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Seasonal Variation of Mutagenic Activity in Drinking Water

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Chemical contamination of water destined for human consumption and the possible health hazards involved are of major concern at present. Over 1000 organic chemicals, including several known or suspected carcinogens, have been found in drinking water (SHACKELFORD & KEITH 1976, KRAYBILL 1976). Epidemiological studies have shown a correlation between drinking water source and incidence of various forms of cancer (WILKINS et al. 1979).

It is very difficult to recover all drinking water contaminants, and of those recovered, only a small percentage have been identified (GARRISON 1977). Bioassay, or the measurement of the effects of a sample in a biological system, provides a means of determining the toxicity of the contaminants in water without the need to isolate and identify each compound. Many short-term bioassays have been developed that predict, with good accuracy, the carcinogenic potential of the test substance (GLATZ 1979). Of these, the Salmonella/mammalian microsome mutagenicity assay, or Ames test, has been used most often (AMES et al. 1975). Mutagenic activity measured in the Ames test has been shown to correlate with about 90% accuracy with carcinogenic activity in tests of over 300 chemicals (McCANN et al. 1975, McCANN & AMES 1976).

Several recent studies have demonstrated the presence of mutagenic contaminants in drinking water. GLATZ et al. (1978) detected mutagenic activity in the organic contaminants recovered from 11 of 14 finished water supplies and from 4 of 14 raw water supplies. Organic residues concentrated by reverse osmosis from the drinking water of six U.S. cities were found to be mutagenic (LOPER et al. 1978) and to cause malignant transformation of mouse cells in vitro (KURZEPA et al. 1979). Mutagenic activity has also been found in concentrated tap water (GRUENER & LOCKWOOD, 1980), in recycled water (NEEMAN et al. 1980, GRUENER & LOCKWOOD 1979) and in effluents from wastewater treatment plants (SAXENA & SCHWARTZ 1979, RAPPAPORT et al. 1979).

Water sources can vary extensively in the type and amount of organic contamination that they carry. Because the formation of halogenated compounds is temperature-dependent (ROOK 1974), the levels of trihalomethanes and other halogenated contaminants formed in the chlorination process can vary with the season of the year. The present study surveys the seasonal changes in mutagenic activity, as measured by the Ames test, of organic contaminants accumulated from raw and finished drinking water.

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MATERIALS AND METHODS

Water source. The water supply of Des Moines, Iowa, was sampled 15 times over the course of one year. Water is obtained from a river as well as from an infiltration gallery in the river valley. The gallery, constructed of segments of concrete cylinders, receives surface water that has percolated through a coarse layer of glacial sand and gravel. Raw water was sampled after sand-gravel filtration and chemical treatment (samples: September, October, March II, April I, April II, June I, June II, July). Finished water was sampled after final chlorination and fluoridation (samples: August, November, December, January, February, March I, March II, April I, April II, May, June I, June II, July).

Resin adsorption procedure. Resin adsorption was performed as described by FRITZ (1977). Organic materials were collected from an average of 550 l finished water and 570 l raw water by adsorption on Amberlite XAD-4 resin (Rohm and Haas, Philadelphia, PA). The sorbent was eluted first with 100 ml diethylether and then with 100 ml absolute ethyl alcohol. The ether and alcohol eluates were reduced to 2.5 ml and 1.5 ml, respectively, by evaporation over a hot water bath, to obtain a final concentration of the accumulated organic material of over 200,000-fold. The ether eluate was exchanged into an equal volume of dimethyl sulfoxide (DMSO, Aldrich Chemical Co., Inc., Milwaukee, WI) before mutagenicity testing.

