Environmental Health Perspectives
Vol. 69, pp. 129-139, 1986

Results of Toxicological Testing of Jefferson Parish Pilot Plant Samples

by Robert G. Miller,* Frederick C. Kopfler,* Lyman W. Condie,* Michael A. Pereira,* John R. Meier,* H. Paul Ringhand,* Merrel Robinson,* and Bruce C. Casto†

Five toxicological tests were performed using concentrated drinking water samples collected at a pilot-scale drinking water treatment plant that had streams treated with different disinfectants (no disinfectant, ozone, chlorine dioxide, monochloramine, or chlorine) before treatment with granular activated carbon (GAC). The toxicological tests used in this study were the Ames Salmonella assay, a subchronic *in vivo* toxicity assay in mice, the SENCAR mouse skin initiation-promotion assay, a rat liver foci assay, and the lung adenoma assay in strain A mice. These tests were conducted to determine the general toxicity and the mutagenic/carcinogenic potential associated with the use of disinfection and/or GAC in the treatment of drinking water. The stability of the mutagenic activity of the samples tested was determined by repeated analysis using the Ames Salmonella assay. Results indicated that the samples remained mutagenic for the duration of the tests.

All the drinking water concentrates (4000×) prepared by the XAD resin adsorption procedure failed to provide statistically significant indication of carcinogenic activity in the SENCAR mouse, rat liver foci, and the lung adenoma assays. However, concentrates of the chlorine, chlorine dioxide, and monochloramine treated waters gave consistent mutagenic responses in the Ames Salmonella assay. GAC was effective for 6 months in removing both the mutagenicity of chlorine-treated water and the potential of water to become mutagenic when treated with chlorine. In the *in vivo*, subchronic 30-day toxicity test in mice, some statistically significant differences in organ weights and body weights of animals exposed to different concentrates of some of the samples were observed. However, a consistent pattern of these differences indicating overt toxicity was not detected.

Introduction

Complex mixtures of organic substances found in surface water and goundwater, as well as wastewaters, defy complete characterization. The best estimate of toxicological effects associated with the exposure of the human population to organic chemicals is the result of biological tests on experimental animals and/or organisms exposed at levels that ensure probable positive responses. To facilitate biological testing of organic contaminants in drinking waters, a process of isolation/concentration is necessary because of the large number of compounds present (both identifiable and nonidentifiable) and their trace occurrence (total organic matter is generally < 10 mg/L).

For the biological testing of concentrated drinking waters treated with different disinfectants and/or granular activated carbon (GAC), a series of toxicological tests were chosen to indicate the general toxicity and mutagenic/carcinogenic activity of these waters. These tests were: the Ames Salmonella assay (*1,2*), the *in vivo* toxicity in mice assay, the SENCAR mouse skin initiation-promotion assay (*3,4*), the rat liver foci assay (*5–7*), and the lung adenoma assay in strain A mice (*8*).

*U.S. Environmental Protection Agency, Health Effects Research Laboratory, Toxicology and Microbiology Division, 26 West St. Clair Street, Cincinnati, OH 45268.

†Environmental Health Research and Testing, Inc., 3235 Omni Drive, Cincinnati, OH 45245.

Methods

Sample Collection and Preparation

The concentrated drinking water samples to be used for toxicological testing were collected and concentrated at a pilot-scale drinking water treatment plant in Jefferson Parish, LA. A detailed description of the facility has been previously described (*9*). The influent stream was split into five separate process streams as indicated in Figure 1. These process streams were untreated, disinfected, using ozone, disinfected with chlorine dioxide, disinfected with monochloramine, and disinfected with chlorine. For this study, the actual sampling sites were 2, 3, 4, 5, 6, 20 + 21, and 36 as shown in Figure 1.

Disinfection after GAC treatment in the chlorine stream (sampling site 36) was accomplished by adjusting

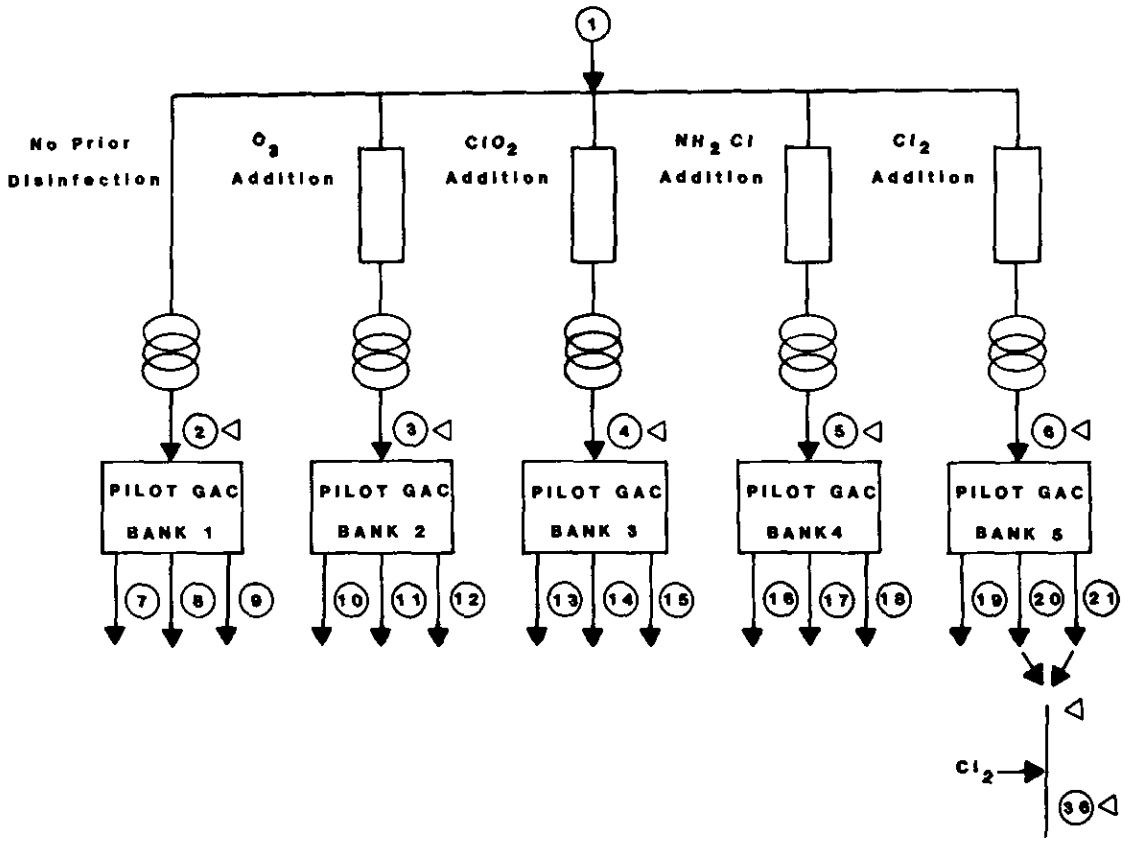

FIGURE 1. Sampling site schematic of the Jefferson Parish, LA, alternative disinfection pilot plant. (△) denotes actual sampling site.

