A CONTINUED STUDY OF POLYMERIC MATERIALS FOR PROTECTION AGAINST CHEMICAL AND BIOLOGICAL CONTAMINANTS AND HALOGEN OXIDANTS FOR IMMOBILIZATION IN PROTECTIVE MATERIALS AND COATINGS

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This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.
This report highlights major findings of a six-year project exploring preparation and use of N-halamines, a class of compounds that can be employed to prepare polymeric materials and coatings for disinfection and detoxification applications:

- Novel halogenated hydantoinyl polystyrene beads quickly disinfect potable water. The new beads are less expensive to prepare than an earlier version prepared by functionalizing polystyrene, but equally effective in gravity-feed water filters. As mild oxidizing agents, they can also be used to detoxify water and to neutralize HD or VX.
- Several N-halamine coating materials regenerable with hypochlorite were prepared and tested that involve attachment of N-chlorohydantoin moieties to surfaces such as cellulose, polyurethanes, and acrylic paints through siloxane and epoxide tethering groups. 6-7 log kills of S. aureus and E. coli O157:H7 in <10 min contact are typical.
- A new acrylamide monomer capable of loading large amounts of halogen and being copolymerized with a variety of other monomers to create a variety of disinfection applications is described. Coatings so prepared showed considerably better resistance to photodecomposition by sunlight than typical N-halamides.
- The mechanism of photodecomposition of N-halamine siloxanes was thoroughly investigated both theoretically and experimentally, and was shown to occur by a Hoffmann -Loefller rearrangement. This limits the utility of siloxane-tethered N-halamine materials.
- N-Halamine polymers can be made water soluble by copolymerization with materials containing charged groups like quaternary ammonium salts, an important step for industrial coating processes as it avoids the use of organic solvents in coating baths.

15. SUBJECT TERMS

chloramides, decontamination, disinfection, hydantoin, hypochlorite, photodecomposition, synthesis, water purification
# TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................................ ii
LIST OF TABLES .......................................................................................................................... ii
PREFACE ...................................................................................................................................... iii
ACKNOWLEDGEMENTS ........................................................................................................... iv

1. SUMMARY ............................................................................................................................1
2. INTRODUCTION ....................................................................................................................2
3. METHODS AND PROCEDURES .........................................................................................4
4. RESULTS AND DISCUSSION .............................................................................................5
   4.1. Major Findings ................................................................................................................5
   4.1.1. Water Disinfection by \(N\)-Halamine Polymers ...............................................................5
   4.1.2. Development of \(N\)-Halamine Antimicrobial Coatings ...................................................6
   4.1.3. Development of a Novel \(N\)-Halamine Monomer for Antimicrobial Coatings ...............12
   4.1.4. Photolytic Decomposition of \(N\)-Halamine Antimicrobial Coatings ...............................13
   4.1.5. Oxidation of Mustard Simulant .................................................................................16
   4.2. Other Findings ..............................................................................................................16
5. CONCLUSIONS ....................................................................................................................17
6. RECOMMENDATIONS .........................................................................................................18
7. REFERENCES .......................................................................................................................19

Appendix: Publications and Presentations Referencing F08637-02-C-7020 and FA8650-07-1-5908 ..........................................................................................................................21

LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS ..................................................29
LIST OF FIGURES

Figure 1. A Disinfecting $N$-Halamine Polymer for Water Disinfection and Detoxification ........ 3
Figure 2. An Improved Disinfecting $N$-Halamine Polymer for Water Disinfection and Detoxification ............................................................................................................... 6
Figure 3. Concept for Preparation of an Antimicrobial Polyurethane ...................................... 7
Figure 4. Preparation of an Antimicrobial $N$-Halamine Diol ................................................ 7
Figure 5. Preparation of an $N$-Halamine Precursor Silane and Polymeric Siloxane ............... 8
Figure 6. Preparation from a Precursor of a Water-soluble $N$-Halamine Copolymer ............. 10
Figure 7. Synthesis of an $N$-Halamine Epoxide Monomer and Formation of Antimicrobial Cellulose ..................................................................................................................... 11
Figure 8. Preparation of a Precursor Epoxide Copolymer ........................................................ 11
Figure 9. An Antimicrobial $N$-Halamine Epoxide Polymer Coating ..................................... 12
Figure 10. Preparation of a Novel Hydantoinyl Acrylamide Monomer .................................... 13
Figure 11. Antimicrobial Paint ($m = 7; n = 3$) ..................................................................... 13
Figure 12. Intramolecular Photorearrangement of Acyclic $N$-Halamides (1,5-Hydrogen Atom Transfer) ...................................................................................................................... 15
Figure 13. Structures of Synthesized Siloxane Coating Materials and Model Compounds ...... 15
Figure 14. Possible Photolytic Rearrangements for 3-Butyl-1-chlorohydantoins ....................... 15
Figure 15. Proposed Sequence for Decomposition of an $N$-Chlorohydantoinylsiloxane ........ 16

