USE OF ORGANIC N-HALAMINES AS WATER DISINFECTANTS

Elisabeth D. Elder, Alisha R. Ward and Jesse B. Scott

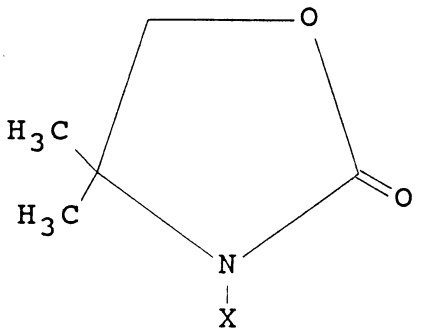
AUTHOR: Biology Department, Georgia Southwestern College, Americus, Georgia 31709.
REFERENCE: Proceedings of the 1991 Georgia Water Resources Conference, held March 19 and 20, 1991, at The University of Georgia, Kathryn J. Hatcher, Editor, Institute of Natural Resources, The University of Georgia, Athens, Georgia.

INTRODUCTION

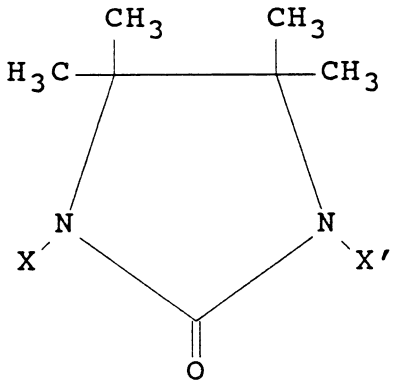
Purpose. For many years potable water has been disinfected primarily through chlorination. A resulting problem has been the production of toxic trihalomethanes through reactions of free chlorine with organic impurities (Vogt and Regli, 1981; Brodtmann and Russo, 1979). Other current disinfectants such as chlorine dioxide, ozone, alkaline hypochlorites, and many of the inorganic and organic halamines lack stability in solid form or in aqueous solutions (Barnela et al., 1986). With industrial development, water recycling, increasing population, and problems caused by chlorination, finding alternative water disinfectants is of increasing importance. The purpose of this study is to determine the efficacies several organic N-halamines as alternative water disinfectants.

Ideally a disinfectant will be stable in solid and aqueous forms, nontoxic, noncorrosive, odorless, tasteless, and effective against a variety of potential pathogens. Some of the organic N-halamines appear to have these qualities (Worley et al., 1985).

Early Studies. Much of the work has focused on 3-chloro-4,4-dimethyl-2-oxazolidinone (compound I). This compound, originally found to be bactericidal by Kaminski et al. (1976) and Kosugi et al. (1976), has been found to be effective against several genera of bacteria including *Staphylococcus, Pseudomonas, Escherichia, Klebsiella, Proteus, Salmonella, Serratia, Enterobacter, and Sphaerotilus* (Elder et al., 1986; Williams et al., 1985; Worley et al., 1985; Worley et al., 1983a; Worley et al., 1981). It has also been found to be effective against several fungal genera including *Candida* and *Rhodotorula*, several protozoal genera including *Giardia* and *Entamoeba*, and poliovirus type I (Worley et al., 1985). Compound I has been found not to be toxic to chickens in drinking water (Mora et al., 1982). In the presence of organic demand compound I reacts much less rapidly with organic impurities than other disinfectants such as calcium hypochlorite (Worley et al., 1984a; Vogt and Regli, 1981; Brodtmann and Russo, 1979). Trihalomethane production is greatly reduced by the decreased reactivity. Compound I is stable in both acidic and neutral aqueous solutions and in solid form (Worley and Burkett, 1984; Worley et al., 1984b; Worley et al., 1983a; Worley et al., 1983b; Burkett et al., 1981).



