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Antimicrobial regulatory efficacy testing of solid copper alloy surfaces in the USA

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ABSTRACT

Recent laboratory studies show that Gram-positive and Gram-negative pathogenic bacteria die when they come into contact with solid copper alloy surfaces at ambient temperature. These results suggest that the antimicrobial effectiveness of copper alloys, which is attributed to the formation of copper ions on alloy surfaces, can assist in reducing the transmission of infectious disease-causing organisms. However, in the United States (US), the approval of the US Environmental Protection Agency (EPA) is required before making antimicrobial claims related to public health. The efficacy testing required by the EPA is described and the test results are summarized. It is anticipated that once regulatory approval is obtained, components made from copper alloy surfaces will be introduced into hospitals, nursing homes and other healthcare facilities, schools, public buildings and onto other highly touched surfaces in public spaces.

Key Words: Copper, nosocomial infections, hospital-acquired infections, Methicillin-Resistant *Staphylococcus aureus*, *Escherichia coli* O157:H7, copper alloys, brass, bronze

INTRODUCTION

Man has exploited the antimicrobial attributes of copper before the nineteenth century, when Louis Pasteur developed his germ theory of disease in which infections are attributed to microbes invading the human body. The Hippocrates Collection, 460 to 380 B.C., to which the father of medicine contributed, recommends the use of copper for leg ulcers related to varicose veins. Pliny, 23 to 79 A.D., used copper oxide with honey to treat intestinal worms. The Aztecs gargled with a mixture containing copper to treat sore throats. In a laboratory study (1), water inoculated with a fecal indicator bacterium, *Escherichia coli*, was stored in water vessels made of brass, a copper alloy, as well as in earthenware vessels. The vessels contained either distilled water or natural water from the Punjab in rural India. No live bacteria were found in the brass vessels after 48 hours. In an earlier study (2) carried out in a hospital, brass doorknobs showed sparse growth of pathogenic bacteria, while stainless steel doorknobs were heavily contaminated.

Previously published results (3-11) show that several bacterial species die on copper alloy surfaces in a matter of minutes to hours, and that Influenza A virus particles become inactivated on copper surfaces (12). This paper discusses tests that were conducted on a range of commercial copper alloys. The objective was to obtain regulatory approval, which will permit public health claims to be made about copper alloys. The tested organisms include *E. coli* O157:H7, a food-borne pathogen associated with several large-scale food recalls, and Methicillin-Resistant *Staphylococcus aureus* (MRSA), a serious hospital-acquired, or nosocomial infection. According to the March 28, 2001 issue of *The New York Times*, 76 million illnesses associated with contaminated food were reported annually in the United States, which resulted in 325,000 hospitalizations and 5,000 deaths. Although most *E. coli* strains are harmless to humans, the US Dept. of Agriculture (USDA) estimates that the cost associated with infectious strains of *E. coli* is US $5 billion annually. In the fall of 2007, *E. coli* O157:H7 contamination resulted in the recall of 9.84 kilotonnes of hamburger meat. Although *E. coli* O157:H7 is commonly associated with processed meat, the recall of spinach during the fall of 2006 indicates that is also a concern in packaged fresh vegetables. According to a July 2004 report by the Infectious Disease Society of America, two million people are infected each year while in the hospital, and 70% of these hospital-acquired or nosocomial infections are resistant to at least one drug. This results in 90,000 deaths and a cost of $5 billion annually. *The New York Times* (July 27, 2007) stated that the US Centers for Disease Control projects the number of deaths to be 99,000 in 2007 and officials estimated that the cost of treatment may approach 1% of the total national cost of healthcare, or $20 billion. In order to be legally permitted to make antimicrobial claims in the United States, products must be approved and registered by the US Environmental Protection Agency (EPA). Antimicrobial claims fall under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). The EPA only requires efficacy testing when registrants wish to make public health claims. Some products, such as the commercially available silver ion-containing coatings, are registered under a special provision in FIFRA, the "treated article exemption." This provision indicates that the antimicrobial ingredient only protects the article containing the ingredient. EPA grants the "treated article exemption" only for non-public health uses of a pesticide that is intended only to protect or preserve the treated article itself. One example of a "treated article exemption" is the addition of a fungicide to paint to prevent mildew. Thus, the fungicide is protecting the paint, and not public health. Therefore, products registered under the "treated article exemption" cannot legally make public health claims. At present, only antimicrobial gases and liquids, such as sterilizers, disinfectants and sanitizers, can make public health claims under FIFRA.