Table 1. The parameters of each process stream at the Jefferson Parish pilot plant.

Parameter	Value
Sampling flow rate	0.5 gal/min
Disinfectant contact time	120 min
Chlorine residual	1.0 mg/L
Chlorine dioxide residual	0.5 mg/L
Monochloramine residual	2.1 mg/L
Ozone residual	0.5 mg/L
Total organic carbon	3.4 mg/L

the sample to a chlorine residual of 1.0 mg/L. The parameters of each stream are shown in Table 1. The contact time for chlorine dioxide, monochloramine, and chlorine streams was increased from 30 min to 2 hr by use of a modified 50-gal stainless-steel drum.

Two methods, reverse osmosis (*10,11*) and macroreticular resin process (*12,13*), were used to collect and concentrate organic compounds from drinking water before toxicological testing.

Reverse Osmosis. By use of a FT-30 reverse osmosis membrane and a Nafion cation exchange membrane, 2000 gal of drinking water were concentrated in the field by batch concentration as shown in Figure 2. In this way, 500 gal were reduced to 10 gal of concentrate with concurrent removal of sufficient calcium and magnesium salts to prevent precipitation. This process was repeated until a total of 40 gal of 50× concentrate was obtained. Figure 3 shows how further concentration was accomplished in the laboratory by repeating a modified dialysis desalting process in conjunction with the usual reverse osmosis process until the salt content was reduced to ≤ 0.85% NaCl and a final volume of 5 gal of 400× concentrate was obtained. The final concentrate was sterile filtered into a sterile, stainless-steel container by use of a series of graded filter elements, of which the last element was a sterile 0.2-μm Poll Ultipor cartridge.

During the reverse osmosis process two continuing chemical adjustments were performed as the samples were being concentrated. These adjustments changed the pH of the water to pH 2 by metering dilute hydrochloric acid into each cycle of sample pumped into the concentrate, and softened the water by use of a Donnan dialysis cation exchange unit (Nafion membrane) with a sodium chloride stripping solution (*11*). The temperature of the sample and concentrate was maintained at 15°C by using heat exchange coils immersed in chilled water baths.

Samples for total organic carbon (TOC) analysis were collected at various stages in the reverse osmosis process to help monitor the progress of the concentration

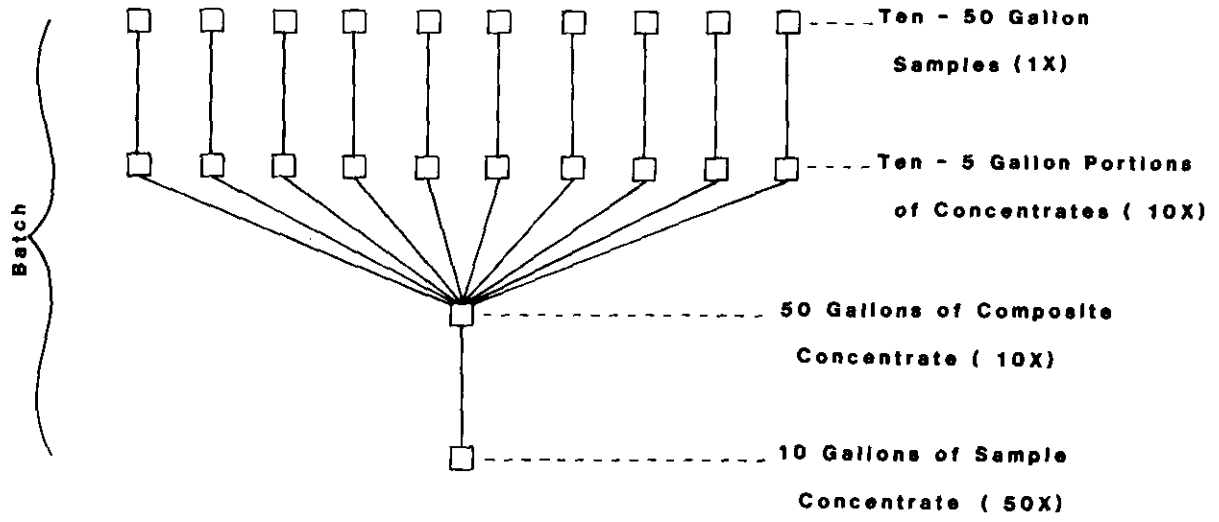

FIGURE 2. Schematic of a typical 50× batch concentration using reverse osmosis procedure.

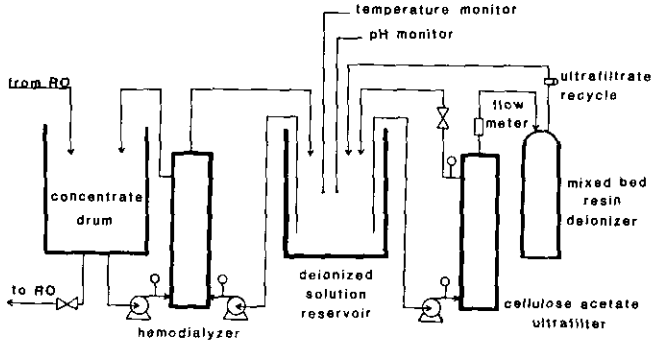

FIGURE 3. Desalting apparatus used in conjunction with reverse osmosis for workup of field concentrates.

and to determine organic recoveries at different steps in the procedure.

