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Effect of Water Treatment on Mutagenic Potential

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In recent years attention has shifted from organic compounds in water which cause taste and odor problems to compounds that are potential health hazards. The presence of compounds in water which are carcinogenic is now recognized (CHEH et al. 1979, CUMMING 1979) although their significance is not yet established (PAGE 1976). Identifying all compounds present in water on a routine basis is not feasible because of the time and expense required. Bioassays are a simple and short-term, widely applicable procedure which can be used to determine if organic compounds in water pose a threat to health.

Positive correlation has been shown between chemical compounds that are positive in the Ames test for mutagenicity and known chemical carcinogens (COMMONER 1976, McCANN & AMES 1976). The application of mutagenicity testing to drinking waters has recently been reviewed by LOPER (1980). Short-term tests by themselves do not give definitive evidence as to whether a substance does or does not pose a carcinogenic hazard to humans. A positive response in these tests is considered suggestive evidence of a carcinogenic hazard (USEPA 1979).

The low concentration of mutagenic materials present requires that, in general, water samples be concentrated for testing (KOOL et al. 1981a, KOOL et al. 1981b). XAD-2 resin has been shown to be a convenient, efficient, and reproducible medium for removing organic compounds from water (JUNK et al. 1974) and has been used to concentrate mutagenic materials from drinking waters (KOOL et al. 1981a, KOOL et al. 1981b).

MATERIALS AND METHODS

Samples were collected from Lake Michigan, the Calumet River, and the Fox River. The samples representing the Lake Michigan water source were collected within the Jardine Water Purification Plant of the City of Chicago. The three points of collection were: (1) the untreated raw water, which is piped from a crib two miles off shore; (2) the settled water, which follows coagulation and sedimentation; and (3) the outlet water, which follows sand filtration and chlorination. Six samples ranging in volume from 100 to 1000 L were collected during the period January, 1979 to July, 1979 for the Lake Michigan source at each of the plant sampling points.

The Calumet River sample was collected from the Indiana

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Harbor Ship Canal at 92nd Street and Dickey Road. The water at this point has passed through one of the most concentrated steel and petroleum manufacturing complexes in the nation, and is heavily polluted with discharges (SNOW 1974). The Fox River sample was collected at Elgin, Illinois at the proposed site on the river for the Elgin water treatment plant. The Fox River is downstream from the Chain of Lakes, a heavily populated recreational area. There is also agricultural and sewage drainage into the river.

River water was collected in 3 L glass bottles and transported to the laboratory. The non-volatile total organic carbon (NVTOC) concentration was measured using a Beckman Instruments Model 915 Total Carbon Analyzer. For each of the two river waters, five samples, 10 L each, were prepared in the laboratory as follows: (1) raw water with no treatment; (2) raw water with chlorine added; (3) chlorinated raw water followed by alum and polymer addition, flocculation, and sedimentation; (4) raw water treated with alum and polymer, followed by flocculation and sedimentation; and (5) raw water treated with alum and polymer as with sample (4), followed by chlorination. The chlorine dosage used was 10 mg/L with a contact time of two hours. Free residual chlorine ranged from 0.2 mg/L to 1.0 mg/L at the end of the contact period. Stock aluminum sulfate and cationic polymer solutions were added at a dosage rate of 15 mg/L of alum and 1.5 mg/L of polymer. The samples were rapid mixed (100 rpm) for 30 sec, and slow mixed (35 rpm) for 30 min. After settling for four hours, the clarified water was decanted.

Each of the samples from the three sources was prepared for testing by passing the water at a flow rate of 100 mL/min through a glass column 3/4-inch diameter by 6-inch length containing XAD-2 resin. Prior to use, the resin was cleaned by the method of JUNK et al. (1974). The adsorbed compounds were eluted from the resin column by passing diethyl ether through the column followed by methanol at a flow rate of 10 mL/min. The eluate was evaporated to dryness and the residue was dissolved in dimethylsulfoxide (DMSO) for testing with the Ames system. The sample concentrates were initially screened with the five Salmonella typhimurium tester strains - TA98, TA100, TA1535, TA1537, and TA1538 both with and without enzymatic activation. Based on these tests, strain TA1538 was used for further testing of the samples when determining the presence or absence of a dose-response effect.