Mutagenicity assays. Assays for mutagenic activity were performed as described by AMES et al. (1975). Strains TA98, TA100, TA1535, TA1537 and TA1538 were used to test all samples by the spot test procedure, but to conserve the samples, only the two most sensitive strains, TA98 and TA100, were used in the plate incorporation procedure. The plate incorporation procedure was performed in smaller petri dishes (60-mm diameter), which allowed all reagents to be reduced in volume and samples to be conserved further. Aroclor 1254 (Monsanto Co., St. Louis, MO) - induced rat livers were prepared as described by AMES et al. (1975). The final preparation (S-9 mix) contained 0.05 ml activated liver per ml and was added at 0.25-0.50 ml per test plate. Sodium azide (Fisher Scientific Co., Fair Lawn, NJ) and 2-nitrofluorene (Aldrich) were used as positive controls and were included in each experiment.

All samples were tested first, in duplicate, in the spot test with all five strains without S-9 mix. A mutagenic response was noted for a sample if it induced more than twice the number of colonies as appeared on the negative (solvent) control plate, and these colonies were concentrated in a ring around the site of sample application.

Regardless of their activity in the spot test, all samples were tested in the plate incorporation procedure with TA98 and TA100. Four sequential 1:4 dilutions of each sample were tested in duplicate in the presence and absence of S-9 mix. A sample was considered mutagenic if a dose-response relationship was observed between the number of revertant colonies per plate and the sample concentration. The degree of mutagenic activity in a sample was determined from the dose-response curve and was defined as the number of revertant colonies induced by the organic material from one l of water. When a sample inhibited a test strain, the lowest dilution that wasn't inhibitory but was mutagenic was used to determine the mutagenic activity per l. Negative

control counts were subtracted from experimental counts before dose-response relationships were calculated.

Gas chromatography. A gas chromatograph (Tracor, Inc., Austin, TX) equipped with an electron capture detector (ECD) was used to detect trihalomethanes. The 38 m x 0.25 mm i.d. column was packed with Carbowax 20 M. The carrier gas was helium at a linear flow rate of 23 cm/sec and a volumetric flow rate of approximately 0.7 ml/min. The oven temperature was programmed from 50° C to 215° C at 4° C/min. Sample injection was 2 μl at approximately 30 to 1 split. Results were recorded by a Fisher Recorder All Series 5000 (Houston Instrument, Austin, TX).

RESULTS AND DISCUSSION

Spot test. Mutagenic activity was detected sporadically in the spot test procedure (data not shown). Because S-9 mix was not included in the assay system, only direct-acting mutagens were detected. Strain TA100 was reverted most frequently by both raw and finished water contaminants. Strains TA98, TA1535 and TA1537 each were reverted by a few samples, but TA1538 was not reverted by any sample. Mutagenic activity was detected in raw water sampled in March II, April I, April II and June I and in finished water sampled in August, November, March I, March II, April I, April II, May, June I, June II and July. With the exception of the November sample, the only mutagenic samples were those obtained in the spring and summer. Whenever a raw water sample was found to contain mutagenic contaminants, finished water sampled at the same time also was found to contain mutagens. In contrast, in several sampling periods mutagens were detected only in the finished water. This pattern was observed previously (GLATZ et al. 1978) and suggests that water treatment may not remove mutagenic contaminants already present but also may create new ones.

Plate incorporation procedure. The plate incorporation procedure was used with strains TA98 and TA100 to quantify the level of mutagenic activity present in each water sample. To compare results more easily, the amount of mutagenic activity in each sample was expressed as the number of revertant colonies induced by the contaminants in one l of water. Results are presented in Fig. 1A-1D.

Mutagenic activity in both raw and finished water was greatest during the spring, summer and early fall and decreased during the winter. The increase in activity from winter to spring probably can be attributed to the contamination of surface waters by agricultural runoff, which is most severe in the early spring when pesticides are being applied and when heavy rains can cause considerable erosion (JUNK et al. 1976). The highest level of mutagenic activity in raw water was measured in the April I sample, which was obtained during a period of flooding. Also, an increase in water temperature and in organic precursor concentration at this time would increase the formation of halogenated compounds during chlorination. This phenomenon is seen in the number of halogenated compounds detected by gas chromatography in the ether eluate fractions of the finished water samples (Fig. 1B). The numbers of peaks recorded by gas chromatography were greatest in the warm-weather samples and were correlated with the level of mutagenic activity

