RESEARCH COMMUNICATION

Mutagenicity of the Drinking Water Supply in Bangkok

Wannee R Kusamran, Nopsarun Tanthasri, Nuntana Meesiripan, Anong Tepsuwan

Abstract

Seventeen samples of tap water in Bangkok and 2 neighboring provinces were collected in winter and summer, concentrated and tested for mutagenic activity using the Ames Salmonella mutagenesis assay. Preliminary results demonstrated that concentrated tap water exhibited clear mutagenicity towards *S. typhimurium* TA100 and YG1029, but not towards TA98 and YG1024, in the absence of S9 mix, and the addition of S9 mix markedly decreased the mutagenicity to both tester strains. Amberlite® XAD-2 resin, but not blue rayon, was able to adsorb mutagens from water at pH 2.

Our data clearly demonstrated that all tap water samples prepared by chlorination of Chao Phraya River water were mutagenic to strain TA100 without S9 mix, inducing 3,351 ± 741 and 2,216 ± 770 revertants/l, in winter and summer, respectively. On the other hand, however, tap water samples prepared from ground water were not mutagenic. Furthermore, it was found that boiling for only 5 min and filtration through home purifying system containing activated charcoal and mixed resin units were very effective to abolish the mutagenicity of water. Storage of water also significantly decreased the mutagenicity, however, it took 2-3 weeks to totally abolish it. Additionally, we also found 1 out of 6 brands of commercially available bottled drinking water to be mutagenic, with about 26 % of the average mutagenicity of tap water.

The results in the present study clearly demonstrated that chlorinated tap water in Bangkok and neighboring provinces contain direct-acting mutagens causing capable of causing base-pair substitution. Boiling and filtration of tap water through home purifying systems may be the most effective means to abolish the mutagenicity. Some brands of commercial bottled waters may also contain mutagens which may be derived from tap water.

Key Words: Chlorinated tap water - chlorinated drinking water - chlorination by-products - *Salmonella typhimurium* - mutagenicity - XAD-2 resin - Bangkok

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Introduction

At present, disinfection of drinking water by chlorination is the principal means of effectively preventing water-borne enteric diseases, such as typhoid fever, cholera and dysentery, in all over the world (IARC, 1991). However, chlorine may react with humic substances in water and generate large numbers of halogenated organic by-products including chlorinated and brominated trihalomethanes (e.g., chloroform and bromodichloromethane), haloacetic acid as well as bacterial mutagens. Several epidemiological studies have associated the consumption of chlorinated drinking water with some forms of human cancer, such as bladder, kidney, stomach and pancreatic cancers (Morris et al., 1993; Koivusalo et al., 1994; Morris, 1995).

There were reports from many countries demonstrating that chlorinated drinking water was mutagenic towards *Salmonella typhimurium* when tested using the *Salmonella*/microsome mutagenicity assay (IARC, 1991). A number of substances found in chlorinated water have been shown to be bacterial mutagens. However, only the chlorinated furanones, i.e. 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) and related compounds, are sufficiently potent and occur in sufficiently high concentrations to account for a significant proportion of the mutagenicity detected in the water (Kronberg and Vartiainen,

Corresponding Author: Dr. Wannee R. Kusamran, Biochemistry and Chemical Carcinogenesis Section, Research Division, National Cancer Institute, Rama VI Road, Bangkok 10400, Thailand. Tel: 66-2246-1300 Ext. 1401-2 Fax: 66-2246-5145, 66-2246-1300 Ext. 1414 E-mail: waroran@health.moph.go.th

1988). Interestingly, MX has recently been reported to induce tumors in various organs of both male and female Wistar rats (Komulainen et al., 1997).