Media preparation and pour plate preparation were according to the method of AMES et al. (1975). Plates were prepared in duplicate and incubated at 35° C for 48 hrs. A positive control prepared by using a known mutagenic compound, 2-nitrofluorene, was run with each set of bioassays.

RESULTS AND DISCUSSION

Two Lake Michigan samples, collected July 14th (600 L volume) and July 27th (800 L volume), produced a response from unactivated compounds with TA1538 which indicated the presence of mutagenic compounds and a change in response that may be attributable to treat-

ment of the water. GRIMM-KIBALO et al. (1981) have also found the presence of mutagenic compounds to vary seasonally. Enzymatic activation was also used in assays of the sample concentrates using the five Salmonella strains and the same volumes of concentrate that were used in the assays without the addition of the S-9 enzyme preparation. Addition of the enzyme preparation decreased the mutagenic response. Concentrates of the three samples collected between January, 1979 and April, 1979 at the three sampling points did not produce responses that would indicate the presence of mutagenic compounds with any of the strains, either with or without enzymatic activation of the compounds. When concentrates of the June sample (1000 L volume) were assayed, the untreated water produced a low number of revertants, indicating the absence of mutagenic compounds. The plates from the settled water header and outlet samples had a decrease in the number of revertants and an absence of background growth as the volume of concentrate was increased. This is characteristic of the effect produced by toxic compounds.

For the July 14th sample an increase in the number of revertants of TA1538 was obtained for each increase in the volume assayed over a two order of magnitude range (Figure 1A). The mutagenic activity ratio (MAR) which expresses the mutagenic activity as a ratio between the experimental counts and the negative control count (COMMONER 1976) was used to evaluate the numerical results. The ratio accounts for the variation from day to day of the spontaneous reversion rate of a given Salmonella strain. Values below 2.0 generally indicate nonmutagenic substances, values between 2.0 and 3.0 are suggestive of the presence of mutagenic substances, and values greater than 3.0 are indicative of the presence of mutagenic activity (COMMONER et al. 1978). A volume of concentrate equivalent to 20 L of water was used for the computation of the MAR. Twenty liters is ten-times the average daily consumption of water (USDHEW 1962). The ratio calculated for each of the three Lake Michigan sampling locations is: crib source, 4.1; settled-water header source, 1.1; and outlet source, 1.8.

The concentrates of the July 27th sample were tested in the same manner (Figure 1B). The MAR calculated for a volume of concentrate containing the equivalent of compounds extracted from 20 L of water is: crib source, 2.6; settled-water header source, 1.3; and outlet source, 2.2.

Frequency distributions of MAR values were calculated for each sampling point from assays of the concentrates of the six samples collected in the period from January through July performed with the five tester strains. The distributions of ratios for the crib and settled-water header samples had maxima at the lowest MAR value of 0-0.5. However, the maximum in the frequency distribution for the outlet samples was obtained at MAR values of 0.5-1.0, indicating slightly higher values for the completely treated water.

Three of the sample concentrates of treatments of Calumet River water representing treatment with chlorine alone, chlorine addition followed by coagulants, and addition of coagulants followed by chlorine addition have MAR values of 5.5, 2.1, and 5.8, respectively, which indicate the presence of mutagenic compounds.