LIST OF TABLES

Table 1. Stability Toward UVA Light of HASA on a Polyester Transparency Slide ................. 14
PREFACE

This document represents the final report for a decade of work at Auburn University on contracts FO8637-02-C-7020 and FA8650-07-5908 sponsored by the Air Force Research Laboratory, (AFRL) at Tyndall Air Force Base (AFB). The technical managers at Tyndall AFB were Drs. Joseph Wander and Jeffery Owens. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily the official policies or endorsements, either expressed or implied, of AFRL or the U. S. Government.
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1. SUMMARY

The work discussed in this final report and in the publications, patents, and presentations cited in the Appendix concerns the use of a class of compounds known as \( N \)-halamines, which can be employed to prepare polymeric materials and coatings for use in disinfection and detoxification applications.

The report highlights the major findings of the work, but details of the work have been discussed extensively in the publications cited in the Appendix. A major finding was that novel halogenated hydantoinyl polystyrene beads can be used to disinfect potable water. The beads discussed are an improvement over prior ones developed in these laboratories in that they are less expensive to prepare than were those developed herein before the start of the project; yet they function equally well in their application for gravity-feed water filters. Because they are mild oxidizing agents, they can also be employed in water detoxification applications.

Several \( N \)-halamine coating materials are discussed. All of these involve attachment of \( N \)-chlorohydantoin moieties to surfaces such as cellulose, polyurethanes, and acrylic paints through tethering groups such as siloxanes and epoxides. The materials generally provide 6–7 log disinfections of \( Staphylococcus aureus \) and \( Escherichia coli \) O157:H7 within 5–10 min of contact. Furthermore, they are capable of recharge by simply exposing them to dilute aqueous household bleach.

A new acrylamide monomer capable of loading large amounts of halogen and being copolymerized with a variety of other monomers to create a variety of disinfection applications is described.

Although \( N \)-halamines are somewhat subject to photolytic decomposition in the presence of direct sunlight or ultraviolet radiation, an \( N \)-halamine copolymer utilizing the acrylamide monomer exhibited minimal decomposition in an acrylic paint application.

The mechanism of photodecomposition of \( N \)-halamine siloxanes has been thoroughly investigated experimentally and with theoretical computations. Our evidence shows that it follows a well-known photorearrangement process known as a Hoffmann–Loeffler rearrangement. This suggests that tethering groups other than siloxanes are probably preferable for use with \( N \)-halamine materials.

It was shown that \( N \)-halamine polymers can be made water soluble by use of copolymers containing charged groups like quaternary ammonium salts of alkali sulfonates. This was an important finding because, for industrial coating processes, one prefers to avoid organic solvents in coating baths.

The \( N \)-halamines employed in these laboratories are less active as oxidizers than other halogenating compounds. They convert organic sulfides into less-toxic sulfoxides rather than to more-toxic sulfones. This finding is important for the detoxification of chemical agents, particularly mustard agent.
2. INTRODUCTION

Work concerning antimicrobial materials has progressed in the laboratories of the principal investigator S. D. Worley and his collaborators for over three decades. Most of this work has focused upon a class of chemical compounds termed \( N \)-halamines or, more precisely, \( N \)-halamides. All compounds developed at Auburn University have been organic and generally heterocyclic, with nitrogen atoms that could be reacted with free chlorine or free bromine to form nitrogen–halogen moieties. In these types of molecules the N–Cl or N–Br bond is polarized such that the halogen is in a +1 oxidation state. Hence the compounds would be expected to (and indeed do) inactivate pathogenic microorganisms and viruses. The compounds studied at Auburn University are generally mild oxidizing agents, which could potentially deactivate chemical agents as well as inactivate pathogens.