In Bangkok, the capital of Thailand, and neighboring province, i.e. Nonthaburi, chlorine has long been used to disinfect water supply prepared from the Chao Phraya River water. Volatile trihalomethanes such as chloroform, bromodichloromethane and chlorodibromomethane have been detected in chlorinated tap water samples both from the treatment plants and from the distribution system in Bangkok Metropolitan area, while water samples from areas using ground water and the raw river water were not found to contain these trihalomethanes (Onodera et al., 1984). However, the mutagenicity of tap water supply has never been investigated. In this communication, we report the mutagenicity of tap water prepared both from chlorinated river water and ground water. The effects of boiling, filtration through home purifying system and storage on the mutagenicity of chlorinated tap water, as well as the mutagenicity of commercial bottled water were also reported.

Materials and Methods

Materials

Amberlite® XAD-2 resin, glucose-6-phosphate (G6P) and benzo(a)pyrene [B(a)P] were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Blue rayon was purchased from Funakoshi Pharmaceutical Co. (Tokyo, Japan), β-NADP from Oriental Yeast Co. Ltd. (Osaka, Japan), polychlorinated biphenyl (PCB-54) from TCI (Tokyo, Japan) and dimethyl sulfoxide (DMSO), spectrophotometric grade, from E. Merck (Darmstard, Germany). 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) was kindly provided by Prof. Dr. T. Matsushima, Japan Bioassay Research Center, Kanagawa, Japan. Bacto agar and Oxoid nutrient broth No. 2 were obtained from Difco Laboratories (Detroit, MI, USA) and Oxoid Ltd., (Hants, UK), respectively. Ethyl acetate, AnalaR® grade, was purchased from BDH Chemical Supplies (Poole, UK) and all other chemicals used were of analytical grade.

Bacterial tester strains

Salmonella typhimurium strains TA98 and TA100 were kindly provided by Prof. Dr. T. Matsushima, Japan Bioassay Research Center, Japan, while strains YG1024 and YG1029 were kindly provided by Dr. T. Nohmi, National Institute of Health Sciences, Tokyo, Japan. Strains YG1024 and YG1029 were produced by introducing plasmids containing the acetyltransferase gene from strain TA1538 into strains TA98 and TA100, respectively (Watanabe et al., 1990). The tester strains were cultured in Oxoid nutrient broth No. 2 for 14-16 h before use.

Collection of water samples

Fifteen tap water samples were collected from 15 locations (13 districts), including 2 treatment plants, in Bangkok, and 1 sample each from Nonthaburi and Pathumthani provinces, as shown in Table 1. Among these tap waters, 15 samples were prepared by chlorination of Chao Phraya River water and the other 2 samples location nos. 13 & 17 were ground water. Most samples were collected both in summer (April-June) and in winter (January-February).

Table 1. Names of Districts in Bangkok and Provinces where Tap Water Samples were Collected from

Location no.	Name of districts in Bangkok/provinces
1	Bangkaen Treatment Plant
2	Bangkapi
3	Bangplad
4	Bangsue
5	Chatuchak
6	Donmueng
7	Dusit
8	Phranakorn
9	Phyathai
10	Pomprab Sattrupai
11	Rajthevi-1 (Rama VI Rd.)
12	Rajthevi-2 (Petchburi Rd.)
13	Tungkru-1 (Pracha-uthit Rd. 54)
14	Tungkru-1 (Pracha-uthit Rd. 76)
15	Samsen Treatment Plant
16	Nonthaburi province
17	Pathumthani province

Bottled drinking water available commercially was also collected. Six brands were bought from local supermarkets in summer.

Concentration of water samples

For most experiments, 4 liters of tap water samples were stirred overnight to remove free chlorine and then their pH was adjusted to 2 with 6 N HCl. Each sample was applied to a column (1.6 cm x 10 cm) containing Amberlite® XAD-2 resin, which was previously successively cleaned with acetone and methanol and then stored in distilled water (Durtson and Ames, 1974; Yamasaki and Ames, 1977). After the water was completely passed through the column, the column was washed with 2 ml distilled water and the adsorbed materials were then eluted from the resin with 150 ml ethyl acetate following the procedure essentially described by Vartiainen et al. (Vartiainen et al., 1988). The eluate was evaporated under reduced pressure at 40-45°C using a rotary evaporator. The residue was dissolved in 1-2 ml DMSO and then tested for mutagenicity.