The initial TOC of influent water was 3.4 mg/L. The mass recoveries, as indicated in Table 2, during field concentrations (1–50×) were generally excellent, and all recoveries were in the 83% to 118% range. Most of the measured membrane (Film Tech FT-30) TOC rejection values were between 92 and 99%. A significant reduction of concentrate TOC mass was lost during laboratory workup of the samples (concentrating from 50× to 400×).

Macroreticular Resins XAD-2 and XAD-8. By use of two resin-filled glass columns in series and subsequent elution and concentration, 2000-gal samples of drinking water were reduced to 2 gal (1000×). The types of resins used were XAD-2 and XAD-8. Each resin was cleaned before use (*14*). The resins were purified by Soxhlet extraction in three separate organic solvents (methylene chlorine, acetone, and methanol) for 24 hr each. A subsample of the column material was then eluted with diethyl ether and the eluate checked before use for purity by using gas chromatographic (GC) analysis. No detectable impurities were found, and the resins were stored at 4°C in methanol until used. An example of a detailed schematic used in the sample collection at sites 2, 3, 4, 5, 6, and 20 + 21 is shown in Figure 4. Each glass column (14 × 49 cm) was filled with 5 L of cleaned resin. Each column pair was rinsed

Table 2. Mass recoveries and membrane TOC rejections for field and laboratory portions of toxicological sample preparation by reverse osmosis.[a]

Disinfectant stream	Sample	TOC mass recovery (field), %	TOC mass recovery (overall), %	Rejection, (field), %	Rejection (lab), %
Untreated	A	109	37	96	93
	B	96	46	99	99
Ozone	A	83	48	98	93
	B	99	43	99	99
Chlorine dioxide	A	100	59	92	85
	B	97	45	99	99
Monochloramine	A	103	56	95	99
	B	96	40	99	99
Chlorine	A	118	61	94	96
	B	102	38	99	99

[a] All samples had same raw water source. Paired samples (A and B) had the same disinfectant but were collected on different dates.

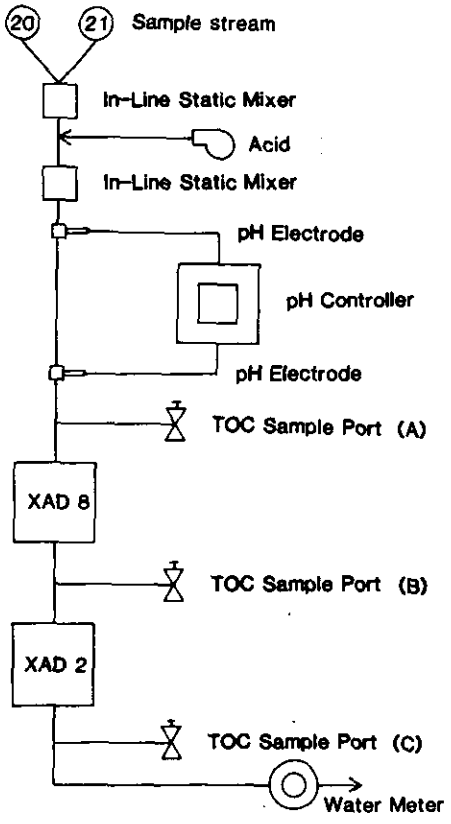

FIGURE 4. Detailed schematic of sampling at sites 20 and 21 (chlorinated and GAC-filtered water). This figure also represents sampling at sites 2, 3, 4, 5, and 6 but before treatment with GAC.

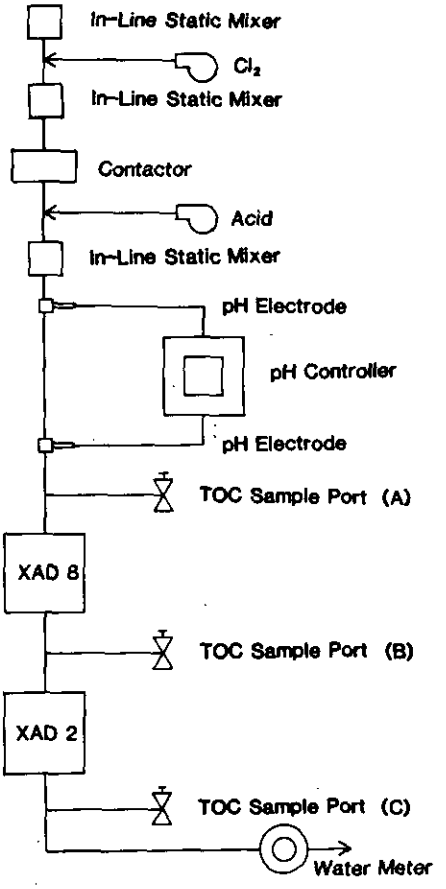

FIGURE 5. Detailed schematic of sampling at site 36 (rechlorinated GAC-filtered water).

with 30 gal of water to flush out the methanol before sample collection. Two thousand gallons of water were processed through the column pairs at a flow rate of 9 to 11 bed volumes/hour (0.4–0.5 gal/min). During the sample collection process, the pH of the influent was monitored and maintained at pH 2 by adding hydrochloric acid. Uniformity of the acid addition was enhanced by an in-line static mixer. Samples for TOC analyses were taken at collection increments of 0, 500, 1000, 1500, and 2000 gal for each process stream collected at points A, B, and C as shown in Figure 4. The sampling schematic of site 36, as shown in Figure 5, differed from the other collected samples in that the chlorine stream was rechlorinated after GAC treatment and had an additional contact time of 120 min before sampling.

An example of TOC data from these process streams is shown in Table 3. Approximately 40% of the TOC was retained by the resins. After sample collection, the XAD-8 and XAD-2 columns were separated, and each column was filled with acetone, agitated to completely wet the resins, and then allowed to soak for 15 min. The columns were each eluted with three resin bed volumes of acetone. Each column extract was separately evaporated to 4L with Buchler continuous flow flash rotary evaporator. These acetone samples (approximately 1 gal from each resin column) were stored at −20°C. The aqueous content of the samples was analyzed to be approximately 20%.