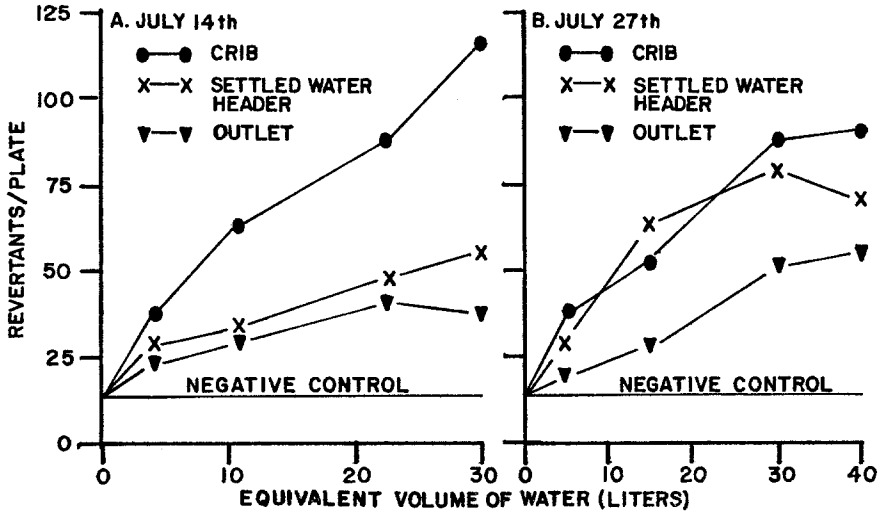

Figure 1. Dose Response of Strain TA1538 with Concentrates of the (A) July 14th and (B) July 27th Lake Michigan Samples.

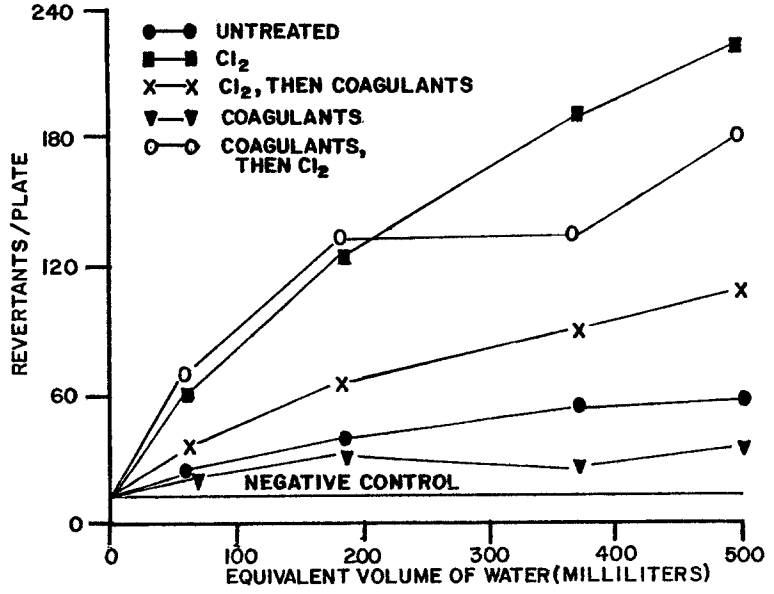

Figure 2. Dose Response of Strain TA1538 with Concentrates of the Calumet River Samples.

Without the addition of chlorine, the maximum MAR was 0.7. The volume of concentrate used for these bioassays was equivalent to testing the compounds extracted from 187 mL of the sample water. The same volume of concentrate produced fewer revertants after enzymatic activation of the compounds.

The five concentrates from the Calumet River water were tested for a dose response effect from strain TA1538. Six volumes of each concentrate, having a two order of magnitude range of response were used for these bioassays. The volumes assayed contained the equivalent of compounds present in 5 to 500 mL of water. The data depicted in Figure 2 show that the concentrate of the water that was treated only with chlorine produced the most revertants per volume of concentrate, followed by the sample that was first treated with coagulants and then chlorine, and the sample that was first treated with chlorine and then coagulants. The concentrates from the untreated water and the water treated with coagulant only produced the least number of revertant organisms per volume of concentrate. Concentrates of the five laboratory-treated Fox River samples did not produce a sufficient number of revertants of any of the five tester strains to indicate the presence of mutagenic compounds either with or without enzymatic activation.

Linear regression was used to assess the effect of treatment on mutagenic potential of the water. The data in Table 1 for the two Lake Michigan water samples that were collected in July show that the mutagenic potential of 3.2 and 2.0 net revertants per liter of the untreated water from the crib source was reduced by treatment processes employed prior to sampling at the settled-water header (SWH) to 0.8 and 1.1, respectively. Water sampled following the final stage of treatment, the outlet sample, shows an increase in net revertants over that produced by the concentrates of the SWH to 1.2 and 1.6 net revertants per liter.