X = Cl, compound I (3-chloro-4, 4-dimethyl-2-oxazolidinone)
X = Br, Compound IB (3-bromo-4, 4-dimethyl-2-oxazolidinone)



X = X'= Cl, Compound A (1,3-dichloro-4, 4, 5, 5-tetramethyl-2-imidazolidinone)
X = X'= Br, Compound AB (1,3-dibromo-4,4,5,5-tetramethyl-2-imidazolidinone)
X = Cl, X' = Br, Compound ABC (1-bromo-3-chloro-4, 4,5,5-tetramethyl-2-imidazolidinone)

Figure 1. Organic N-Halamines

The key to the stability of compound I is the presence of the two methyl substituents at the 4 position of the oxazolidinone ring. When chlorine is released, forming either hypochlorous acid or hypochlorite, the electron donating methyl groups cause destabilization of any

anionic character developing at the nitrogen moiety. The effect is the stabilization of the N-Cl bond so that chlorine is released slowly under chlorine demand-free conditions. As free chlorine is lost through reaction or vaporization in the system, the equilibrium will be maintained by slow formation of additional free chlorine. Based on an estimated hydrolysis Keg of 10^{-9}, insufficient free chlorine would be present to kill organisms at normal disinfection concentrations of 1 to 10 mg/l. Compound I itself appears to be the active biocide (Barnela et al., 1986).

Current studies. The assumption that the stability of N-halamines is enhanced by electron-donor substituents adjacent to the N-X moieties led to the development of the compound A series. Based on stability testing, the compound A series should be more rapid disinfectants than compound I under acidic and neutral chlorine demand-free conditions and in chlorine demand conditions. Compounds AB and ABC should generally be faster disinfectants than compound A. The reason for the increased rate of disinfection is the presence of bromine. Since bromine is larger than chlorine, the N-Br bond is longer and weaker than a N-Cl bond. Bromine can therefore be released more readily.

With the need for better approaches to disinfection, the technical promise of the compounds, and the possible production of organic N-halamines during existing chlorination procedures, these compounds merit further study. The purpose of this study was to determine the efficacy of the compound A series as water disinfectants.

METHODS

Bacterial Selection. Reasons for selecting *Escherichia coli, Legionella pneumophila, Pseudomonas aeruginosa*, and *Streptococcus faecalis*, were their involvements in nosocomial infections, use as indicators of fecal pollution, involvement in Legionnaire's disease, and germicide resistance. The bacteria were grown and maintained as recommended by the American Type Culture Collection (ATCC). For use in the experiments, bacteria from plates held 24 hours at 37° C were suspended in sterile saline. A spectrophotometer was used to calibrate the suspension to 1.0 to 2.0 x 10^8 CFU/ml.

Disinfectant Protocol. The disinfectants were obtained from Dr. S. D. Worley at Auburn University. Stock solutions of approximately 200 mg/l Cl^+ (or the molar equivalents with bromine) were made in chlorine demand-free water. Exact concentrations were determined using standard iodometric titrations. The stock solutions were utilized to form 2.5 (7.05 x 10^{-5} M) and 5.0 (1.41 x 10^{-4} M) mg/l Cl^+ (or the molar equivalents of bromine) solutions in the experimental flasks.

Bactericidal activity was tested in 0.05 M acetate buffer for pH 4.5, 0.05 M phosphate buffer for pH 7.0, and 0.01 M borate buffer for pH 9.5. These buffers were selected to assure maximum buffering capacities at the desired pH levels. To remove any chlorine demand in the buffers, sodium hypochlorite was added to form 2 to 3 mg/l Cl solutions. Dechlorination was accomplished by exposure to sunlight for 18 to 24 hours. The buffers were sterilized by autoclaving for 15 min at 15 psi and 121° C. An Orion pH meter with a glass combination electrode was used to determine the pH.

Glassware used in the experiments were made chlorine demand-free by a 24 hour soak in a 3 to 5 mg/l Cl solution, rinses with deionized and chlorine demand-free water, and drying in direct sunlight. Chlorine demand-free water was prepared by combining distilled, deionized water with 1 to 2 mg/l sodium hypochlorite for 24 hours followed by dechlorination in sunlight for 18 to 24 hours.

The variables included in the experiment were disinfectants, temperature, pH, organism, and concentration of the disinfectant. The experiments were run with a split plot design with duplicate trials for each set of conditions. Each trial included two experimental flasks, one with 2.5 mg/l and one with 5.0 mg/l Cl^+ (or the molar equivalent with bromine), and one flask with buffer only.

