabolic activation and the higher mutagenicity towards YG strains than TA strains, the contaminants adsorbed to blue-rayon from the water (samples Nos. 1 to 8) in the Thames might have amino-aromatic structures.

All samples from the Asahi (samples Nos. 11, 12 and 13) and the Katsura (sample No. 14) in Japan showed mutagenicity toward *S. typhimurium* YG1024 in the presence of S9 mix (Table 1). Water samples from the Thames and from the Japanese rivers showed mutagenicity in YG1024 in the presence of metabolic activation, suggesting the presence of similar types of mutagenic contaminants. The samples (Nos. 12, 13 and 14) were also mutagenic towards *S. typhimurium* YG1024 in the absence of S9 mix (Table 1). These water sample from the Japanese rivers might include some direct-acting mutagens as well. The samples from the Asahi (No. 13) and from the Katsura (No. 14) also exerted mutagenicity in *S. typhimurium* YG1029 and TA98 in the presence or in the absence of S9 mix. The values of His⁺ revertant per liter of sample with *S. typhimurium* YG1029 in the presence of S9 mix were less than a quarter of those obtained with *S. typhimurium* YG1024. Higher mutagenicity of the water samples (Nos. 12, 13 and 14) were observed towards YG strains than TA strains in the Ames test. Major mutagens found in the samples from the Asahi and the Katsura (samples Nos. 11 to 14) might be a mixture of frameshift mutagens and those having an amino- or nitro-group in the polycyclic structures. Since the mutagenicity of sample No. 13 is higher towards YG 1024 than YG1021, amino-group is more likely than nitro-group in the structure of mutagenic contaminants in sample No. 13, for examples, amino-PAHs and heterocyclic amines in Table 1.

It has been reported that heterocyclic amines (18) and nitro-PAHs (23) were detected in the samples from the river Yodo. Benzo[*a*]pyrene was also detected in the water from the Asahi in 1994 and 1995 (19). PBTA-type mutagens were found in the river Nishitakase, Uji and Nikko (25) and PCB derivatives from the river Waka (26). Mutagens in the Katsura was investigated in detail as PBTA-1, -2, -3, -6, -7, and -8 and found to have

originated from the azo dyes, industrial chemicals used in textile dyeing, *via* chlorination in the sewage works (14–17). Although the sewage process with chlorination was partly converted to ozone-treatment, the water from the Katsura still had mutagenic contaminants in 2005. Mutagenic contaminants in the Asahi also remained in 2005. Regular and continuous monitoring of the mutagenicity of river water is necessary to maintain the aquatic environment around the world.