The first decade of research at Auburn University focused upon small, water-soluble, monomeric \( N \)-halamine compounds. The thrust of the research was to develop compounds that were very stable in aqueous solution over time, i.e., those with very low hydrolysis constants (e.g., less than \( 10^{-11} \)), which released negligible amounts of corrosive free halogen into their water solutions. It was found that derivatization of the \( N \)-halamine heterocyclic structure adjacent to the N–X moiety with alkyl groups (generally methyl groups) considerably enhanced the stability of the N–X bond toward release of free halogen. There are at least three reasons for this. One, alkyl groups are electron donors which push electron density toward the developing negative charge on the nitrogen as the positive halogen atom is released. This is a destabilizing interaction which causes a strengthening of the N–X bond. Second, the alkyl groups in close proximity to the N–X moiety sterically hinder the approach of solvent water molecules. And third, and probably most important, two alkyl groups adjacent to the N–X moiety prevent all possibilities of a dehydrohalogenation reaction, i.e., loss of HX, which would consume the oxidative halogen and destroy any chance of an antimicrobial event. Several such monomeric \( N \)-halamine compounds were developed at Auburn University which were very stable in water, killed various species of bacteria, and, in fact, detoxified toxic mustard agent.\(^1\) The mechanism of action of the \( N \)-halamine compounds was thought to be a direct transfer of the oxidative halogen atom to a receptor site in a cell, followed by inactivation by oxidation, as would be the case for free halogen, e.g., hypochlorite in household bleach.

In about 1990, we realized that commercialization of the water soluble \( N \)-halamine materials was problematic, since numerous expensive toxicity tests would be necessary to secure governmental regulatory approval, as disinfected water would indeed contain the soluble compounds. Yet, development of new disinfecting materials for potable water was the primary goal in these laboratories at the time given that water-borne disease was (and still is) a principal cause of mortality in developing nations. Thus the decade of the ‘90s was devoted to creating an insoluble \( N \)-halamine material that could be employed in water-disinfecting filtration applications. The obvious choice was an insoluble \( N \)-halamine polymer. This part of the work was a tremendous success, as we were able to derivatize polystyrene with a hydantoin moiety that could be reacted with solutions containing chlorine or bromine as shown in Figure 1 and thus rendered antimicrobial.
Extensive testing of filters containing the new material (in either bead form or as an amorphous solid) showed that it could kill all bacterial species and viruses tested in a few seconds of contact time in a gravity feed application.\textsuperscript{2,3} Once the halogen was exhausted through antimicrobial events or reaction with impurities and reducing agents in the water, the polymer could be replenished by merely exposing it to a solution of free halogen. The material is currently being marketed by HaloSource, Inc., in disinfecting gravity-feed water filtration devices in several developing nations.

Once it was evident that an $N$-halamine polymer could provide useful disinfecting applications for water, we began to explore the possibility of extending the work to polymer coatings of various materials for which a disinfection or detoxification event would add value. The new applications—all of which should be of use to the military—then became the focus for the work discussed in this final report.
3. METHODS AND PROCEDURES

In this project the synthetic methods employed were all straightforward and will be elaborated upon where necessary in the next section. Characterization of the various materials developed was generally performed by $^1$H and $^{13}$C nuclear magnetic resonance (NMR) using a Bruker 400-MHz spectrometer, Fourier-transform infrared spectroscopy (FTIR) using a Nicolet 6700 FTIR spectrometer with an attenuated total reflectance accessory, thermal analyses using TA Instruments Q500 and Q2000, and scanning electron microscopy (SEM) using a Zeiss DSM 940. Coatings on materials, e.g., cotton, were commonly created from synthesized polymers by dipping the material in a solution of the polymer—in some cases in water, in others cases in an organic solvent—for a prescribed amount of time, removing the wet material, drying at ambient temperature, curing at elevated temperature, rinsing with tap water, drying in air, and then further treatment with aqueous bleach (or not for controls). Halogenation was commonly effected by soaking the cured, coated material in 10 % by weight aqueous household bleach in water at a pH controlled at 7–10, or in a dilute solution of bromine. The loading of the halogen on the coatings was determined using iodometric/thiosulfate titration. Weight percent Cl$^+$ on the samples was calculated using Equation 1 below:

$$\text{wt}-\% \, \text{Cl}^+ = \left(35.45 \frac{N \cdot V}{W} \right) \times 100/(2 \, W)$$

(1)

where $N$ and $V$ are the normality (equiv/L) and volume (L) of the sodium thiosulfate titrant, and $W$ is the weight of the sample (g). For wt-% Br$^+$ 79.90 replaces 35.45 in Equation (1). Typically, for cellulose as an example, $W$ is 0.1–0.15 g, $N$ is 0.00375 equiv/L, and $V$ is of the order of 5 mL. For nonporous samples the halogen loadings were determined as Cl$^+$ or Br$^+$ atoms per unit surface area.

Stability testing for the halogen on the coatings was generally conducted by three means: with light exclusion, under laboratory lighting, and in an accelerated weathering tester (Q-Panel Co., Cleveland, OH). The latter testing was done to evaluate stability of the halogen in the presence of UVA photons at controlled temperature (37.6 °C) and humidity (17 %).