Bacterial Disinfection Protocol. In each experiment 50 ml of the appropriate buffer was placed in a 125 ml Erlenmeyer flask and covered with a gauze-cotton plug. The flasks were placed in a temperature controlled water bath and agitated at 160 rpm for 15 minutes to allow for temperature equilibration. For the experimental flasks an appropriate aliquot of buffer was removed and replaced with the disinfectant to obtain the desired concentration while maintaining a 50 ml volume. After adding a 0.5 ml aliquot of the bacterial suspension to each flask, the mixture was agitated for 5 minutes to allow for dispersal of bacterial cells. An initial 1 ml sample was collected to obtain a baseline count for each flask. The timed sampling procedure was initated concurrently with the addition of the disinfectant. One ml aliquots were transferred from the flasks to tubes containing 1.0 ml of sterile 0.02 N thiosulfate (buffered to pH 7.0) to quench the disinfectant. After the addition of the disinfectants samples were taken at 0.25, 0.5, 1, 2, 5, 10, 30, 60, 120, and 240 minutes as required to achieve a 6 log (99.9999%) decrease in viable CFU/ml.

To facilitate counting serial dilutions were made from each sample. Spot plates were made on the ATCC recommended agar using three 25 µl aliquots per dilution. The plates were incubated at 37° C with counts made at 24 and 48 hours (96 and 120 hours for *L. pneumophila*) to allow for the growth of injured or weakened cells. Each colony contained in a 25 µl thiosulfate/sample suspension represented 80 CFU/ml in the original reaction flask.

RESULTS AND CONCLUSIONS

The basis of comparison was the time required to observe a 6 log (99.9999%) decrease in viable CFU/ml. Results are presented in Tables 1-3.

Compounds AB and ABC are generally more efficient disinfectants than compound A. Use of a combination of the compounds could yield immediate and long term water disinfection.

Disinfection rates were faster under acidic and alkaline conditions (pH 4.5 and pH 9.5, respectively) than under neutral conditons. The range of effectiveness would allow for use in softener-treated and untreated water.

Disinfection rates were more rapid at 25° and 37° C, temperatures likely to be encountered in distribution systems, than at 4° C.

The higher concentration tested (5.0 mg/l Cl^+ or the molar equivalents with bromine) resulted in slightly faster disinfection than the lower concentration (2.5 mg/l Cl^+ or the molar equivalents with bromine). Both concentrations are within the range commonly used in water disinfection.

The organic N-halamines tested are potentially effective against a broad range of potential pathogens. After further testing to determine the most effective compounds and to increase the toxicity data, these compounds should be effective in water distribution systems, swimming pools, hot tubs, air conditioning cooling towers, and possibly other applications.

ACKNOWLEDGEMENTS

This work was supported through grants by the National Science Foundation and the National Institutes of Health. The contributions of Edward Shaw, John Jones, Greg Sanders, and Tommy Walton are greatly appreciated.

LITERATURE CITED

Barnela, S. D. et al. 1986. Syntheses of new N-halamine water disinfectants. Journal of Phamaceutical Science 76:245-247.

Brodtmann, N. V. and P. J. Russo. 1979. The use of chloramine for reduction of tri-halomethanes and disinfection of drinking water. Journal of the American Water Works Association 71:40-42.

Burkett, H. D. et al. 1981. A novel chlora-mine compound for water disinfection. Water Resources Bulletin 17:874-879.

Elder, E. D. et al. 1986. Bactericidal properties of an organic N-halamine formed *in situ*. Journal of Applied Bacteriology 62:457-462.

Kaminski, J. J. et al. 1976. N-halo derivatives. V. Comparative antimicrobial activity of soft N-chloramine systems. Journal of Pharmaceutical Science 65:1737-1742.

Kosugi, M. et al. 1976. N-halo derivatives. VI. Microbiological and chemical evaluations of 3-chloro-2-oxazolidinones. Journal of Pharmaceutical Science 65:1743-1746.

Mora, E. C. et al. 1982. Properties of a new chloramine disinfectant and detoxicant. Poultry Science 61:1968-1971.

Vogt, C. and S. Regli. 1981. Controlling trihalomethanes while attaining disinfection. Journal of the American Water Works Association 73:33-40.

Williams, D. E. et al. 1985. Bactericidal properties of a new water disinfectant. Applied and Environmental Microbiology 49:637-643.