In case of using blue rayon as an adsorbent to concentrate tap water, 1 gram of blue rayon was filled in a plastic net bag and hung in a beaker containing 4 liters of treated water and then the water was stirred for 24 hr. The process was repeated once with another 1 g of blue rayon. Two lots of blue rayon were combined and washed 3 times with distilled water and dried with paper towels. The adsorbed materials

were then eluted from blue rayon with 150 ml of ethyl acetate by the method modified from that described by Sakamoto and Hayatsu (Sakamoto and Hayatsu, 1990). After that, the extract was evaporated and dissolved in DMSO as described above.

Mutagenesis assay

Mutagenicity was determined by standard plate incorporation assay on *Salmonella typhimurium* strains TA98, TA100, YG1024 and YG1029 in the presence and in the absence of S9 mix as described by Maron and Ames (Maron and Ames, 1983). S9 mix used in this study contained 50 µl of S9 (PCB-54-induced rat liver S9) per 500 µl.

Mutagenic potency (number of His^+ revertants/liter) was calculated using the Microsoft Excel program. The criteria for positivity or negativity of samples were considered according to Maron and Ames (Maron and Ames, 1983).

Results

Mutagenicity of tap water extracted by acetone-hexane, ethyl acetate and methanol towards Salmonella typhimurium in the presence and in the absence of S9 mix

Various kinds of solvents, i.e. acetone, acetone in hexane, dichloromethane, DMSO, ethyl acetate, ethyl ether and methanol have been used to elute the mutagenic compounds in the water that adsorbed to XAD resin (IARC, 1991). In our preliminary study, the mutagenicities of organic materials present in one water sample which were adsorbed to XAD-2 columns and eluted by 3 kinds of solvent, namely, 15 % acetone in hexane, ethyl acetate and methanol were compared by determining in *S. typhimurium* strains TA98, TA100, YG1024 and YG1029 both in the presence and in the absence of S9 mix. Results in Table 2 showed that materials eluted by all 3 kinds of solvent exhibited significant mutagenicities towards only strains TA100 and YG1029 in the absence of S9 mix, but strain TA100 was slightly more sensitive than YG1029. However, the addition of S9 mix markedly decreased the mutagenicities to both tester strains. Results shown in Table 2 also demonstrated that the mutagenicities of adsorbed materials eluted by 15 % acetone in hexane and ethyl acetate were somewhat quite equal, but were a little stronger than those eluted by methanol. We therefore used ethyl acetate to elute the organic compounds in tap water that adsorbed to XAD-2 resin and used *S. typhimurium* only strain TA100 for testing the mutagenicities of tap water samples in the absence of S9 mix in the rest of experiments.

Comparison of the efficiency of blue rayon with that of XAD-2 resin to extract the mutagenic compounds from tap water at 3 different pH

Blue rayon has been successfully used for extracting the mutagenic compounds from river water (Sakamoto and Hayatsu, 1990; Kusamran et al., 1994; Kataoka et al., 2000). The method using this adsorbent is simpler than that using XAD-2. The pH of water is also important for extraction of mutagenic compounds. It has been shown that high mutagenicity was obtained when pH of water was adjusted to be in the acid range especially at pH 2 (IARC, 1991). However, there were some investigators who could also extract mutagens from chlorinated water in the neutral or alkaline ranges (Monarca et al., 1985; Vartiainen et al., 1987; Wei et al., 1984). In this study, we therefore compared the

Table 2. Mutagenicity of Tap Water Extracted by 3 Kinds of Solvent Towards *S. typhimurium* TA98, TA100, YG1024 and YG1029 in the Presence and in the Absence of S9 Mix

