MUTAGENIC ACTIVITY OF SELECTED ORGANIC COMPOUNDS TREATED WITH OZONE

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Introduction

In the early 1970's it was discovered by several groups of investigators that trihalomethanes (THM) were present in some American drinking water supplies, and that their presence was due primarily to the process of chlorination. Bellar and co-workers (1974) pinpointed the appearance of trihalomethanes to the addition of chlorine in the water treatment process. Symons and co-workers, in the National Organics Reconnaissance Survey (1975), reached the conclusion that treated water contained higher levels of trihalomethanes than untreated water supplies, as surveyed in cities across the United States. In 1978 Glatz and co-workers reported finding increased mutagenic activity in chlorine-treated water in several U.S. cities.

In 1976 and 1977 Rook published evidence that certain natural organic substances, such as humic and fulvic acids, might be serving as precursors for trihalomethane formation. He pointed out that aromatic compounds with meta dihydroxy groups produce a high level of chloroform subsequent to chlorination. Other investigators have succeeded in demonstrating formation of chloroform and other chlorinated organics in treated waters (Cheh et al., 1980; Maruoka and Yamanaka, 1980).

Because of these findings, alternatives to chlorination are being considered and studied. One of these alternatives is ozone treatment. Ozone is a proven water disinfectant, but little is known about the products of its reaction with complex organic compounds. This project examined one aspect of the effect of ozone on natural water; that is, the effect on the mutagenic activity of organic compounds that may be present in ozone-treated waters.

Model Compounds

To study this effect, we chose six organic compounds to serve as models for organic material in natural or industrial process water. These compounds include (figure 1):

1. Phenol.
2. Resorcinol.
3. Rutin, chosen to represent fulvic acid because of its meta-dihydroxylated benzene ring. Quercetin, a known mutagenic flavanol, is obtained by cleavage of the glycosidic bond of rutin.
4. Lignin, represented here by coniferyl alcohol which is one of its monomers. Lignin is present in pulp mill effluent and in leachate from woody plants.
5. Tannic acid, a hydrolyzable tannin, which is an ester of a sugar (usually glucose). Tannic acid is also present in leachate.
6. Humic acid, the last model compound, is not represented in the figure, since it is a large complex molecule containing many phenolic, quinoid and benzenecarboxylic acid groups. For this project a commercially prepared salt of humic acid was used (Aldrich sodium salt).

Experimental

These compounds were tested for mutagenic activity in the Ames *Salmonella*/mammalian microsome assay. The Ames test is a reverse mutation assay utilizing specially developed strains of *Salmonella typhimurium*. These strains require histidine for growth, but can be reverted to histidine prototrophy by certain mutagens. The strains used in these experiments were TA98 which detects frameshift mutation, and TA100 which detects base-pair substitution types of mutation.

A standard agar incorporation assay was done (Ames et al., 1975), with bioactivation supplied by rat liver S9 fraction (induced with phenobarbital). Each compound was tested over a range of concentrations with no ozone treatment; this was a dose-response experiment. Next an ozonation experiment was

done for each compound: a single concentration (100 ppm) of the compound was tested for mutagenic activity against varying times of ozonation. Positive controls were N-methyl-N'-nitro-N-nitrosoguanidine, an alkylating agent that is positive in strain TA100 with and without bioactivation, and benzidine, an aromatic amine which is positive in strain TA98 with bioactivation. The results of each experiment were expressed as the mutation index: this is the ratio of revertant colonies on the test plate to revertant colonies on the control plate. This ratio must be greater than 2.0 in order to demonstrate an increase in mutagenicity over control. An analysis of variance was performed on all data from dose-response experiments and from the ozonation experiments.

For the ozonation experiments ozone was generated from oxygen by means of electrical discharge. The ozone was passed into a 200-ml aqueous solution of the organic compound for a period of 2, 5, 10 or 15 minutes. Ozone output was measured by a standard iodometric titration (Standard Methods for the Examination of Water and Wastewater, 14th edition). The ozone available to react with the model compounds was determined by measuring the amount of ozone that was not utilized by a water blank in a given time period. The amount of ozone that reacted with the model compound was determined by the difference between the excess ozone given off by the organic solution and by the water blank.