Immediately before use in toxicity tests, aliquots of each sample were concentrated an additional 5 times by Kuderna-Danish apparatus, whereby 10% Emulphor in water was added in small measured aliquots (to minimize precipitation). Evaporation was continued until all acetone was evaporated and a sample of 2% Emulphor aqueous solution remained, leaving a concentrate equal to 4000× the original water sample.

Results

Testing of Reverse Osmosis Concentrates

Two toxicological tests were performed on drinking water samples collected at sample points 2, 3, 4, 5, and

Table 3. Typical TOC data by resin process.

TOC sampling port[a]	TOC, mg/L				
	0 gal	500 gal	1000 gal	1500 gal	2000 gal
A	3.6	3.5	3.4	3.4	3.4
B	11.0[b]	2.2	2.7	2.7	2.5
C	30.0[b]	1.6	2.0	2.1	1.9

[a] Designations refer to Fig. 4.
[b] Resin contaminated with methanol (MeOH) even after prerinse with 50 gal of water.

6 (Figure 1) and concentrated (400×) by reverse osmosis process, the Ames Salmonella assay and the *in vivo* toxicity assay.

Ames Salmonella Assay. The Ames Salmonella assay was used as a screening process to determine the potential carcinogenic activity of the samples. The results of the Ames assay are shown in Table 4. No indications of a mutagenic response for strains TA 98 or TA 100 were apparent with any of the reverse osmosis concentrates when tested either in the presence or the absence of a metabolic activation system (−/+ S9).

In Vivo *Toxicity Assay.* The subchronic toxicity of the reverse osmosis samples was evaluated in a 30-day study using CD-1 mice, 10 males and 10 females per dose group, in which the aqueous samples were administered as drinking water in concentrations equal to the original concentrate (400×) and one-fourth of the original concentrate (100×). At the end of the exposure period, a necropsy was performed on each animal to examine it for gross pathological changes. The results of this study are shown in Tables 5 and 6. Taste aversion to the reverse osmosis concentrates was not seen in the study. In fact, significant increases in fluid consumption were detected in four of the female exposure groups. Statistically significant differences in the body weights were found at sacrifice of two male and two female exposure groups, but the differences in body weights were caused by differences in initial body weights, since the rate of growth of the mice in each experimental group was the same throughout the study. Alterations in the ratios of organ weights to body weights are also depicted in Tables 5 and 6. Since these data and necropsy reports did not reveal any overt, subchronic toxicity, histopathological examination of major organs was not performed.

Testing of Macroreticular Resin Concentrates

Drinking waters that were collected and concentrated by the macroreticular resin process were used as samples for toxicological testing. An outline of these tests and sample identification is shown in Table 7. As noted,

Table 4. Evaluation of mutagenicity of reverse osmosis concentrates of Jefferson Parish water samples.

		His$^+$ revertants/plate[a]			
		TA 98		TA 100	
Test compound	Dose level	−S9	+S9	−S9	+S9
Negative control	—	24	26	146	135
Positive controls (μg)					
Sodium azide	1			702	
2-Nitrofluorene	5	841			
2-Aminoanthracene	1		302		587
Reverse osmosis concentrates (mL)					
Untreated	0.025	25	30	169	161
	0.050	22	29	154	143
	0.100	23	31	153	151
	0.250	25	30	152	131
	0.500	17	26	130	115
	1.000	19	28	128	122
Ozone	0.025	29	32	161	160
	0.050	21	32	153	148
	0.100	24	32	146	132
	0.250	25	30	150	151
	0.500	20	23	133	126
	1.000	22	22	136	113
Monochloramine	0.025	26	36	167	167
	0.050	24	37	144	162
	0.100	24	37	150	177
	0.250	20	39	142	147
	0.500	21	29	130	159
	1.000	18	27	146	134
Chlorine dioxide	0.025	26	36	171	150
	0.050	27	29	146	155
	0.100	23	35	159	150
	0.250	25	32	143	140
	0.500	23	28	150	135
	1.000	19	29	130	135
Chlorine	0.025	26	39	185	156
	0.050	25	39	159	175
	0.100	21	36	162	178
	0.250	21	31	147	140
	0.500	21	34	144	125
	1.000	22	29	135	139

[a] Duplicate experiments using two plates per dose.

Table 5. Summary of a 30-day subchronic toxicity study in which CD-1 female mice received various RO concentrates for drinking water for 4 weeks.[a]

Group	Concentration	Fluid consumed	Final body weight	Ratio of organ weights to body weight					
				Brain	Kidney	Liver	Lung	Ovaries	Spleen
Ozone	100×	ns	ns	ns	ns	ns	↑	↓	ns
	400×	↑	↑	ns	ns	ns	ns	ns	ns
Chlorine dioxide	100×	↑	ns	↓	ns	ns	ns	ns	ns
	400×	ns	ns	ns	ns	ns	ns	ns	ns
Monochloramine	100×	↑	↑	↓	↓	ns	↓	ns	ns
	400×	ns	ns	ns	ns	ns	ns	↑	ns
Chlorine	100×	ns	ns	ns	ns	ns	ns	ns	ns
	400×	↑	ns	ns	ns	↑	ns	ns	ns
Nondisinfected	100×	ns	ns	ns	ns	ns	↓	↓	ns
	400×	ns	ns	ns	ns	ns	↓	↓	↓

[a] Disinfectants were compared to corresponding nondisinfected groups compared to distilled water control group by orthogonal contrasts following ANOVA. ↑ = statistically significant increase or ↓ = statistically significant decrease ($p < 0.05$). ns = no significant difference.

Table 6. Summary of a 30-day subchronic toxicity study in which CD-1 male mice received various RO concentrations for drinking water for 4 weeks.[a]

Group	Concentration	Fluid consumed	Final body weight	Ratio of organ weights to body weight				
				Spleen/brain	Kidney	Liver	Lung	Testes
Ozone	100×	ns	ns	ns	ns	ns	ns	ns
	400×	ns	ns	ns	ns	↓	ns	ns
Chlorine dioxide	100×	ns	ns	ns	ns	ns	ns	ns
	400×	ns	↓	ns	ns	ns	ns	ns
Monochloramine	100×	ns	ns	ns	ns	↑	↓	ns
	400×	ns	ns	ns	↓	↓	ns	ns
Chlorine	100×	ns	ns	ns	ns	ns	↓	ns
	400×	ns	↓	ns	ns	ns	↓	↓
Nondisinfected	100×	ns	ns	ns	ns	↓	ns	ns
	400×	ns	ns	ns	ns	ns	ns	ns

[a] Disinfectants were compared to corresponding nondisinfected groups and nondisinfected groups compared to distilled water control group by orthogonal contrasts following ANOVA. ↑ = statistically significant increase or ↓ = statistically significant decrease ($p < 0.05$). ns = no significant difference.