Solvent	Volume		Number of His^+ revertants/plate*							
	E (µl)	water (ml)	TA98		YG1024		TA100		YG1029	
			-S9 mix	+S9 mix	-S9 mix	+S9 mix	-S9 mix	+S9 mix	-S9 mix	+S9 mix
15 % Acetone	40	80	45	54	91	163	329	224	231	202
in hexane	100	200	73	63	127	177	544	254	407	236
Ethyl acetate	40	80	50	59	82	126	331	163	234	228
	100	200	61	77	108	168	555	277	374	301
Methanol	40	80	58	64	74	145	300	211	224	226
	100	200	55	63	99	167	506	261	319	272
AF-2	-	-	782	ND	914	ND	615	ND	704	ND
B(a)P	-	-	ND	972	ND	1213	ND	1150	ND	1205
DMSO	-	-	47	61	83	129	182	183	135	176

Three water samples from location no.11 were, after adjusting the pH to 2, applied to XAD-2 columns and the adsorbed materials were eluted with 150 ml of either 15 % acetone in hexane, ethyl acetate or methanol. The eluates were then processed for mutagenicity testing as described in Materials and Methods.

*Values are averages obtained from 2 independent experiments.

The amount of AF-2 used for strains TA98 and YG1024 was 0.25 µg/plate and for strains TA100 and YG1029 was 0.025 µg/plate, and that of B(a)P used for all strains was 5 µg/plate.

E, water extract; ND, not determined.

Table 3. Mutagenicities of Tap Waters Extracted by Amberlite® XAD-2 Resin and Blue Rayon at 3 Different pH Towards *S. typhimurium* TA 100 in the Presence and in the Absence of S9 Mix

Adsorbent	pH	Volume		Number of His^+ revertants/plate*	
		E(µl)	water (ml)	-S9 mix	+S9 mix
XAD-2	2	40	100	236	102
		100	250	571	138
	7.9	40	100	36	106
		100	250	0	105
	11	40	100	21	70
		100	250	-18	58
Blue rayon	2	40	100	-2	53
		100	250	4	78
	7.9	40	100	-9	118
		100	250	40	129
	11	40	100	8	-2
		100	250	-11	-18

The pH of water samples from location no. 3 was adjusted to 2 and 11 using 6 N HCl and 1M NaOH, respectively, while the pH of 7.9 was the original one of water. The water samples were then either applied to the XAD-2 columns or brought to extract the mutagenic materials by using blue rayon as described in Materials and Methods.

*Values are averages obtained from 2 independent experiments and have already been corrected for average numbers of spontaneous revertants: 172 and 158 revertants/plate for –S9 mix and +S9 mix, respectively.

[a]E, water extract.

mutagenicity of water extracted by XAD-2 resin with that by blue rayon at 3 different pH, i.e. 2, 7.9 and 11. Table 3 shows that tap water exhibited significant mutagenicity towards *S. typhimurium* TA100 when XAD-2 resin was used as adsorbent and the pH of water was 2, while water having pH 7.9 (original pH of water sample) and pH 11 did not show any mutagenicity. In contrast, water samples at all 3 pH did not exhibit any mutagenicity when blue rayon was used for extracting the organic materials.

Mutagenicities of tap waters collected from different locations in Bangkok Metropolitan and neighboring provinces

Seventeen samples of tap water were collected as described in Materials and Methods. Table 4 shows the mutagenicities of all samples collected in both winter (January-February) and summer (April-June) towards *S. typhimurium* TA100 in the absence of S9 mix. Water samples collected in both seasons from most locations, except location nos. 13 and 17, exhibited clear mutagenicities. These 15 samples were prepared by chlorination of Chao Phraya River water while those from location nos. 13 and 17 were ground water. The mutagenic activities of these 15 samples varied considerably from place to place. For samples collected in winter, 9 samples (from