The results presented represent a summary of the required EPA-approved efficacy tests. They were submitted along with the other required information, in pursuit of FIFRA registration. When registration is granted, antimicrobial copper alloys will be the first solid materials legally permitted to make public health claims.

MATERIALS AND METHODS

Table I: Nominal Alloy Composition (Weight %)

Alloy UNS Number	Cu	Zn	Sn	Ni	Fe	Cr	P
C11000: Copper:	99.90						
C26000: Brass	70	30					
C51000: Bronze	95		5				0.2
C70600: Cu-Ni	90			10			
C75200: Cu-Ni-Zn	65	17		18			
S30400: Stainless Steel				8	74	18	

The nominal chemical compositions of the alloys tested are shown in Table I. Although alloys are identified with the UNS five-digit alloy numbering system, a three digit designation is used for brevity. For example, C26000 is referred to as C260. The alloys tested range from copper (C110) to brass (C260) and bronze (C510), copper-nickel (C706) and copper-nickel-zinc (C752). Although alloy C752 is commonly referred to as a nickel-silver because of its silver color, it does not contain silver. Each of the five alloys is representative of a major family of alloys. The experimental control is type UNS S304 stainless steel, a material widely used in food processing and healthcare applications, which does not exhibit antimicrobial efficacy.

The tests were conducted in accordance with EPA Good Laboratory Practices (GLP). Adherence to EPA GLP testing insures integrity and accuracy of the data required for registering products for public health use under FIFRA, and facilitates EPA audits of the test data. GLP tests were conducted at ambient temperature on two or three separately manufactured lots of five copper alloys, which range in copper content from 65% to 100%. Specifically, three lots of each alloy were mandated for the *S. aureus* and *E. aerogenes* tests, and two lots were required for the Methicillin-Resistant *S. aureus* (MRSA), *P. aeruginosa* and *E. coli* O157:H7 tests. Standard microbiological techniques were used to culture, recover and enumerate bacteria. Coupons of each alloy were inoculated with concentrations of bacteria and exposed for a pre-determined time period as described in the EPA-approved test protocols. After the exposure times, the remaining viable bacteria were recovered and enumerated. Each type of bacteria was cultured for 48+/- 4 hours in the identified growth media: *E. aerogenes* (ATCC#13048) in tryptic in soy broth, *P. aeruginosa* (ATCC#15442) in nutrient broth, and *S. aureus* (ATCC#6538), MRSA (ATCC#33592) and *E. coli* O157:H7 (ATCC#35150) in synthetic broth. Inocula of 5-20 µL consisting of the culture plus a soil load of 5% fetal bovine serum plus 0.01% Triton X-100 (to aid in spreading) were spread on 2.54 cm square carriers. Five carriers were tested per alloy per organism per time point. Following exposure at 20 C, the carriers were neutralized in Letheen broth (0.07% Lecithin and 0.5% Tween 80), sonicated, plated onto Agar Plate Medium (Tryptic Soy Agar with 5% Sheep Blood), and incubated at 35-37 C for 48+/-4 hours prior to visual enumeration of the number of colonies.

The three EPA-approved GLP test protocols are:

- Efficacy as a Sanitizer, which measures surviving bacteria on alloy surfaces after two hours (inoculum: 1×10^5 - 2×10^8 CFU/carrier (median ~ 4×10^7))
- Residual Self-Sanitizing Activity, which measures surviving bacteria on alloy surfaces before and after six wet and dry wear cycles over 24 hours in a standard wear apparatus, depicted schematically in Figure 1(inoculum: 1×10^5 - 7×10^7 CFU/carrier (median ~ 4×10^7)), and
- Continuous Reduction of Bacterial Contamination, which measures surviving bacteria after repeatedly inoculating alloy surfaces eight times in a 24-hour period without intermediate cleaning and wiping (inoculum: 1×10^5 - 3×10^7 CFU/carrier (median ~ 1×10^7)).

