Bull. Environm. Contam. Toxicol. 20,522-526 (1978)

Mutagenicity of Ozonated, Recycled Water

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Ozonation is being considered as a partial replacement for chlorination to disinfect water. Advantages of the process include:

1. Strong oxidative reactivity.
2. Fast decomposition of ozone in water (which also has disadvantages).

The possibility that the ozonated compounds may be more toxic, carcinogenic, or mutagenic has not been evaluated until now. Chemical analyses of ozonated mixtures and pure compounds in water have been recently undertaken in a number of laboratories. AMES and coworkers (1975) developed a mutagenicity test, the Salmonella/microsome test, which measures the reversion rate of specially constructed Salmonella strains unable to grow without histidine. The test has been applied to over 300 compounds (MCCANN, et al., 1975); carcinogenic effects on animals for two-thirds of these compounds have been well documented. A high degree of correlation (90%) was found between the known carcinogenic or non-carcinogenic compounds and their positive or negative end points in the Salmonella test. This paper reports the results of the mutagenicity of ozonated and non-ozonated recycled water tested via the Salmonella/microsome test.

METHODS

Water samples were drawn from high ground water (50 m deep) at the edge of the ponds of the Dan Region Sewerage Reclamation Project. In this project, municipal wastewater is subjected to biological and chemical treatment, after which ground water recharge is carried out by slow filtration through natural sand dunes. The sampling well used for this study was located only 50 m from the edge of the pond. The total organic carbon (TOC) content of the water ranged between 15 and 20 mg/l.

Concentration of this water was carried out by low pressure, low temperature (37-38°C) distillation, and the recovery of organics (measured as TOC) in the concentrates

0007-4861/78/0020-0522 $01.00

was 95%. The pH of the concentrated water was in the range of 9-10. The pH was brought to 7-7.4, and the concentrate sterilized by ultrafiltration prior to testing.

Ozonation was done with a Fisher OZ-3 instrument. Oxygen flow was 40 liters/hour, and the generator amperage was 200 mA. The system was stabilized for 15 minutes before starting the experiments. Ozonated samples were tested 24 hours after treatment.

The mutagenicity tests were done on two new strains of Salmonella typhimurium (TA100 and TA98) kindly supplied by Dr. B. N. Ames. These strains, which have been described by AMES, et al. (1975), are more sensitive for detection of carcinogens than previous strains. Cultures were grown overnight in complete medium (Difco Nutrient Broth 0.8%, NaCl 0.5%) containing ampicillin 2.5 µg/5 ml. Platings with soft agar technique were made on complete medium (CM plates) and on minimal medium (MM) described by VOGEL and BONNE (1956) with the supplements according to AMES, et al. (1975). Liver fractions from male rats (S_9) were prepared according to the method of GARNER, et al. (1972) after induction by either Aroclor 1254 (500 mg/kg), 3-methylcholanthrene (80 mg/kg), or sodium phenobarbital (0.1% in drinking water). Concentrated water samples were added in different volumes (up to 1 ml) to the top agar. When samples larger than 0.1 ml were introduced to the system, a higher concentration of Vogel and Bonner Medium E was used and the appropriate dilution made. A result was considered positive when the number of the induced revertants was two-fold greater than the controls.

RESULTS AND DISCUSSION

Initially, the toxicity of the concentrated water to the bacteria was checked. It was found that there was no effect on the viability of the bacteria for up to 250 µg TOC per plate. From this level, the toxicity increased steeply, and at 500 µg of TOC, only 50% of the bacteria remained after 24 hours; at 1,000 µg, no viable cell was detectable. At this stage of experimentation, it is not possible to determine which constituent is responsible for the effect. The increased osmotic pressure apparently is not the cause, since the salt concentration is about 1/3M NaCl solution at the highest concentration tested. In other experiments (N. Gruener, unpublished data), samples of NaCl solutions up to 3M did not show any effect on the viability or on the mutagenicity of known mutagens in the Salmonella test. The detergents, which amount to about one-fourth of the total organic carbon content in the water, are possible toxins.