Table 4. Mutagenicities of Tap Waters Collected from Different Locations in Bangkok and 2 Neighboring Provinces in Winter and Summer Towards *S. typhimurium* TA100 in the Absence of S9 Mix

Location no.	Winter			Summer		
	Volume of water (ml)	No. of His^+ revertants* Per plate	Per liter	Volume of water (ml)	No. of His^+ revertants* Per plate	Per liter
1	0	159		NA		
	80	511				
	160	833				
	400	1,750	3,970			
2	NA			0	194	
				50	267	
				100	347	
				250	531	1,347
3	0	198		0	212	
	40	251		100	400	
	80	462		250	812	2,407
	200	895	3,602			
4	0	163		0	195	
	80	516		80	381	
	160	904		160	543	
	400	1,857	4,244	400	1,053	2,139
5	0	225		NA		
	80	443				
	160	806				
	400	1,588	3,444			
6	0	152		NA		
	100	409				
	200	734				
	400	1,312	2,916			
7	0	159		0	213	
	80	298		80	486	
	160	425		160	739	
	400	1,177	2,542	400	1,564	3,373
8	0	159		0	213	
	80	473		80	342	
	160	782		160	497	
	400	1,801	4,104	400	964	1,884
9	0	163		0	176	
	80	446		80	359	
	160	610		160	488	
	400	1,532	3,393	400	1,098	2,292
10	0	163		NA		
	80	446				
	160	712				
	400	1,648	3,708			
11	0	225		0	177	
	80	444		100	361	
	160	690		250	655	1,913
	400	1,527	3,262			

Table 4. Continued

Location no.	Winter			Summer		
	Volume of water (ml)	No. of His⁺ revertants*		Volume of water (ml)	No. of His⁺ revertants*	
		Per plate	Per liter		Per plate	Per liter
12	0	202		0	213	
	80	321		80	308	
	160	480		160	519	
	400	1,048	2,125	400	914	1,782
13	0	167		0	194	
	80	182		50	217	
	160	167		100	218	
	400	183	33(NM)	250	245	192(NM)
14	0	225		0	195	
	80	487		80	311	
	160	898		160	457	
	400	2,009	4,497	400	914	1,805
15	0	175		0	213	
	80	345		80	328	
	160	630		160	445	
	400	1,324	2,901	400	831	1,546
16	0	152		0	182	
	100	306		80	442	
	200	611		200	956	3,883
	400	1,017	2,204			
17	0	198		NA		
	40	192				
	80	203				
	200	210	72(NM)			

Four liters of each water sample were, after adjusting the pH to 2,applied to Amberlite® XAD-2 column and the adsorbed materials were eluted with ethyl acetate and processed for mutagenicity testing as described in Materials and Methods.
*Values are averages obtained from 2 independent experiments.
NA, not available; NM, not mutagenic

location nos. 1, 3-5, 8-11 and 14) exhibited quite strong mutagenicities, being 3,262-4,497 revertants/l, while the other 5 samples showed moderate mutagenicities, being 2,125-2,916 revertants/l. The average mutagenicities of these 14 samples was 3,351 ± 741 revertants/l. However, for samples collected in summer, there were only 2 (from location nos. 7 and 16) out of 11 samples showed mutagenicities greater than 3,000 revertants/l. Interestingly, most samples, except those from location nos. 7 and 16, showed less mutagenic activities than those collected from the same locations in winter. The average mutagenicities of these 11 samples was 2,216 ± 770 revertants/l and significantly lower than that of samples collected in winter (P=0.0012).

Most water samples collected in both seasons were also tested for mutagenic activities using strain YG1024 in the presence and in the absence of S9 mix, and found to be not mutagenic (data not shown).