Table 7. Toxicity testing performed on samples prepared by macroreticular resin process.

Sample description (stream and dates)	Assay performed				
	Ames/Salmonella	Liver foci	Lung adenoma	SENCAR mouse	*In vivo* toxicity
July 1983					
Nondisinfected	X	X	X	X	
Chlorine	X	X	X	X	
December 1983					
Nondisinfected	X	X	X	X	
Ozone	X	X	X	X	
Chlorine dioxide	X	X	X	X	
Monochloramine	X	X	X	X	
Chlorine	X	X	X	X	
Chlorine + GAC	X	X	X	X	
Chlorine + GAC rechlorinated	X	X	X	X	
August 1984					
Nondisinfected	X	X			X
Monochloramine	X	X			X
Chlorine	X	X			X
Chlorine + GAC	X	X			X
Chlorine + GAC rechlorinated	X	X			X

all samples tested were collected on three different dates.

Ames Salmonella Assay. Since all the tests for toxicity could not be conducted simultaneously, the stability of the toxic potential of the samples was evaluated using bacterial mutagenicity in the Ames assay as an end point. These data are presented in Figure 6. For those samples which were initially positive in the Ames

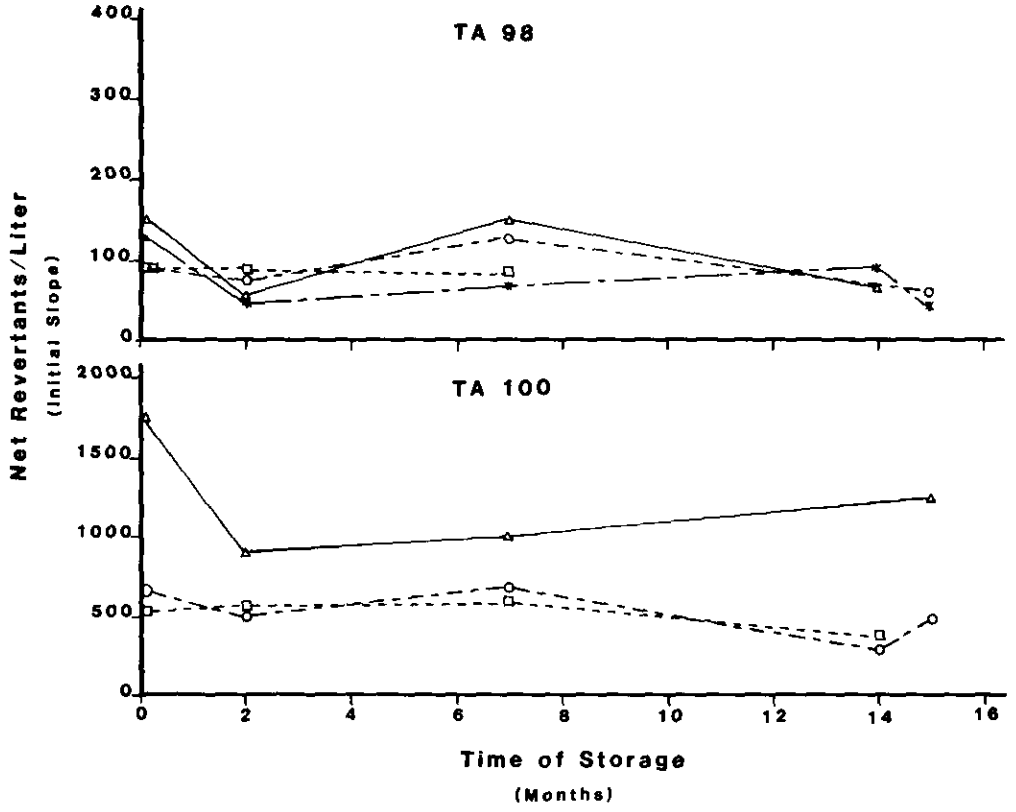

FIGURE 6. Stability of mutagenic activity in drinking water concentrates as determined by Ames Salmonella assay: (*) chlorine dioxide; (○) monochloramine; (□) chlorine + GAC + rechlorinated; (△) chlorine.

assay, mutagenicity in both TA 98 and TA 100 strains was consistently detectable throughout the course of the toxicological tests when stored at 4°C in 2% Emulphor. Some differences in the levels of mutagenicity were noted, but these differences were probably attributable to between-experiment variability in the assay. Although data are not shown, no differences in mutagenic activity were found when the mutagenic activity of samples stored for 4 months in acetone at −20°C was compared to that of samples stored in 2% Emulphor at 4°C.

In addition to the stability studies, other samples collected on four different occasions, spanning a period of 14 months were also tested for mutagenicity in the Ames test. The results of tests on these samples assayed without S9 are shown in Table 8. When assayed with S9, the results of all samples gave fewer revertants/liter equivalent dose than those without S9. The concentrates of untreated and ozone-treated water at all sample collection dates were nonmutagenic in the Ames test. Monochloramine- and chlorine-treated water concentrates were consistently mutagenic in both TA 98 and TA 100 strains; chlorine-dioxide-treated water was mutagenic only for strain TA 98. The overall order of mutagenic activity was chlorine > monochloramine > chlorine dioxide. Treatment of the chlorine-disinfected water with GAC was initially effective in removing both the mutagenicity and the mutagen-forming potential of the water. After 6 months use, the GAC only partially removed the mutagen-forming potential but was still effective in removing the mutagenicity of the water. The TA 98 data also indicate that after being used for an extended period (14 months) the GAC may become ineffective in removing the mutagenicity of chlorinated water.