Using the same method of analysis with the dose response data obtained for the Calumet River concentrates, the untreated water yielded 90 revertants/L. With chlorine addition as the only method of treatment, the value increases to 419 revertants/L. With the combination of coagulation followed by chlorination, the value is 299 revertants/L. Both values are 3 to 4½ times the value obtained from the untreated water concentrate. If the untreated sample (90 rev/L) and the coagulant-treated sample (31 rev/L) are compared, it is seen that the coagulation process removes a large portion of the mutagen-producing compounds. Further analysis shows that chlorination of these two water samples does not produce proportional increases in the number of net revertants. When chlorine was added to the untreated water, the number of revertants increased to 419/L, a four-fold increase over 90. When chlorine was added to the water that had been treated with coagulants, the number of net revertants increased to 299/L, a ten-fold increase over 31. This suggests that coagulation may not be an effective remover of precursors of potent mutagenic compounds. Other reports have stated that water treatment is not effective in removing mutagens present in raw water and also may create new ones (GRIMM-KIBALO et al. 1981). CHEH et al. (1980)

Table 1. Revertant Organisms Per Milligram of NVTOC and Per Liter Calculated from Dose-Response Assays of the Lake Michigan, Calumet River, and Fox River Sample Concentrates

Sample	Correlation coefficient r^2	Net revertants per mg NVTOC	Net revertants per liter
Lake Michigan - July 14th			
Crib	0.99	1.3	3.2
Settled water header	0.94	0.3	0.8
Outlet	0.97	0.5	1.2
Lake Michigan - July 27th			
Crib	0.98	1.0	2.0
Settled water header	0.98	0.5	1.1
Outlet	0.91	0.8	1.6
Calumet River			
Untreated	0.98	21.0	90.0
Cl_2	0.99	96.0	419.0
Cl_2, then coagulants	0.98	43.0	189.0
Coagulants	0.76	7.0	31.0
Coagulants, then Cl_2	0.95	68.0	299.0
Fox River			
Untreated	0.62	0.9	14.0
Cl_2	0.66	1.8	28.0
Cl_2, then coagulants	0.67	1.1	17.0
Coagulants	0.58	0.9	14.0
Coagulants, then Cl_2	0.74	1.7	27.0

concluded that chlorination was responsible for non-volatile mutagen production. They found that chlorination increased mutagenic activity two to ten times that of the unchlorinated sample. They also determined that the mutagenic activity seemed to be a result of chlorination of the water constituents and did not appear to result from mutagens preexisting in the chlorine or from reactions of the chlorine with the experimental apparatus.

Comparison of the Fox River and Calumet River results shows no correlation between non-volatile total organic carbon concentration and the degree of mutagenic response. The untreated Fox River water had a NVTOC concentration of 15.6 mg/L, approximately four times greater than the Calumet River water which was 4.4 mg/L. However, the untreated Fox River sample concentrate produced only 0.9 revertants/mg NVTOC while the untreated Calumet River sample concentrate produced 21 revertants/mg NVTOC. Chlorination increased the value for the Fox River sample to only 1.8 revertants/mg NVTOC but increased that for the Calumet River sample to 96 revertants/mg NVTOC (Table 1).

Two-factor analysis of variance (ANOVA) was used to determine

if the difference in the number of revertants obtained from sample concentrates representing the various treatment methods was significant and attributable to the treatment method. The data from each set of Lake Michigan samples and the Calumet River samples all have calculated F values that show that at the 95% level of confidence, there are significant differences among the responses for the different treatment methods.

SUMMARY AND CONCLUSIONS

Enzymatic activation did not convert the compounds in the sample concentrates into mutagens. These results are in agreement with those of CHEH et al. (1979) that the mutagenic activity was two to three times greater without the activating system than with it.

The dose response relationships demonstrated a low level of mutagenic activity for the concentrates of the Lake Michigan samples. The MAR increases for the completely treated potable water.

The concentrates of the Calumet River water to which chlorine had been added produced more revertants per volume of concentrate than did the concentrates of water which had not been chlorinated. Chlorination of the water with no additional treatment produced the highest degree of activity.

For the Calumet River samples, treatment with coagulants reduced the net number of revertants to one-third the value for the raw water. When chlorine was added as the final treatment step, the net number of revertants increased by a factor of ten.

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