Table 5. Effect of Boiling on the Mutagenicities of Tap Waters Towards *S. typhimurium* TA100 in the Absence of S9 Mix

Location no.	Boiling time (min)	Volume of water (ml)	Number of His⁺ revertants*			
			Before boiling		After boiling	
			Per plate	Per liter	Per plate	Per liter
6-W	5	100	257		14	
		200	582		48	
		400	1,160	2,916	66	173 (NM)
16-W	5	100	154		19	
		200	459		55	
		400	865	2,204	58	156 (NM)
11-W	15	80	208		-27	
		160	417		17	
		400	1,463	3,658	41	120 (NM)

Four liters of each water sample were boiled for either 5 or 15 minutes and then extracted and tested for mutagenicity as described in Table 4.
*Values are averages obtained from 2 independent experiments and have already been corrected for average numbers of spontaneous revertants: 152 revertants/plate.
W, sample collected in winter; NM, not mutagenic.

Effects of boiling, filtration and storage on the mutagenicities of tap waters

Table 5 shows the effect of boiling on the mutagenicities of tap waters towards *S. typhimurium* TA100. After boiling for 5 min, both samples from location nos. 6 and 16 showed no mutagenicity, as well as the other sample from location no. 11 which was boiled for 15 min. These results clearly demonstrated that boiling for only 5 minutes completely abolished the mutagenic activities of chlorinated tap waters. Results shown in Table 6 revealed that two samples of tap waters which were filtered through home purifying system

Table 6. Effect of Filtration on the Mutagenicities of Tap Waters Towards *S. typhimurium* TA100 in the Absence of S9 Mix

Location no.	Volume of water (ml)	Number of His⁺ revertants*			
		Before filtration		After filtration	
		Per plate	Per liter	Per plate	Per liter
5-W	80	218		11	
	160	581		11	
	400	1,363	3,444	28	67 (NM)
11-S	80	122		-22	
	160	224		5	
	400	695	1,733	17	55 (NM)

Four liters of each water sample were filtered through home purifying system containing activated charcoal and mixed resin units and then processed as described in Table 4.
*Values are averages obtained from 2 independent experiments and have already been corrected for average numbers of spontaneous revertants: 179 revertants/plate.
S and W; samples collected in summer and winter, respectively.
NM, not mutagenic.

containing activated charcoal and mixed resin units were also not mutagenic to TA100, comparing with strong mutagenicities of waters before filtration. These results indicated that filtration through home purifying system could totally remove mutagens from tap waters.

Table 7 shows the effect of storage of tap water at room temperature on the mutagenicity towards TA100. The mutagenic activities of water samples from location no. 3 collected in both winter and summer decreased significantly after storage for 7 days and were almost completely destroyed after 21 days. The decline rate of mutagenicities in both samples was quite similar, loosing about 53, 75, 87 and 61, 72, 85 percent for winter and summer samples after 7, 14 and 21 days, respectively.

Mutagenicities of commercial bottled drinking waters

Six brands of bottled drinking waters available in the markets were determined for mutagenic activities towards *S. typhimurium* TA100 in the absence of S9 mix. Results shown in Table 8 demonstrated that only brand B exhibited clear and significant mutagenicity. The mutagenic activity was about 26 % of the average mutagenicity of tap water samples collected in summer (2,216 ± 770 revertants/l).

Discussion

Our preliminary results in the present study demonstrated that tap water sample was mutagenic towards *S. typhimurium* only strains TA100 and YG1029 when tested in the absence of S9 mix, indicating that mutagens present in tap water were direct-acting type causing base-pair substitution. In addition, our results showed that Amberlite® XAD-2 resin, but not blue rayon, was able to adsorb mutagens from tap water, and all three kinds of solvent, namely, 15 % acetone in hexane, ethyl acetate and methanol showed comparable efficiencies to elute the mutagenic compounds from XAD-2 resin, although the efficiencies of 15 % acetone in hexane and ethyl acetate were slightly higher than that of methanol. Due to strong smell and toxicity of hexane, we then used ethyl acetate as an eluent in all following studies. These results together with the finding that XAD-2 resin was able to extract mutagens from water only when its pH was 2, but not at all at pH 7.9 or pH 11, seemed to agree well with those reported by other investigators that XAD type resin could adsorb mutagens from chlorinated water and the greatest mutagenicity was observed when the concentration was performed at low pH (IARC, 1991).