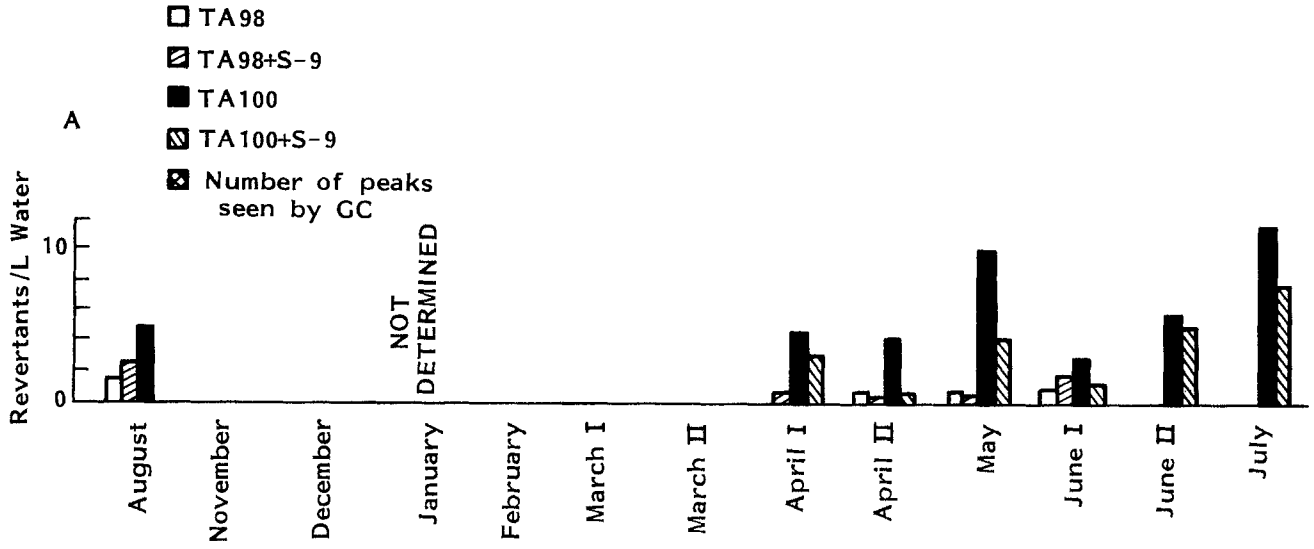

Fig. 1. *Mutagenic activity measured in organic materials accumulated from raw and finished drinking water samples.* A. Finished water, alcohol eluate fraction. B. Finished water, ether eluate fraction. C. Raw water, alcohol eluate fraction. D. Raw water, ether eluate fraction. The number of trihalomethane peaks detected by gas chromatography was determined for the ether eluate of finished water, and is shown in (B).

B
Revertants/L Water or #GC Peaks
60
50
30
20
10
August
November
December
January
February
March I
March II
April I NOT DETERMINED
April II
May
June I
June II
July