Results And Discussion

Ozone Utilization

The results of ozone utilization are shown in figure 2; ozone consumption was recorded as moles ozone utilized per mole of organic substrate. The amount of ozone consumed by each model compound appears to fall into a natural grouping based on the structural complexities of the compunds. Phenol and resorcinol utilized the least amount of ozone and their rate of ozone consumption had stabilized within 15 minutes. At the other end of the spectrum humic acid utilized more ozone than any other model compound and its rate was still increasing after 15 minutes of ozone treatment. This is probably a conservative estimate of ozone utilization by humic acid, because a conservative value was used for the molecular weight when calculating the molar ratio of ozone consumption.

The decrease of model compound concentration during the course of ozonation was measured by the Folin-Ciocalteu test for phenolics (Standard Methods for the Examination of Water and Wastewater, 14th edition). This was done for four model compounds and the results are shown in figure 3. The decrease in concentration of phenol was nearly linear over 15 minutes and ozone had destroyed virtually all measurable phenol. The other compounds exhibited a rapid decrease to a concentration between 10 to 30 ppm, followed by a much slower rate of decrease.

Mutagenicity Studies

In the dose-response experiments (in which compounds were tested for mutagenicity without ozone treatment) the analysis of variance detected no correlation between increasing concentration and increase in mutagenic activity. All compounds (except rutin) exhibited levels of mutagenic activity that were similar to control levels (The analysis of variance did indicate a significant interaction between strain, bioactivating system and compound; this interaction is due to slightly increased mutagenic activity in strain TA100 (with S9) on the part of rutin (figure 4). This mutagenic activity was not reproducible between experiments, and the increase in activity was attributed to an unidentified impurity). The lack of a dose-response relationship indicates that, under the conditions of this study, these model compounds are not mutagenic prior to water treatment.

In the ozonation experiments the model compounds demonstrated no increase in mutagenic activity after ozone treatment, with one exception. This exception was lignin, which did exhibit an increase in mutagenic activity after two minutes of treatment with ozone; this activity returned to control levels with longer ozone treatment. The analysis of variance clearly indicates the increased response of lignin at two minutes (figure 5).

One interesting point about this observed increase in mutagenic activity is that the responses of the bacterial tester strains differed. Lignin treated with ozone for two minutes exhibited increased mutagenicity in strain TA98 with and without bioactivation, but the response without the bioactivating system was somewhat greater (figure 6). Tested against strain TA100, the treated lignin demonstrated a weak response only in the absence of the bioactivating system; there was no increase in mutagenic activity in the presence of the bioactivating system (figure 7). It appears from this that a transient direct-acting mutagen is being formed and then rapidly destroyed by ozonation. While these increases in mutagenic acitivity are statistically significant, they represent only a weak mutagenic response and may not have biological significance; but it will require further testing to determine this.

This project has demonstrated several things: first, the usefulness of the Ames test in assessing the potential mutagenic effects of a water treatment process, and second, the production of a transient mutagenic species upon ozonation of lignin. The model system presented here could be used to study more completely the effect of ozone treatment on lignin in water supplies.

Acknowledgment

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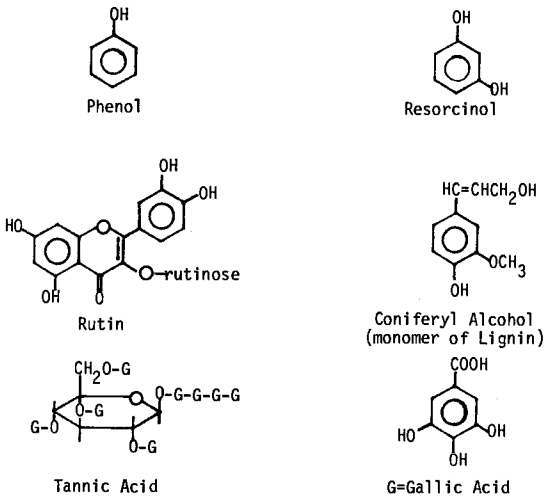


Figure 1. Model compounds. Humic acid (Aldrich sodium salt) is the only model compound not shown.