Blue rayon was unable to extract mutagens from water

Table 7. Effect of Storage on the Mutagenicities of Tap Waters Towards *S. typhimurium* TA100 in the Absence of S9 Mix

Storage time (day)	Volume of water (ml)	Number of His⁺ revertants*			
		W Sample		S Sample	
		Per plate	Per liter	Per plate	Per liter
0	80	179		129	
	160	330		264	
	400	847	2,111	614	1,537
7	80	140		53	
	160	221		80	
	400	403	993	209	516
14	80	63		20	
	160	91		84	
	400	214	526	167	429 (WM)
21	80	34		29	
	160	52		46	
	400	111	274 (WM)	93	230 (NM)

Water sample no. 3 was collected and stored at room temperature. At time indicated, the samples were extracted and tested for mutagenicity as detailed in Table 4.
*Values are averages obtained from 2 independent experiments and have already been corrected for average numbers of spontaneous revertants: 151 and 177 revertants/plate for W and S samples, respectively.
S and W, samples collected in summer and winter, respectively
NM, not mutagenic; WM, weakly mutagenic

Table 8. Mutagenicities of Commercial Bottled Drinking Waters Towards *S. typhimurium* TA100 in the Absence of S9 Mix

Sample no.	Volume of water (ml)	Number of His⁺ revertants*	
		Per plate	Per liter
A	80	10	
	160	-4	
	240	12	
	400	11	26 (NM)
B	80	44	
	160	78	
	240	126	
	400	231	568
C	80	25	
	160	-13	
	240	31	
	400	0	7 (NM)
D	80	13	
	160	22	
	240	-11	
	400	11	8 (NM)
E	80	1	
	160	-14	
	240	20	
	400	22	61 (NM)
F	80	17	
	160	69	
	240	82	
	400	81	230 (NM)

For details of the extraction of organic materials and mutagenicity testing, see Table 4.
*Values are averages obtained from 2 independent experiments and have already been corrected for average numbers of spontaneous revertants: 150 revertants/plate.
NM, not mutagenic

sample at all 3 pH. This adsorbent has been successfully used for extracting mutagenic compounds from river water (Sakamoto and Hayatsu, 1990; Kusamran et al., 1994; Nukaya et al., 1997; Kataoka et al., 2000) and cooked food (Kusamran, unpublished observation), and blue cotton, which was prepared before blue rayon and contains blue pigment copper phthalocyaninetrisulfonate ligands 2-3 times less than blue rayon per unit weight, has also been widely used for extracting mutagens from cooked foods (Hayatsu et al., 1983; Takahashi et al., 1985). Mutagens adsorbed by blue rayon and blue cotton are mainly multicyclic planar compounds, such as heterocyclic amines and polycyclic aromatic hydrocarbons (Hayatsu et al., 1983; Takahashi et al., 1985; Hayatsu, 1992), and those found in river water and cooked food have been shown to be indirect-acting frameshift mutagens causing mutation to *S. typhimurium* strain YG1024 in the presence of S9 mix (Takahashi et al., 1985; Kusamran et al., 1994; Nukaya et al., 1997). Mutagens in chlorinated water may have different structures from above compounds, thereby could not be adsorbed by blue rayon.

Our data in this study clearly demonstrated that all tap water samples, which were collected in Bangkok Metropolitan area and Nonthaburi province and were prepared by chlorination of Chao Phraya River water, contained direct-acting mutagens causing base-pair substitution. On the other hand, however, tap water preparing from ground water such as in one location in Bangkok and in Pathumthani province exhibited no mutagenicity. These results agree quite well with those from some other countries which demonstrated that concentrated chlorinated surface water was mutagenic towards *S. typhimurium* TA100 and the inclusion of a metabolic activation system usually resulted in a reduced response or totally abolished it (see Table 8-10 in Ref. 1).