Fractionation of the soluble compounds to different groups will permit study of this problem in detail.

Salmonella mutagenicity tests were performed on water samples in the range of 20-250 μg TOC/plate, with and without microsomes. Results were negative at these levels, and higher levels of TOC could not be tested because of toxicity. A characteristic experiment is represented by the results in Table 1. As shown, the concentrated water did not cause any increase in the number of revertants. There was a small increase in the total number of viable cells in the presence of the concentrated water. Preliminary experiments showed that preincubation of bacteria and the concentrated water for 10-20 minutes prior to the addition of the agar increased the number of colonies per plate. With TOC levels of 120-240 μg/plate, 250-300 colonies were found, compared to 150-180 colonies in the control (using strain TA100). This phenomenon has yet to be confirmed. Strain TA98 gave results similar to those presented in Table 1 for TA100.

TABLE 1

SALMONELLA MUTAGENICITY TEST OF CONCENTRATED RECYCLED WATER*

Source	TOC Level (μg/plate)	Activation System (S_9 Mix)	Number of Viable Cells/ Plate X 10^{-7} (mean value of 2 plates)	Number of Mutants/ Plate
Control (distilled H_2O)	0	-	8.0	149
		+	8.0	169
Concentrated Water	120	-	11.2	155
		+	11.2	186
	240	-	10.8	177
		+	10.8	189

*The bacteria strain was TA100, and the S_9 fraction was prepared from Aroclor 1254 induced rats.

The effect of ozonation on the TOC content is given in Table 2. A yellowish color observed in the concentrate disappeared seconds after the start of ozonation. However, the organic content decreased by only 25% after 15 minutes and by less than 50% after one hour. It is apparent that the organic compounds first change form to other solubilized compounds; only after some time does a fraction of the TOC oxidize completely to CO_2.

TABLE 2

REDUCTION OF TOTAL ORGANIC CONTENT IN WATER AFTER OZONATION

Ozonation Time (min)	TOC Content (mg/l)
0	385
15	270
30	260
60	260
90	175

The results presented in Table 3 show that water ozonated for 15 minutes is mutagenic to both strains: TA100 (base pair substitution) and TA98 (frameshift). No activation by microsomal fractions was needed to cause mutations in TA100. With strain TA98, too, there was an effect in the absence of the liver fraction (about three times the control values); in the presence of the liver fraction, there was an additional increase in the number of mutants.

TABLE 3

MUTAGENICITY OF OZONATED RECYCLED WATER IN SALMONELLA STRAINS

Treatment	Number of Mutants Per Plate TA100	TA98	Microsomes
--	159	37	--
--	223	73	Methylcholanthrene
--	167	38	Phenobarbital
--	160	35	Aroclor 1254
15 min ozonation	595	120	--
"	700	352	Methylcholanthrene
"	610	170	Phenobarbitol
"	660	380	Aroclor 1254
Control (distilled water)	147	30	--
"	172	86	Methylcholanthrene
"	204	40	Phenobarbital
"	163	66	Aroclor 1254

The specific mutagens formed during ozonation have yet to be determined. MOYER and FALK (1976) recently reviewed work done on the chemical reaction occurring between ozone and different compounds. Most of the ozonated products have not yet been tested for mutagen-

icity or carcinogenicity. One exception is malonaldehyde, a product of ozonated fatty acids (ROEHM, et al., 1971), which was found to be mutagenic in certain Salmonella typhimurium (MUKAI and GOLDSTEIN, 1976). Another group of chemicals, the N-oxide purines, have been shown to be carcinogenic in rats (SUGIURA, et al., 1970). Parallel chemical and biological studies may identify the mutagenic products formed in ozonated water.

ACKNOWLEDGEMENT

This study was done under Research Agreement No. 167 with the United States-Israel Binational Science Foundation.

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