Lung Adenoma Assay. One of the toxicological tests used for determining the tumor-initiating potential of the concentrated drinking water samples is the mice lung adenoma assay. Nine concentrated water samples in 2% Emulphor were tested at two dose levels (4000× and 2000×) in both sexes of 6-week-old strain A mice. The three control groups used were a negative control, a vehicle control (2% sterile Emulphor), and a positive control (10 mg urethane/mouse by gavage, single dose). There were 20 animals per group, for a total of 840 mice in 42 groups. After 2 weeks in quarantine, 0.25 mL of sample was administered by gavage three times a week for 8 weeks with toxicological observations for an additional 16 weeks. After lung perfusion, the adenomas were counted independently by two technicians, and histological confirmation was performed on 10% of the positive specimens. The number of animals within each group that had lung adenomas is presented in Table 9. The positive control animals all had lung adenomas with

Table 8. Mutagenic activity of organic concentrates of raw and treated Mississippi River water assayed without S9.

	His⁺revertants/L equivalent dose[a]							
	6–15–83[b]		7–6–83[b]		12–5–83[b]		8–24–84[b]	
Sample	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100	TA 98	TA 100
Nondisinfected	ns	ns	ns	ns	ns	ns	ns	ns
Ozone	ns	ns	ns	ns	ns	ns	—	—
Chlorine dioxide	117 ± 17	ns	142 ± 19	ns	126 ± 39	ns	—	—
Monochloramine	291 ± 11	771 ± 42	327 ± 24	794 ± 57	92 ± 12	656 ± 28	ns	ns
Chlorine	351 ± 22	1338 ± 57	160 ± 14	912 ± 47	151 ± 21	1746 ± 65	162 ± 15	475 ± 73
Chlorine + GAC	ns	ns	—	—	ns	ns	34 ± 9	ns
Chlorine + GAC rechlorinated	ns	ns	—	—	90 ± 20	521 ± 35	55 ± 7	213 ± 36

[a] Values calculated from the linear portion (initial slope) of the dose-response curves. ns = nonsignificant (less than 2-fold above background).
[b] Refers to the date the samples were collected.

a mean of 11.5 adenomas per animal, the vehicle control had 0.1 adenoma per animal, and all other treated groups had a mean of ≤ 0.4 adenoma per animal.

The body weight changes and the mortality data within the exposure groups and controls gave further indication that no treatment-related effect was evident during the study.

SENCAR Mouse Initiation-Promotion Assay. A mouse skin initiation-promotion assay of the drinking water concentrates (4000×) was conducted in SENCAR mice. Each test sample of 0.5 mL was administered orally three times per week for 2 weeks. Two weeks after the final initiating dose, 1.0 μg of 12-tetradecanylphorbal-13-acetate (TPA) in 0.2 mL acetone was administered by topical application to the dorsal skin of ½ of the experimental animals three times per week for 20 weeks, whereas the remaining animals received acetone only (0.2 mL/mouse) for the same duration. The control groups for this study were a vehicle control (2% Emulphor) and a positive control (urethane at 500 mg/kg). The data presented in Table 10 represent the skin tumor incidence at 30 experimental weeks.

The treatment with the vehicle control (2% Emulphor) yielded 0.09/9 (Study I), 0.25/25 (Study II), and 0.38/36 (Study III) tumors per animal/animals with tumor. Only the group of animals receiving the chlorine/GAC exposure had more tumors, at 30 weeks into the study (0.33/33), than did the corresponding control group. The high background activity associated with the oral Emulphor treatment, when averaged for the three studies, is consistent with previous results from this laboratory. The positive control group (urethane at 500 mg/kg) had 0.9 to 1.4 tumors per animal, whereas results from earlier studies in this laboratory with the same oral dose yielded 3.0–3.3 tumors per animal after 30 weeks on study. None of the concentrates of disinfectant-treated water induced tumors on the backs of the mice.

Rat Liver Foci Study. The carcinogenic activity of the organic substances present in the concentrated drinking water samples was also evaluated in the rat liver foci assay. This short-term bioassay determines the ability of the sample to initiate γ-glutamyltranspeptidase (GGT)-positive foci (*5–7*). Ten rats per group

Table 9. Results of lung adenoma assay in strain A mice when tested with two dose levels of concentrated drinking water.

	Female				Male			
	2000 × Concentrate		4000 × Concentrate		2000 × Concentrate		4000 × Concentrate	
Sample description (stream and place)	Animals with tumors	Tumors per animal	Animals with tumors	Tumors per animal	Animals with tumors	Tumors per animal	Animals with tumors	Tumors per animal
July 1983								
Nondisinfected	5/20	0.30	5/19	0.32	1/20	0.05	2/29	0.11
Chlorine	1/20	0.05	5/16	0.38	0/20	0.00	3/20	0.15
December 1983								
Nondisinfected	4/20	0.20	3/18	0.17	5/18	0.28	1/17	0.06
Ozone	1/20	0.05	1/15	0.07	7/20	0.40	1/19	0.05
Chlorine dioxide	0/20	0.00	2/17	0.12	3/20	0.15	4/16	0.31
Monochloramine	2/18	0.11	1/13	0.08	3/20	0.20	1/20	0.05
Chlorine	0/19	0.00	1/18	0.06	1/20	0.05	1/20	0.05
Chlorine + GAC	0/20	0.00	1/19	0.05	0/20	0.00	1/19	0.05
Chlorine + GAC rechlorinated	1/20	0.05	2/19	0.11	2/20	0.15	2/20	0.10
Control (neg.)	1/20	0.05			1/19	0.05		
Control (veh.) (2% Emulphor)	1/20	0.05			2/15	0.13		
Control (pos.) (10 mg urethane)	19/19	11.47			20/20	11.55		

Table 10. Results of mouse skin initiation-promotion assay in SENCAR mice treated with concentrates of alternative disinfectant solutions.