Worley, S. D. and H. D. Burkett. 1984. The stability in water of a new chloramine disinfectant as a function of pH, temperature, and water quality. Water Resources Bulletin 20:365-368.

Worley, S. D. et al. 1981. A novel bactericidal agent for treatment of water, *IN*: R. L. Jolley et al. (eds). Water Chlorination Environmental Impact and Health Effects, v. 4., pp.1105-1113. Ann Arbor: Ann Arbor Science.

Worley, S. D. et al. 1983a. A new water disinfectant: a comparative study. Industrial and Engineering Chemical Product Research and Development 22:716-718.

Worley, S. D. et al. 1983b. The stability in water of a new chloramine disinfectant. Water Resources Bulletin 19:97-100.

Worley, S. D. et al. 1984a. The tendency of a water disinfectant to produce toxic trihalomethanes. Water Resources Bulletin 20:369-371.

Worley, S. D. et al. 1984b. New disinfection agents for water. Engineering and Services Laboratory, Air Force Engineering and Services Center, ESL-TR-83-68.

Worley, S. D. et al. 1985. Comparisons of a new chloramine compound with free chlorine as disinfectants for water, *IN*: G. E. Janaeur (ed.), Proceedings of the Conference on Chemical Disinfectants, II. SUNY,Binghamton, pp. 45-60.

TABLE 1: EFFICACY OF COMPOUND A

pH	Conc (mg/l)	Temp (oC)	Time (min) Ec	Lp	Pa	Sf
4.5	2.5	4	480	30	60	240
		25	60	10	10	1440
		37	30	0.5	2	240
	5.0	4	240	30	30	240
		25	30	5	5	1440
		37	10	0.5	1	240
7.0	2.5	4	240	120	60	2160
		25	60	30	30	1440
		37	30	10	5	480
	5.0	4	240	120	60	2160
		25	30	30	10	1440
		37	10	5	2	480
9.5	2.5	4	60	60	60	2160
		25	30	5	30	480
		37	10	5	2	120
	5.0	4	30	60	30	1440
		25	10	2	10	240
		37	5	2	2	60

Ec = Escherichia coli, Lp = Legionella pneumophila, Pa = Pseudomonas aeruginosa, Sf = Streptococcus faecalis

TABLE 2: EFFICACY OF COMPOUND AB

pH	Conc (mg/l)	Temp (oC)	Time (min) Ec	Lp	Pa	Sf
4.5	2.5	4	10	5	5	2
		25	2	0.5	2	2
		37	2	0.25	0.25	0.25
	5.0	4	10	2	2	1
		25	2	0.25	1	1
		37	0.25	0.25	0.25	0.25
7.0	2.5	4	30	30	10	60
		25	5	5	10	10
		37	2	1	2	5
	5.0	4	30	10	10	30
		25	5	2	5	5
		37	1	0.5	0.5	2
9.5	2.5	4	10	10	5	10
		25	1	2	1	5
		37	0.5	0.5	1	2
	5.0	4	10	5	5	10
		25	1	1	1	2
		37	0.25	0.25	0.5	1

Ec = Escherichia coli, Lp = Legionella pneumophila, Pa = Pseudomonas aeruginosa, Sf = Streptococcus faecalis

TABLE 3: EFFICACY OF COMPOUND ABC

pH	Conc (mg/l)	Temp (oC)	Time (min) Ec	Lp	Pa	Sf
4.5	2.5	4	10	5	10	10
		25	1	0.5	2	10
		37	1	0.25	1	5
	5.0	4	5	5	5	5
		25	0.5	0.25	1	5
		37	0.5	0.25	0.5	2
7.0	2.5	4	120	30	30	120
		25	10	10	30	30
		37	5	5	5	10
	5.0	4	60	30	30	60
		25	5	5	10	10
		37	2	2	2	5
9.5	2.5	4	5	10	10	10
		25	1	1	2	1
		37	0. 5	0.25	0.5	0.5
	5.0	4	5	10	10	5
		25	0.5	0.5	1	2
		37	0.25	0.25	0.25	0.5

Ec = Escherichia coli, Lp = Legionella pneumophila, Pa = Pseudomonas aeruginosa, Sf = Streptococcus faecalis