Fig. 1B

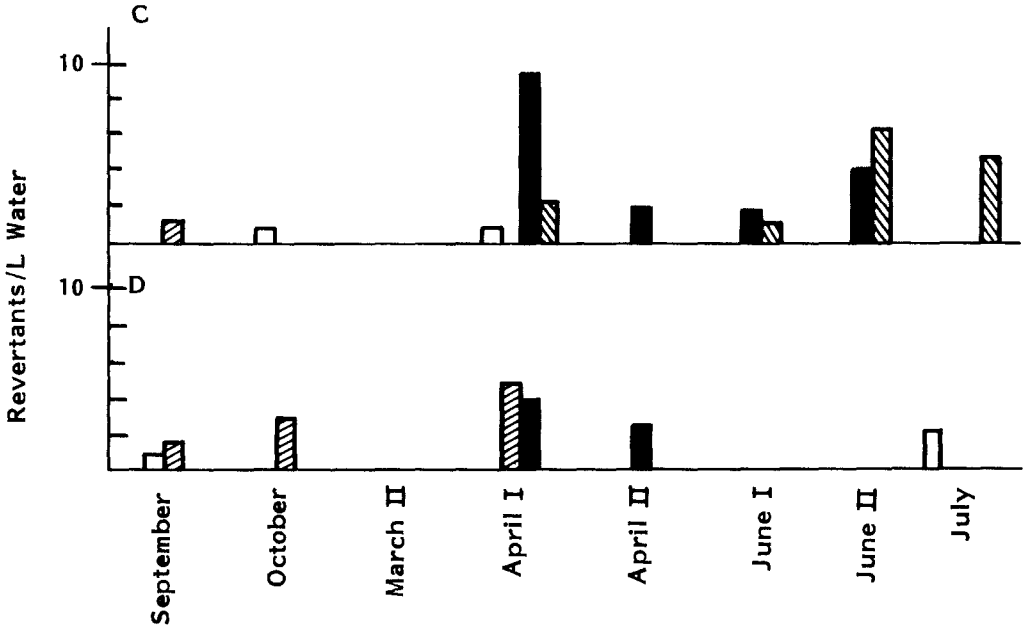
C
10
10
D
Revertants/L Water
September
October
March II
April I
April II
June I
June II
July

Fig. 1 C and D

detected in the ether eluate fractions. These peaks represent the volatile trihalomethanes, which usually are not active in the Ames test. An increase in their numbers, however, indicates an increase in larger halogenated molecules that are mutagenic.

The high mutagenic activity measured in the late summer and fall samples may be due to carry-over of organic material accumulated over the spring and summer within the filter beds of the treatment plant. Mutagenic activity during the winter may be due to chlorinated or nonchlorinated pollutant chemicals introduced into the raw water throughout the year.

Mutagenic activity was detected more often, and at higher levels, in finished water than in raw water. The nature of the mutagens may be different between raw and finished water, however. Activity in raw water was low and was found in both the alcohol eluate fraction, which contains relatively polar compounds, and in the ether eluate fraction, which contains the more nonpolar compounds. Strain TA100, which responds to base-pair substitution mutagens and to some frameshift mutagens, was reverted most frequently by the alcohol eluate. The ether eluate was more mutagenic for strain TA98, which is reverted by frameshift mutagens. For finished water, the ether eluate was more mutagenic more often than was the alcohol eluate, and strain TA100 was reverted more than strain TA98. These data suggest that a varied population of mutagens are present in raw water but that most of the mutagenic activity in finished water may be attributed to nonpolar compounds that cause base-pair substitutions.

Usually, mutagenic activity decreased in the presence of S-9 mix. The oxidative mammalian enzymes in this preparation could inactivate direct-acting mutagens as well as convert promutagens to an active form. Our results suggest that direct-acting mutagens are the most significant in this water supply.

Inhibition of the test strains was observed occasionally for both raw and finished water samples. Inhibitory agents found in raw water usually were not removed during treatment, but were found in the simultaneously sampled finished water. The addition of S-9 mix decreased the observed inhibition.

These results agree with other reports that mutagens are present in raw water supplies, and that water treatment not only does not effectively remove mutagens, but also contributes significantly to their measured activity. A distinct seasonal variation was observed in the level of measured mutagenic activity, which correlated well with the presence of contaminants in the raw water, the water temperature, and the number of halogenated compounds observed.

The samples tested in this study, however, contained relatively low mutagenic activity. We have calculated, for example, that one cigarette contains over 1000 times the level of mutagenic activity as would be found in the normal consumption of 2 l/day of this drinking water (HOOPER et al. 1977). However, improved methods of water treatment and alternatives to chlorination should be sought to lower the levels of potentially harmful contaminants in drinking water.

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