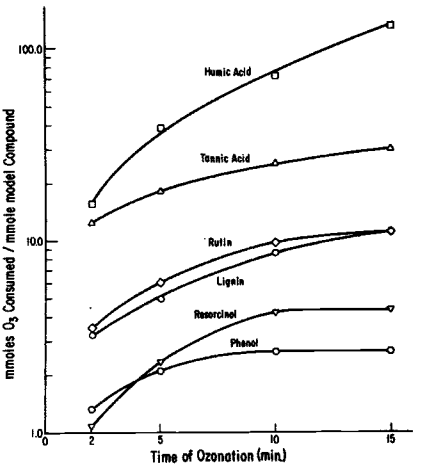
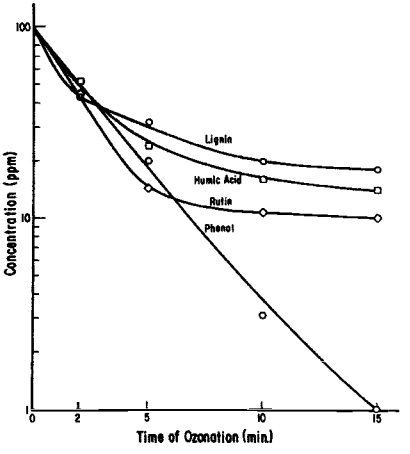


Figure 2. Utilization of ozone by model organic compounds. Ozone utilization is calculated as a molar ratio. All conditions of ozonation were held constant except for time. Ozone dose = 7.1 mg/L.

Figure 3. Decrease in model compound concentration during ozonation. Standard curves of model compound concentration were constructed using the Folin-Ciocalteu (F-C) test for hydroxylated aromatics; the same compounds were then measured by the F-C test subsequent to ozonation.



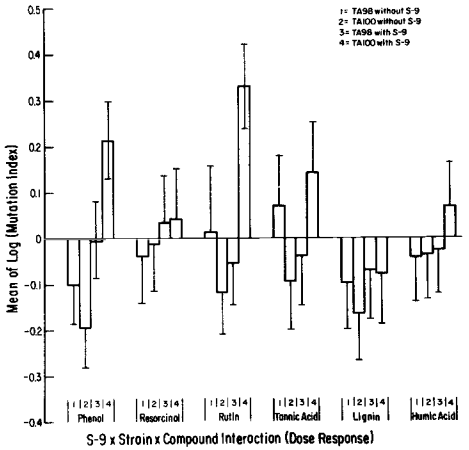


Figure 4. Analysis of variance for dose-response experiment. The significant interaction (S9 x strain x compound) does not involve concentration, but indicated a weak mutagenic response in strain TA100 with bioactivation when tested against rutin. Mutation index must exceed 2.0 to indicate a significant increase in mutagenic activity (log 2 = 0.301).

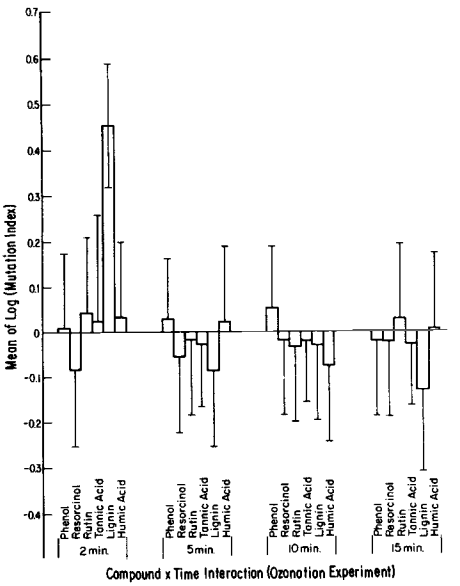


Figure 5. Analysis of variance for ozonation experiment. Only lignin at two minutes of ozone treatment exceeded level of significance. Further ozonation of lignin caused mutagenic activity to return to control levels.

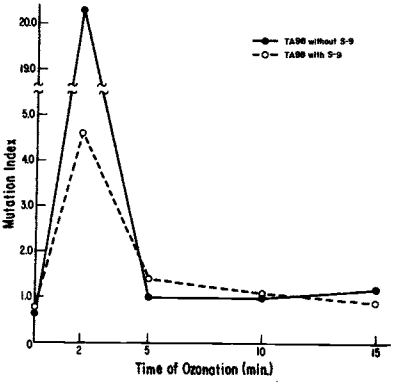


Figure 6. Mutagenic response of strain TA98 to ozone-treated lignin. The mutagenic activity is greater in the absence of the bioactivating system than in its presence.

Figure 7. Mutagenic response of strain TA100 to ozone-treated lignin. The mutagenic activity is increased over control only in the absence of the bioactivating system.