For microbiology testing, generally Gram-positive $S$. aureus (ATCC 6538) and Gram-negative $E$. coli $O157:H7$ (ATCC 43895) were employed as pathogens. A “sandwich test” was used for fabrics, in which 25 µL of a known concentration ($10^6$–$10^7$ CFU) of bacteria was placed in the center of a small swatch, typically $2.54 \times 2.54$ cm, and an identical swatch was placed on top. After a given contact time under a sterile weight employed to hold the swatches in close contact with the bacteria, the swatches were placed into a sterile tube containing 0.02 N sodium thiosulfate to quench any further disinfectant action, and the mixture was vortexed to remove the bacterial cells. Then serial dilutions were prepared using 100 mM phosphate buffer solution (pH 7) and plated onto trypticase soy agar plates, which were incubated at 37 °C for 24 h, and the viable bacteria were counted for antimicrobial assessment. Nonporous solid samples were handled in a similar manner.
4. RESULTS AND DISCUSSION

4.1. Major Findings

4.1.1. Water Disinfection by *N*-Halamine Polymers

Refinements to the hydantoinyl polystyrene bead system were made in hopes that a disinfecting, detoxifying potable water filter could be developed for use by the military in remote locations. It was found that the weight percent loading of halogen was very dependent upon the pH at which the beads were treated with halogen.\(^3\)\(^4\) For example, it was found that a dichlorohydantoinyl polystyrene bead containing about 20 wt-% chlorine could be formed by chlorination at pH lower than 8.0. However, beads with this high a chlorine loading can be hazardous in terms of flammability in contact with moisture upon storage. If the chlorination was performed between pH 8–8.5, the chlorine loading was 14–18 wt-%, which is a safer loading for use, although these beads should be kept dry in storage. Beads chlorinated with sodium hypochlorite at a pH above 8.8 were monosubstituted with about 13 wt-% chlorine content. The FTIR spectrum of these beads (prominent band at 1602 cm\(^{-1}\)) showed them to be the sodium salt (at the imide nitrogen) with the chlorine bonded to the amide nitrogen, the thermodynamically more stable position. These beads were hygroscopic, but could be treated with dilute acid to protonate the imide nitrogen, which rendered them easier to maintain dry. Dibromo and monobromo beads were prepared by similar pH-controlled methods. This was significant because the monobromo beads were shown to be as effective antimicrobials as the dichloro beads. It was demonstrated in the 1980s in these laboratories that bromine is about 50 times more effective in a biocidal event than is chlorine.\(^1\) In practice, the monobromo beads are the form that has been commercialized by HaloSource, Inc., and is being marketed in developing nations. The beads elute only 0.1–0.2 mg/L of free halogen into flowing water—well below the U.S. Environmental Protection Agency standard requirement for safe drinking water. The antimicrobial event occurs within the bead bed itself through a direct contact mechanism that transfers oxidative halogen to pathogenic cells.

It was recognized that the polymer beads illustrated in Figure 1, albeit very effective in an antimicrobial potable water application, are fairly expensive to produce, primarily because of the use of cyanide in the synthetic process. Thus considerable effort was expended to develop an alternate polymer that worked as well in an antimicrobial application, but avoided the use of cyanide in the industrial synthesis process. The result was the development of a hydantoinyl polystyrene bead material utilizing the reaction scheme shown in Figure 2.\(^5\)\(^6\) In this case commercial chloromethylated polystyrene beads (Merrifield resin) were reacted with the potassium salt of 5,5-dimethylhydantoin.

Because the Friedel–Crafts acylation reaction and the use of cyanide (Figure 1) are avoided for the synthesis of this polymer, the expense of the industrial process is reduced by about two-thirds. The performances of the two types of halogenated hydantoinyl polystyrene beads in antimicrobial events are equivalent, although the new polymer does liberate slightly more free halogen. HaloSource, Inc., is scaling up the new process for commercialization in gravity-feed water filtration devices. During the course of the research the analogous polymeric quaternary ammonium salt was produced by reacting the Merrifield resin beads with methyldecylamine. Whereas complete (5–6 log) reductions of *S. aureus* and *E. coli* were obtained within 1–2 s for the *N*-chloramine functionalized beads, only ~1.5-log kill of *S. aureus* and almost no attenuation...
Figure 2. An Improved Disinfecting N-Halamine Polymer for Water Disinfection and Detoxification

of *E. coli* were observed after 5 s of contact time in a flowing water experiment for the quaternary-ammonium-functionalized derivative. This illustrated the superior antimicrobial performance of an N-halamine polymer compared to an analogous quaternary ammonium polymer.