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References

- Ohe T, White PA, DeMarini DM. Mutagenic characteristics of river waters flowing through large metropolitan areas in North America. *Mutat Res.* 2003; 534: 101–12.
- Ohe T, Watanabe T., Wakabayashi K. Mutagens in surface waters: a review. *Mutat Res.* 2004; 567: 109–49.
- Chen G, White PA. The mutagenic hazards of aquatic sediments: a review. *Mutat Res.* 2004; 567: 151–225.
- DeMarini DM, Plewa MJ., Brockman HE. Use of four short-term tests to evaluate the mutagenicity of municipal water. *J Toxicol Environ Health.* 1982; 9: 127–40.
- Hayatsu H, Oka T, Wakata A, Ohara Y, Hayatsu T, Kobayashi H, Arimoto S. Adsorption of mutagens to cotton bearing covalently bound trisulfo-copperphthalocyanine, *Mutat Res.* 1983; 119: 233–8.
- Hayatsu H. Cellulose bearing covalently linked copper phthalocyanine trisulphonate as an adsorbent selective for polycyclic compounds and its use in studies of environmental mutagens and carcinogens. *J Chromatogr.* 1992; 597: 37–56.
- Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. *Mutat Res.* 1983; 113: 173–215.
- Harvey JS, Lyons BP, Waldock M, Parry JM. The application of the ³²P-postlabelling assay to aquatic biomonitoring. *Mutat Res.* 1997; 378: 77–88.
- Lyons BP, Harvey JS, Parry JM. An initial assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in the intertidal teleost *Lipophrys pholis*. *Mutat Res.* 1997; 390: 263–8.
- Lyons BP, Stewart C, Kirby MF. The detection of biomarkers of genotoxin exposure in the European flounder (*Platichthys flesus*) collected from the River Tyne Estuary. *Mutat Res.* 1999; 446: 111–9.
- Watanabe M, Ishidate M, Nohmi T. Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated O-acetyltransferase levels. *Mutat Res.* 1990; 234: 337–48.
- Watanabe M, Ishidate M, Nohmi T. A sensitive method for the detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of *Salmonella typhimurium* strains TA98 and TA100. *Mutat Res.* 1989; 216: 211–20.
- Yahagi T, Nagao M, Seino Y, Matsushima T, Sugimura T, Okada M. Mutagenicities of N-nitrosamines on *Salmonella*. *Mutat Res.* 1977; 48: 121–30.
- Ohe T, Takeuchi N, Watanabe T, Tada A, Nukaya H, Terao Y, Sawanishi H, Hirayama T, Sugimura T, Wakabayashi K. Quantification of two aromatic amine mutagens, PBTA-1 and PBTA-2, in the yodo river system. *Environ Health Perspect.* 1999; 107: 701–4.
- Shiozawa T, Tada A, Nukaya H, Watanabe T, Takahashi Y, Asanoma M, Ohe T, Sawanishi H, Katsuhara T, Sugimura T, Wakabayashi K, Terao Y. Isolation and identification of a new 2-phenylbenzotriazole-type mutagen (PBTA-3) in the Nikko river in Aichi, Japan. *Chem Res Toxicol.* 2000; 13: 535–40.
- Watanabe T, Nukaya H, Terao Y, Takahashi Y, Tada A, Takamura T, Sawanishi H, Ohe T, Hirayama T, Sugimura T, Wakabayashi K. Synthesis of 2-phenylbenzotriazole-type mutagens, PBTA-5 and PBTA-6, and their detection in river water from Japan. *Mutat Res.* 2001; 498: 107–15.
- Watanabe T, Takahashi Y, Takahashi T, Terao Y, Nukaya H, Takamura T, Sawanishi H, Ohe T, Hirayama T, Wakabayashi K. Mutagenicity of two 2-phenylbenzotriazole derivatives, 2-[2-(acetyl-amino)-4-(diethylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole and 2-[2-(acetyl-amino)-4-(diallylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole and their detection in river water in Japan. *Mutagenesis* 2002; 17: 293–9.
- Ohe T. Quantitation of mutagenic and carcinogenic heterocyclic amines, MeIQx, Trp-P-1, Trp-P-2 and PhIP, contributing highly to genotoxicity of river water. *Mutat Res.* 1997; 371: 79–85.
- Kira S, Nogami Y, Ito T, Hayatsu H. Detection of benzo(a)pyrene and mutagenicity in water of Lake Baikal (Russia) and rivers in Okayama (Japan) using the blue rayon method: A simplified handling and transportation of samples from remote sites. *Environ Toxicol.* 1999; 14: 279–84.
- Nagai A, Kano Y, Funasaka R, Nakamuro K. Mutagenic characteristics and contribution of polycyclic aromatic hydrocarbons to mutagenicity of concentrates from municipal river water by blue chitin column. *J Health Sci.* 2002; 48: 232–41.
- Kataoka H, Hayatsu T, Hietsch G, Steinkellner H, Nishioka S, Narimatsu S, Knasmüller S, Hayatsu H. Identification of mutagenic heterocyclic amines (IQ, Trp-P-1 and AαC) in the water of the Danube river. *Mutat Res.* 2000; 466: 27–35.
- Ono Y, Somiya I, Oda Y. Identification of a carcinogenic heterocyclic amine in river water. *Water Res.* 2000; 34: 890–4.
- Ohe T, Nukaya H. Genotoxic activity of 1-nitropyrene in water from the Yodo River, Japan. *Sci Total Environ.* 1996; 181: 7–12.

- 24 Umbuzeiro GA, Freeman HS, Warren SH, Oliveira DP, Terao Y, Watanabe T, Claxton LD. The contribution of azo dyes to the mutagenic activity of the Cristais river. *Chemosphere*. 2005; 50: 55–64.
- 25 Nukaya H, Yamashita J, Tsuji K, Terao Y, Ohe T, Sawanishi H, Kasuhara T, Kiyokawa K, Tezuka M, Oguri A, Sugimura T, Wakabayashi K. Isolation and chemical-structural determination of novel aromatic amine mutagen in water from Nishitakase river in Kyoto. *Chem Res Toxicol*. 1997; 10: 1061–6.
- 26 Takamura-Enya T, Watanabe T, Tada A, Hirayama T, Nukaya H, Sugimura T, Wakabayashi K. Identification of a new mutagenic polychlorinated biphenyl derivative in the Waka River, Wakayama, Japan, showing activation of an aryl hydrocarbon receptor-dependent transcription. *Chem Res Toxicol*. 2002; 15: 419–25.