Study	Treatment	Promotion (TPA)	No. of animals with tumor, %	Total tumors	Tumors/animal
I	Nondisinfected	Yes	5/13 (16)	5	0.16
	Nondisinfected	No	0/16 (0)	0	0
	Ozone	Yes	5/31 (16)	5	0.16
	Ozone	No	0/19 (0)	0	0
	Chlorine dioxide	Yes	4/34 (12)	5	0.15
	Chlorine dioxide	No	0/14 (0)	0	0
	2% Emulphor	Yes	3/35 (9)	3	0.09
	2% Emulphor	No	0/37 (0)	0	0
	Urethane	Yes	13/20 (65)	18	0.90
II	Monochloramine	Yes	6/38 (16)	6	0.16
	Monochloramine	No	0/20 (0)	0	0
	Chlorine	Yes	3/40 (8)	3	0.08
	Chlorine	No	0/19 (0)	0	0
	Chlorine/GAC	Yes	13/39 (33)	13	0.33
	Chlorine/GAC	No	0/17 (0)	0	0
	2% Emulphor	Yes	10/40 (25)	10	0.25
	2% Emulphor	No	0/39 (0)	0	0
	Urethane	Yes	14/20 (70)	28	1.40
III	Chlorine/GAC/chlorine	Yes	5/39 (13)	7	0.18
	Chlorine/GAC/chlorine	No	0/19 (0)	0	0
	Nondisinfected/July	Yes	6/38 (16)	6	0.16
	Nondisinfected/July	No	0/18 (0)	0	0
	Chlorine/July	Yes	5/40 (13)	5	0.13
	Chlorine/July	No	0/20 (0)	0	0
	2% Emulphor	Yes	14/39 (36)	15	0.38
	2% Emulphor	No	0/39 (0)	0	0
	Urethane	Yes	13/20 (65)	26	1.30

were used for each of the test samples, vehicle control (2% Emulphor), and positive control (50 mg diethylnitrosamine/kg body weight). All rats were hepatectomized on day 0 (⅔ partial hepatectomy), and treated 24 hr later by oral administration of the test and control materials. One week later (day 7), the rats started to receive 500 ppm sodium phenobarbital in their drinking water for a total of 56 days. All animals were sacrificed at day 70. Frozen liver sections were prepared for the histochemical detection and quantitation of GGT foci. Results of these experiments are shown in Table 11. None of the concentrates of alternative disinfectant-treated water initiated an incidence of GGT foci above that of the vehicle control group. The positive control diethylnitrosamine (DENA) induced a high incidence of GGT foci, which indicated that the test was functioning properly.

Table 11. Incidence of GGT foci in rats treated with concentrates of alternative disinfection solutions.

Expt.	Sample description (stream)	*N*	Mean ± SD no. of GGT-foci/cm^2
I	Nondisinfected	9	150 ± 93
	Monochloramine	10	121 ± 40
	Chlorine	10	140 ± 63
	Chlorine + GAC	9	46 ± 46
	Chlorine + GAC rechlorinated	10	62 ± 31
	Control (veh.) (2% Emulphor)	9	64 ± 35
	Control (pos.) (50 mg DENA/kg)	9	2111 ± 246
II	Nondisinfected	9	21.0 ± 21.0
	Ozone	10	0.00
	Chlorine dioxide	7	0.00
	Monochloramine	10	0.00
	Chlorine	10	17.1 ± 17.1
	Chlorine + GAC	4	0.00
	Chlorine + GAC rechlorinated	9	3.33 ± 3.33
	Nondisinfected	9	0.00
	Chlorine	9	0.00
	Control (veh.) (2% Emulphor)	7	0.00
	Control (pos.) (50 mg DENA/kg)	7	383 ± 97

In Vivo *Toxicity*. Five of the prepared concentrated samples were used in an *in vivo* toxicity test. Two dose levels (4000× and 2000×) of each concentrated sample were administered to 10 male and 10 female CD-1 mice per dose. Each 0.3 mL dose was administered via gavage three times per week for a total of 4 weeks. Body weights were measured once per week for 4 weeks, and mice were observed for gross physical pathological changes. On day 30, all animals were sacrificed, and a gross necropsy was performed. Summary data from this study are shown in Tables 12 and 13.

Table 12 indicates that few changes were detected in the chlorine and monochloramine groups when compared to the corresponding nondisinfected groups. However, numerous changes were detected when the toxicity of material passing through "spent" GAC was evaluated before and after rechlorination. These changes between the chlorine + GAC ("spent") and rechlorinated chlorine + GAC ("spent") groups are shown in Table 13. The specific organ weights to body weight ratios were larger in the rechlorinated chlorine

Table 12. Summary of a study in which CD-1 mice received XAD extracts by gavage (0.3 mL, three times weekly for 4 weeks).[a]

Group	Sex	Concentration	Final body weight	Ratio of organ weights to body weight: Brain/liver/lung/ovaries/testes/spleen	Ratio of organ weights to body weight: Kidney
Nondisinfected	M	1000×	ns	ns	ns
	M	4000×	↓	ns	ns
Monochloramine	M	1000×	ns	ns	ns
	M	4000×	ns	ns	ns
Chlorine	M	1000×	ns	ns	ns
	M	4000×	ns	ns	ns
Nondisinfected	F	1000×	↓	ns	↑
	F	4000×	↓	ns	↑
Monochloramine	F	1000×	ns	ns	ns
	F	4000×	ns	ns	ns
Chlorine	F	1000×	ns	ns	ns
	F	4000×	ns	ns	ns

[a]Monochloramine and chlorine groups compared to corresponding high or low nondisinfected groups and nondisinfected groups compared to corresponding vehicle control groups by Tukey's multiple comparison test following ANOVA. ↑ = statistically significant increase or ↓ = statistically significant decrease ($p < 0.05$). ns = no significant difference.

+ GAC groups in every instance where significant differences were detected.

Discussion

The two methods (reverse osmosis and macroreticular resins) used for the collection and concentration of these alternative disinfected drinking water samples have been shown to be reliable in the past when preparing samples for identifying specific constituent organic compounds. However, the results of this study suggest that because of concentration factor limitations (maximum 400× because of salt interferences), reverse osmosis is not a good method for concentrating drinking water for use in toxicological testing. These results also suggest that the concentration of drinking water samples beyond 4000× or a better collection/concentration method is warranted if the toxicity and the mutagenic/carcinogenic activity of trace organic pollutants are to be detected in general animal toxicological experiments. This is particularly true for the rat liver foci assay, lung adenoma assay in strain A mice, and SENCAR mouse initiation-promotion assay, since these methods have previously demonstrated their ability to detect the carcinogenic activity of complex mixtures.