4.1.2. Development of N-Halamine Antimicrobial Coatings

Given the success experienced in N-halamine antimicrobial polymers for potable water disinfection, we decided to attempt to extend the technology to the preparation of N-halamine antimicrobial coatings for a variety of surface applications.

4.1.2.1. Polyurethane Coatings

The first successful effort involved preparation of an N-halamine monomer that could be formulated into a commercial, water-borne, acrylic polyol, thus producing a polyurethane for use in paints that, upon treatment with dilute bleach, became antimicrobial. The general concept for
The synthesis involved reaction of commercial 3-hydroxymethyl-5,5-dimethylhydantoin with diethanolamine in methanol solvent at 75 °C. The viscous residue produced after removal of the methanol was dissolved in ethyl acetate and precipitated as a white solid at 4 °C. The solid was stirred into the water-borne acrylic at a weight ratio of 0.7 g to 10 g. The formulation was spread onto various surfaces and allowed to dry. Chlorination with a 10 % aqueous household bleach solution (about 0.5 % sodium hypochlorite) provided a Cl$^+$ loading of about $1.3 \times 10^{17}$ atoms/cm$^2$. This chlorinated surface achieved a complete reduction (>4.5 logs) of S. aureus over a 2-h contact period. Scientists at Tyndall AFB performed an experiment with the material in which they painted portions of a restroom stall door, chlorinated portions leaving other portions as unchlorinated controls, and three months later challenged the surfaces with about $10^6$ CFU of Pseudomonas pseudoalcaligenes. After a 5-min contact time, the bacteria on the chlorinated surfaces were completely inactivated. The experiment was repeated after six months (without rechlorination) with the same result. In another experiment scientists at the Center for Biofilm...
Engineering at Montana State University coated polycarbonate slides with our material and tested them (chlorinated versus unchlorinated) in a biofilm reactor. With about 1 mg/L free chlorine present, the slides containing the chlorinated polyurethane coating yielded 1–2 logs fewer biofilm organisms than did slides not containing the coating.

4.1.2.2. Siloxane Coatings

We have found that N-halamine siloxanes are very versatile building blocks for antimicrobial surface coatings. Alkoxysilanes, upon conversion into hydroxysilanes in the presence of moisture and traces of acid, can react with any surface containing oxide or –OH moieties, e.g., cellulose; they also undergo facile homopolymerization and co-polymerization with other monomers. Thus they are logical candidate antimicrobial coatings. Our first effort to exploit these materials involved the reaction of the potassium salt of 5,5-dimethylhydantoin with 3-chloropropyltriethoxysilane in dimethyl formamide at 60–90 °C using the procedure of Berger. The monomeric product, 3-triethoxysilylpropyl-5,5-dimethylhydantoin (Figure 5), has often been nicknamed BA-1 in these laboratories and elsewhere. The monomer can be homopolymerized as shown in Figure 5. Alternatively, the starting material 3-chloropropyltriethoxysilane can be homopolymerized in the presence of HCl, followed by a nucleophilic substitution reaction using the potassium salt of 5,5-dimethylhydantoin. The monomer BA-1 and the two versions of polymer can all be reacted with surfaces such as cellulose to produce N-halamine precursor structures, which, upon exposure to dilute aqueous bleach, become antimicrobial.

Figure 5. Preparation of an N-Halamine Precursor Silane and Polymeric Siloxane
BA-1 or its polymers could be dissolved in ethanol/water mixtures (50/50) and then applied to surfaces such as cotton fabric by dipping the fabric into a bath of the dissolved material. Alternatively, the material in solution could be sprayed onto the surface of the substrate. The treated surfaces were then cured at elevated temperature, e.g., 130–150 °C. Halogenation then was accomplished at ambient temperature followed by rinsing and then drying at a temperature no higher than 45 °C.

Office paper treated with chlorinated BA-1 (0.5–0.8 wt-% Cl⁺) completely inactivated *S. aureus* (>5.4 logs) within 10 min of contact. The treated paper lost only 5 % of its chlorine upon storage under ambient conditions during a 36-d period. *S. aureus* was inactivated (>5.7 logs) on treated cotton fabric (at a chlorine loading of about 0.4 wt-%) in the contact time interval 10–30 min; for *E. coli* O157:H7 the time interval for equivalent performance was about 60 min. Testing in a Launder-Ometer showed that the coatings, especially the polymeric ones, were fairly stable to repeated washing cycles. For example, a polymer-coated sample chlorinated before and after washing lost only about 42 % of its coating during 50 washing cycles; in contrast, a monomer-coated sample treated identically lost about 64 % of its coating, implying that the polymer is bound more strongly to the cotton than is the monomer. It was also shown that the BA-1 technology could be utilized with silicon dioxide and silica gel to produce antimicrobial sand and silica gel antimicrobial water filters, albeit chlorine loadings are much lower (0.3–2.0 wt-% Cl⁺) leading to considerably longer contact times for flowing water (min) to achieve antimicrobial activity than was the case for the hydantoinyl polystyrene beads (~1 s) discussed above.¹⁰,¹¹