The average mutagenicity of chlorinated tap waters in this study was found to be about 2,800 net revertants/l; 3,351 and 2,216 net revertants/l in winter and summer, respectively. This activity is about the same level in drinking water (3,000 net revertants/l) that found to be related to an increased risk of some cancers in the consumers of chlorinated drinking water (Koivusalo et al., 1994). Thus, a possibility of an increased risk of some forms of cancer in people living in Bangkok should be our great concern.

The mutagenicities of water samples from most locations collected in summer, including that from the treatment plant (location no. 15), were significantly lower than those collected in winter. The reason for this is not known. However, it may not be due to the evaporation of mutagenic compounds at higher temperature since it has been shown that the mutagens in chlorinated drinking water were mainly non-volatile compounds (Cheh, et al., 1980). It is worth noted that the Chao Phraya River water in winter is more cloudy than that in summer since winter in Thailand comes after the rainy season which usually washouts clay and humus substances from the forest in the northern part of Thailand down to the river. Thus, one reason for higher mutagenicity of chlorinated water in winter might possibly be the higher concentration of humic acid in the raw water.

The mutagens present in our tap water were different from those found in the Chao Phraya River water, the source of tap water. The mutagenic compounds in river water have been shown to be indirect-acting frameshift mutagens (Kusamran et al., 1994). Thus, these mutagens were not derived from raw water used for the preparation of tap water. It has been shown that bacterial mutagenicity in chlorinated tap water is probably due to chlorination of natural constituents, such as humic and fulvic acids. The mutagenic activity in chlorinated water samples may not be due to volatile trihalomethanes, but much of the mutagenicity is due to nonvolatile acidic and polar substances (Cheh et al., 1980; IARC, 1991 and references therein). MX, one of the chlorination by-products, has been shown to be mutagenic to *S. typhimurium* TA100 and responsible for a significant portion of the bacterial mutagenicity of some concentrated chlorinated surface water (Kronberg and Vartiainen, 1988). Thus, one of the major mutagens present in our tap water may also likely to be MX. MX has been detected in many countries (Meier et al., 1987; Kronberg and Vartiainen, 1988; Horth et al., 1989; Andrews et al., 1990; Suzuki and Nakanishi, 1990; Huizian et al., 1995) and has recently been reported to cause cancers in several organs in male and female Wistar rats (Komulainen et al., 1997).

One of the main objectives of the present study was to find simple methods to destroy or to abolish the mutagenicity of chlorinated tap water. We found that boiling for only 5 min and filtration through home purifying system consisting of activated charcoal and mixed resin units were very effective to abolish the mutagenicity of water. Storage of water also significantly decreased the mutagenicity, however, it took 2-3 weeks to totally abolish it. However, boiling may be a better method to prepare drinking water since it has been also shown that the levels of some carcinogenic trihalomethanes, such as chloroform, bromodichloromethane and chlorodibromomethane which were found in tap waters in Bangkok (Onodera et al., 1984), greatly decreased in boiled water (IARC, 1991), while these volatile compounds were not effectively removed by activated carbon treatment. Moreover, boiling can kill microorganisms, which may contaminate the water after being piped into the distribution system.

At present, bottled drinking water is widely consumed by a large number of people. In this study, 1 out of 6 brands of bottled water available in the marketplaces in Bangkok was found to contain direct-acting mutagens causing base-pair substitution, the type found in tap water. This bottled water was collected in summer, its mutagenicity was then compared with those of tap water samples collected also in summer and found to be about 26 % of the average mutagenicity. This result indicated that this brand of bottled water may be prepared from chlorinated water and some steps of preparation especially filtration might not be properly done, thereby leaving some mutagenic by-products left in the finished water.

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