The subchronic *in vivo* studies with CD-1 mice were designed to detect only the overt toxicities of the disinfected streams. The extracts produced little, if any, obvious toxicological effects. If subtle toxicities had occurred, they would not have been detected by the experimental design. The most significant changes in this study occurred between the chlorine + GAC and rechlorinated chlorine + GAC groups of mice. Apparently, some material passed through the GAC treat-

Table 13. Summary of a study in which CD-1 mice received XAD extracts by gavage (0.3 mL three times weekly for 4 weeks).[a]

Group	Sex	Concentration	Final body weight	Ratio of organ weights to body weight: Brain/kidney	Liver	Lung	Ovaries/testes	Spleen
Chlorine	M	1000×	—	—	—	—	—	—
	M	4000×	—	—	—	—	—	—
Chlorine + GAC	M	1000×	ns[b]	ns[b]	ns[b]	ns[b]	ns[b]	↓
	M	4000×	↓[b]	ns	ns[b]	ns	ns	ns[b]
Chlorine + GAC *rechlorinated*	M	1000×	↓[b]	ns[b]	↑[b]	ns[b]	ns	ns
	M	4000×	ns[b]	ns	↑[b]	↓	ns	ns[b]
Chlorine	F	1000×	—	—	—	—	—	—
	F	4000×	—	—	—	—	—	—
Chlorine + GAC	F	1000×	ns[b]	ns	ns	ns	↓	ns
	F	4000×	ns	ns	ns	ns	ns	ns
Chlorine + GAC *rechlorinated*	F	1000×	↑[b]	ns	ns	ns	↓	ns
	F	4000×	ns	ns	ns	ns	↓	ns

[a]Each high and low dose group was compared to others by sex by Tukey's multiple comparison test following ANOVA. ↑ = statistically significant increase or ↓ = statistically significant decrease ($p < 0.05$). ns = no significant difference when compared to chlorine group.
[b]Indicates significant differences between chlorine + GAC and chlorine + GAC rechlorinated treatment groups.

ment that may have exerted a biological effect during rechlorination. It is not clear why the organ sizes increased in the rechlorinated chlorine + GAC groups.

The most significant results of all tests performed were those of the Ames Salmonella assay on the concentrates derived from the macroreticular resin process. This assay demonstrated that the concentrated samples (in 2% Emulphor) remained active with respect to mutagenic activity throughout the 15-month experimental period. This finding indicates that 2% Emulphor may be used as a long-term storage matrix for concentrated aqueous samples. In the Ames Salmonella assay, the chlorine dioxide-, monochloramine-, and chlorine-treated water concentrates were consistently mutagenic. Also, according to the Ames assay, GAC is effective in removing the mutagenicity of chlorinated drinking water and the potential of water to become mutagenic when treated with chlorine. However, after 6 months' use in the chlorine stream, the GAC was only partially effective in removing the mutagen-forming potential but was still effective in removing the mutagenicity of the water.

The results of these short-term animal toxicological studies reveal the difficulties in analyzing actual drinking waters for detectable toxic effects.

The research described in this report was supported by U.S. Environmental Protection Agency (EPA) Contract 68-03-3164 with Gulf South Research Institute, Contract 68-03-3173 with James M. Montgomery Consulting Engineers, and Contract 68-03-1840 with Environmental Health Research and Testing Inc.

This paper has been subject to the Agency's review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The authors would like to thank Judy Stober of the U.S. EPA for the statistical assistance and Keith Kelty and Melda Hirth of the U.S. EPA for their assistance in the preparation of the manuscript.

REFERENCES

1. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. 31: 347–363 (1985).
2. de Serres, F. J., and Shelby, M. D. Recommendations on data production and analysis using the *Salmonella*/microsome mutagenicity assay. Mutat. Res. 64: 159–165 (1979).
3. Boutwell, R. K., Verma, A. K., Ashendel, C. L., and Astrup, E. Mouse skin: a useful system for studying the mechanism of chemical carcinogenesis. Carcinogenesis 7: 1–12 (1982).
4. Hecker, E. H., Fusenig, N. E., Kunz, W., and Marks, F. (Eds.). Carcinogenesis, A Comprehensive Survey, Vol. 7. Raven Press, New York, 1981.
5. Pereira, M. A. Rat liver foci assay. In: Handbook on Carcinogen Testing (H. A. Milman and E. K. Weisburger, Eds.), Noyes Publications, Park Ridge, NJ, 1985.
6. Ford, J. D., and Pereira, M. A. Short-term *in vivo* initiation/promotion bioassay for chemical carcinogens. J. Environ. Pathol. Toxicol. 4: 39–46 (1980).
7. Pilot, H. C., and Sirica, A. E. The stages of initiation and promotion in hepata-carcinogenesis. Biochim. Biophys. Acta 604: 191–215 (1980).
8. Shimkin, M. B., and Stoner, G. D. Lung tumor in mice: application to carcinogenesis bioassay. Adv. Cancer Res. 21: 1-58 (1975).
9. Lykins, B. W., and Koffskey, W. Products identified at an alternative disinfection pilot plant. Environ. Health Perspect. 69: 000-000 (1986).
10. Kopfler, F. C., Coleman, W. E., Melton, R. G., Tardiff, R. G., Lynch, S. C., and Smith, J. K. Extraction and identification of organic micropollutants: reverse osmosis method. Ann. N.Y. Acad. Sci. 298: 20–30 (1977).
11. Smith, J. K., Lynch, S. C., and Kopfler, F. C. Concentration of organics for toxicity testing using membrane process. In: Chemistry in Water Reuse, Vol. 2 (W. J. Cooper, Ed.), Ann Arbor Science, Ann Arbor, MI 1981, Chap. 10.
12. Kool, H. J., van Kreijl, C. F., van Kranen, H. J., and deGreef, E. The use of XAD-resins for the detection of mutagenic activity in water. I. Studies with surface water. Chemosphere 10: 85–98 (1981).
13. Kool, H. J., van Kreijl, C. F., and van Kranen, H. J. The use of XAD-resins for the detection of mutagenic activity in water. II. Studies with drinking water. Chemosphere 10: 99–108 (1981).
14. Aiken, G. R., Thurman, E. M., and Malcolm, R. L. Comparison of XAD macroporous resins for the concentration of fulvic acid from aqueous solution. Anal. Chem. 51: 1799–1803 (1979).