A possible limitation of the BA-1 technology is the fact that its utilization requires a solvent containing alcohol, which can cause problems in industrial settings. Thus, an effort was made to modify the compound as to render it soluble in water. The successful reaction scheme is shown in Figure 6. The homopolymer of 3-chloropropylsilane was synthesized as shown in Figure 6. It was then reacted with the potassium salt of 5,5-dimethylhydantoin and trimethylamine, either sequentially or in one pot, to produce the hydantoin/quaternary ammonium copolymer. The values of *n* and *m* in Figure 6 were easily controlled by setting the feed ratios. It was found that the copolymer that contained the largest proportion of *N*-halamine precursor for disinfection, together with an adequate proportion of quaternary ammonium groups to achieve water solubility for practical uses, had values of *n* and *m* of 9 and 1, respectively.¹² This copolymer could be loaded onto cotton with 0.69 wt-% Cl⁺ from a 5 wt-% coating solution. Its performance when bonded to cotton was very good, providing a 7-log reduction of both *S. aureus* and *E. coli* O157:H7 after a contact time interval of 1–5 min. Its stability on the surface of the cotton to washing was similar to that for the BA-1 homopolymer.¹²
4.1.2.3. Epoxide Coatings

Another logical path for producing an \( N \)-halamine functionalized surface, particularly on cellulose, is the use of epoxide derivatives. Our first entry into this area is illustrated by the reaction scheme in Figure 7.13 First, 5,5-dimethylhydantoin was converted into its potassium salt by stirring in an aqueous solution of KOH. Then, an equimolar concentration of commercial epichlorohydrin was added to the solution followed by simply stirring at ambient temperature for 10 h. After removal of most of the water by evaporation, the product was extracted from the remainder of the water with acetone. After evaporation of the acetone, the hydantoinyl epoxide was obtained as an oil.

Extensive purification of the product yielded a white solid. However, the oil could be used for coating, thus eliminating the need of a tedious chromatographic purification step. For treatment of cotton, an aqueous coating bath containing 5 wt-% of the epoxide was prepared. Cotton swatches were held in the bath for 15 min, followed by drying at 95 °C for 1 h, and curing at 145 °C for 20 min. After rinsing, the swatches were chlorinated with 10 % aqueous bleach at pH 7 and ambient temperature for 45 min. Drying at 45 °C was performed to remove any occluded free chlorine. Testing included stability in storage of the treatment solution and stability toward...
washing and antimicrobial efficacy for the coated swatches. The treatment solution performed the same, i.e., led to the same chlorine loadings on the swatches, after 30 d as on day 1. Washing stability for the coatings was excellent, as the same chlorine loadings could be obtained after 50 washing cycles as were obtained before washing. Complete inactivation of \textit{S. aureus} and \textit{E. coli} O157:H7 occurred within 10–30 min contact time.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7}
\caption{Synthesis of an \textit{N}-Halamine Epoxide Monomer and Formation of Antimicrobial Cellulose}
\end{figure}

In general, polymers provide more-stable coatings than do monomers. A polymeric \textit{N}-halamine epoxide that was developed during this project is shown in Figure 8 and Figure 9.\textsuperscript{14}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{Preparation of a Precursor Epoxide Copolymer}
\end{figure}
A commercial monomer (3-chloro-2-hydroxypropyl methacrylate, CM) was copolymerized with glycidyl methacrylate (GM) in methanol at 65 °C for 2 h. The product copolymer in acetone was padded onto cotton using a laboratory wringer, and then was cured at 165 °C for 1 h. The coated cotton was then immersed in a solution of the potassium salt of 5,5-dimethylhydantoin in ethanol for 5 min under reflux. After chlorination at pH 7 for 1 h, rinsing in water, and drying at 45 °C for 1 h, stability toward washing and exposure to UVA light and antimicrobial efficacy were tested. The coated material showed remarkable stability toward washing; no coating was lost in 50 machine washing cycles, i.e., the same chlorine loading was obtained at the end as at the beginning. Although the samples lost most of their chlorine content over a period of 120 h of exposure to UVA, 94 % of the chlorine could be recovered after the exposure upon rechlorination. Complete inactivation of both S. aureus and E. coli O157:H7 (6.75 logs) were obtained within 5 min of contact.

4.1.3. Development of a Novel N-Halamine Monomer for Antimicrobial Coatings

Recent work in these laboratories focused on a new hydantoin acrylamide monomer (Figure 10), which can copolymerize with a variety of other monomers for use in novel applications. This monomer, which contains three nitrogen atoms that can be halogenated—none of which is subject to a dehydrohalogenation process—was prepared by reacting commercial N-(1,1-dimethyl-3-oxobutyl)acrylamide with KCN and ammonium carbonate in ethanol–water (1:1 by volume) solvent. The white solid product was formed with the vinyl group intact (proof by NMR and FTIR) and thus could be copolymerized with other monomers useful for tethering to surfaces such as siloxanes and epoxides. Of particular interest to us was copolymerization with a water-soluble acrylamide for use in a latex paint (Figure 11). Different feed ratios (m and n in Figure 11) were used. Optimum values of m and n proved to be 7 and 3, respectively. This formulation was soluble in water and thus provided excellent dispersion in a commercial latex paint. The treated paint was spread on a polyester transparency slide surface and allowed to dry. Chlorination was effected with dilute bleach (10 wt-% in aqueous solution). Chlorine loadings of the order of 10¹⁷ chlorine atoms/cm² were obtained. The painted surface provided > 6-log inactivation of both S. aureus and E. coli O157:H7 within 5 min contact. In contrast, unchlorinated control coatings provided less than 0.2-log inactivations at the same contact time.
4.1.4. Photolytic Decomposition of N-Halamine Antimicrobial Coatings

The primary limitation of N-halamine coatings and materials is slow decomposition in the presence of ultraviolet photons. This, of course, limits their use in direct sunlight. Not only is the nitrogen–halogen bond sensitive to dissociation accelerated by UV photons, most of the materials themselves decompose slowly over time of exposure. This can be shown by repeated UV exposure and N-halamine regeneration cycles, as the halogen loadings slowly decrease with
the cycling. The mechanism of this decomposition process seems to be dependent upon the tethering group, e.g., siloxane, epoxide, etc. One of the materials studied in these laboratories that showed better stability toward UV decomposition is the paint labeled HASA in Figure 11. Its performance during repeated UVA–rechlorination cycles is shown in Table 1. This could be because the paint matrix partially protects the N-halamine from decomposition. However, some of the materials such as the siloxanes are less stable toward UV exposure. For example, BA-1 loses all of its chlorine within 24 h exposure to UVA photons and can be only partially regenerated (to about 35 % of its initial value) at that time.19 We decided to perform an experimental/theoretical study of this decomposition process.

Table 1. Stability Toward UVA Light of HASA on a Polyester Transparency Slide

<table>
<thead>
<tr>
<th>Exposure Time (d)</th>
<th>% Cl(^+) Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.30</td>
</tr>
<tr>
<td>1</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>0.19</td>
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<tr>
<td>3</td>
<td>0.18</td>
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<tr>
<td>4</td>
<td>0.18</td>
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<tr>
<td>5</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>0.13</td>
</tr>
<tr>
<td>7</td>
<td>0.13</td>
</tr>
<tr>
<td>14</td>
<td>0.12</td>
</tr>
<tr>
<td>Rechlorination</td>
<td>0.28</td>
</tr>
<tr>
<td>28</td>
<td>0.16</td>
</tr>
<tr>
<td>Rechlorination</td>
<td>0.30</td>
</tr>
<tr>
<td>42</td>
<td>0.16</td>
</tr>
<tr>
<td>Rechlorination</td>
<td>0.27</td>
</tr>
<tr>
<td>70</td>
<td>0.11</td>
</tr>
<tr>
<td>Rechlorination</td>
<td>0.26</td>
</tr>
</tbody>
</table>

N-Halamine precursor coatings exhibit little or no decomposition upon exposure to UV light, so obviously the presence of halogen accentuates the process. A search of the literature revealed that acyclic N-halamides are subject to an intramolecular photorearrangement process known as the Hoffmann–Loeffler rearrangement (Figure 12).20 We thought that the cyclic N-halamide BA-1 might undergo a similar photorearrangement. Thus we initiated a model compound study using mass spectrometry to identify products produced under UV photolysis and Density Functional Theory \textit{ab initio} computations.21 The structures in the study are shown in Figure 13. We found that compounds identified in Figure 14 as A and B were isolated in the model compound study (mass spectral identification), and the computations showed the transition states represented by 1,5- or 1,6- hydrogen transfer to be energetically favorable. Thus it is likely that under UV photolysis the N–Cl bond breaks in a radical process, followed by a hydrogen atom migration from C\(_7\) and/or C\(_8\) (Figure 15). Subsequent recombination of the radical centers with chlorine...
atoms would leave the products illustrated in Figure 14. Thus for the BA-1-derived siloxane the chlorine atom would migrate to positions either $\alpha$ or $\beta$ relative to the silicon atom. It is well known that when halogen atoms are bonded to carbon atoms in $\alpha$ or $\beta$ positions relative to a silicon atom, the C–Si bond is notoriously susceptible to bond scission.\textsuperscript{22} Thus, the hydantoin group would be lost before and during an attempted rechlorination, accompanied by loss of antimicrobial efficacy as illustrated in Figure 15.

**Figure 12. Intramolecular Photorearrangement of Acyclic $N$-Halamides (1,5-Hydrogen Atom Transfer)**

**Figure 13. Structures of Synthesized Siloxane Coating Materials and Model Compounds**

**Figure 14. Possible Photolytic Rearrangements for 3-Butyl-1-chlorohydantoins**
4.1.5. Oxidation of Mustard Simulant
It was shown that N-halamine compounds, such as the chlorinated polystyrene hydantoin beads, oxidize organic sulfides, such as mustard simulant, to the less toxic sulfoxides rather than to the more toxic sulfones, as other halogenating agents, such as free chlorine, do. The various N-halamine coatings discussed in this report should do the same.

4.2. Other Findings
This report has emphasized certain major findings during the project. However, much other work was accomplished during the 10-year period including both experimental and theoretical studies. All of the studies have been published, and full citations are given in the Appendix.
5. CONCLUSIONS

A massive amount of work has been performed on the various projects supported by contract F08637-02-C-7020 and grant FA8650-07-1-5908. The work has demonstrated that \( N \)-halamine chemistry can be of great benefit to the military. It has been shown that the \( N \)-halamine technology can be employed for disinfection of potable water, and because the materials are oxidizing agents, they can also be used for detoxification of contaminated water. Much of the work on the project has focused on antimicrobial coatings for such surfaces as cellulose, but paints and fabrics such as polyester can also be rendered antimicrobial by the \( N \)-halamine materials. Various polymerization techniques such as admicellar polymerization and layer-by-layer deposition of \( N \)-halamine materials have also been addressed in publications cited in the Appendix. Likewise, theoretical studies have been conducted to support the experimental work and rationalize observations. Sixty-one publications/patents and numerous presentations at national and international meetings have highlighted the work and are referenced in the Appendix. Furthermore, the work has served as a training ground for several post-doctoral fellows and graduate students at Auburn University. Several of the materials developed during the study such as BA-1, the antimicrobial polyurethane, and some alkylated hydantoin compounds have been supplied to the U.S. Air Force for testing and evaluation.
6. RECOMMENDATIONS

We recommend that the military seriously consider adaptation of the halogenated hydantoinyl polystyrene beads for disinfection and detoxification of potable water in remote locations. The beads have been proven to work well in developing nations by HaloSource, Inc., the company which markets them. They would be far superior to the methods currently used by the military in remote locations. Also, work on actual applications of the N-halamine coatings should be pursued by the U.S. military services with the intent of adopting them for real field use. At one time the BA-1 technology seemed to be moving toward adoption by the military. We do not know where that stands at this time, but during the project we have developed N-halamine coatings superior to BA-1 in terms of photostability, washing fastness, and antimicrobial efficacy.
7. REFERENCES


Appendix: Publications and Presentations Referencing F08637-02-C-7020 and FA8650-07-1-5908

Publications


Patents


Presentations


### LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AFRL</td>
<td>Air Force Research Laboratory</td>
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<tr>
<td>AIBN</td>
<td>azobis(isobutyronitrile)</td>
</tr>
<tr>
<td>aq</td>
<td>aqueous</td>
</tr>
<tr>
<td>BA-1</td>
<td>3-triethoxysilylpropyl-5,5-dimethylhydantoin</td>
</tr>
<tr>
<td>cell</td>
<td>cellulose (cotton)</td>
</tr>
<tr>
<td>CM</td>
<td>commercial monomer, 3-chloro-2-hydroxypropyl methacrylate</td>
</tr>
<tr>
<td>cm</td>
<td>centimeters</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>wavenumbers</td>
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<td>days</td>
</tr>
<tr>
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<td>equivalents</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier-transform infrared spectrometry</td>
</tr>
<tr>
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<td>GM</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance spectrometry</td>
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<td>SEM</td>
<td>scanning electron microscopy</td>
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<tr>
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</tr>
<tr>
<td>UVA</td>
<td>lowest-energy UV light</td>
</tr>
<tr>
<td>µL</td>
<td>microliters</td>
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