RESULTS AND DISCUSSION

The results of the 180 GLP tests, involving three test protocols, five different alloys, and five bacteria, are summarized in Table II. In both the Efficacy as a Sanitizer test and Residual Self-Sanitizing test, a reduction in live bacteria >99.9% is seen on copper in all sixty tests compared to S304 stainless steel. In the Continuous Reduction of Bacterial Contamination test, a reduction of >99.9% is achieved in 54 out of 60 tests. In five of the tests, all related to *S. aureus*, the reduction was 99.3% on one lot of C260, 99.7% on two lots of C260, and 99.6% on two lots of C752. In the sixth test, MRSA on C706, the reduction was 99.9%. In many of the Continuous Reduction tests, there were no survivors. An exception, shown in Figure 2, is MRSA on C110.

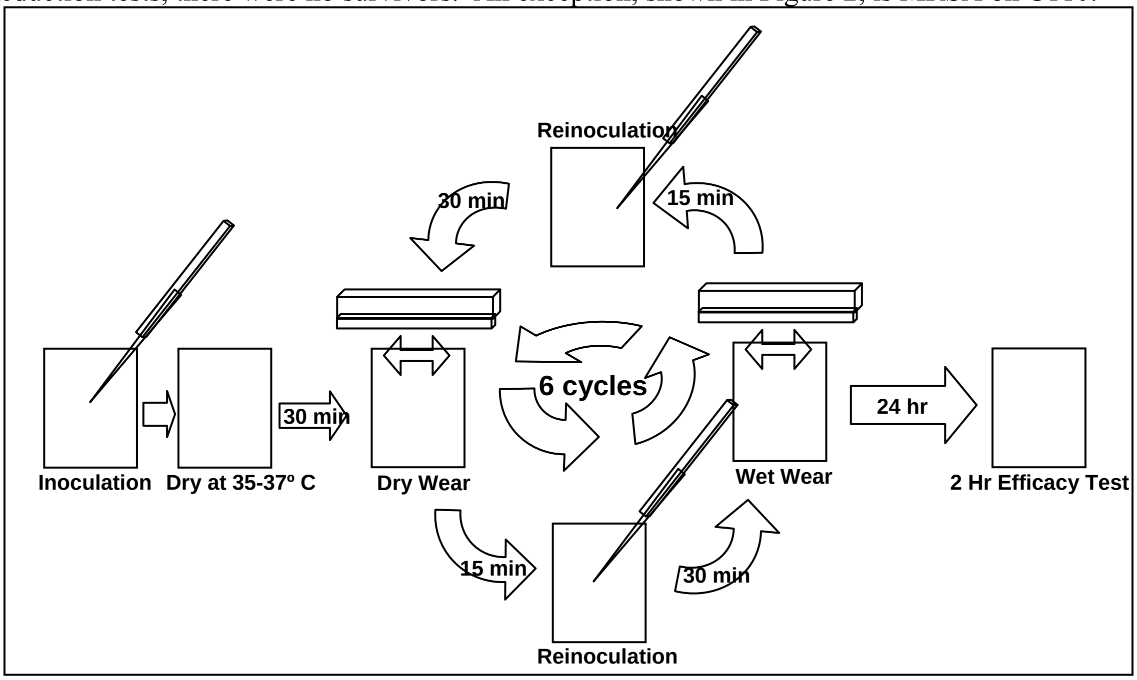

Figure 1: Residual Self-Sanitizing Test

Table II – Summary of Antimicrobial Efficacy Test Results

Alloy	*S. aureus*	*E. aerogenes*	MRSA	*P. aeruginosa*	*E.coli* O157:H7
Efficacy as a Sanitizer					
C110	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C510	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C706	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C260	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C752	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
Residual Self-Sanitizing					
C110	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C510	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C706	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C260	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C752	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
Continuous Reduction					
C110	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C510	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 >99.9	>99.9 >99.9	>99.9 >99.9
C706	>99.9 >99.9 >99.9	>99.9 >99.9 >99.9	>99.9 99.9	>99.9 >99.9	>99.9 >99.9

C260	99.3	99.7	99.7	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9
C752	>99.9	99.6	99.6	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9	>99.9

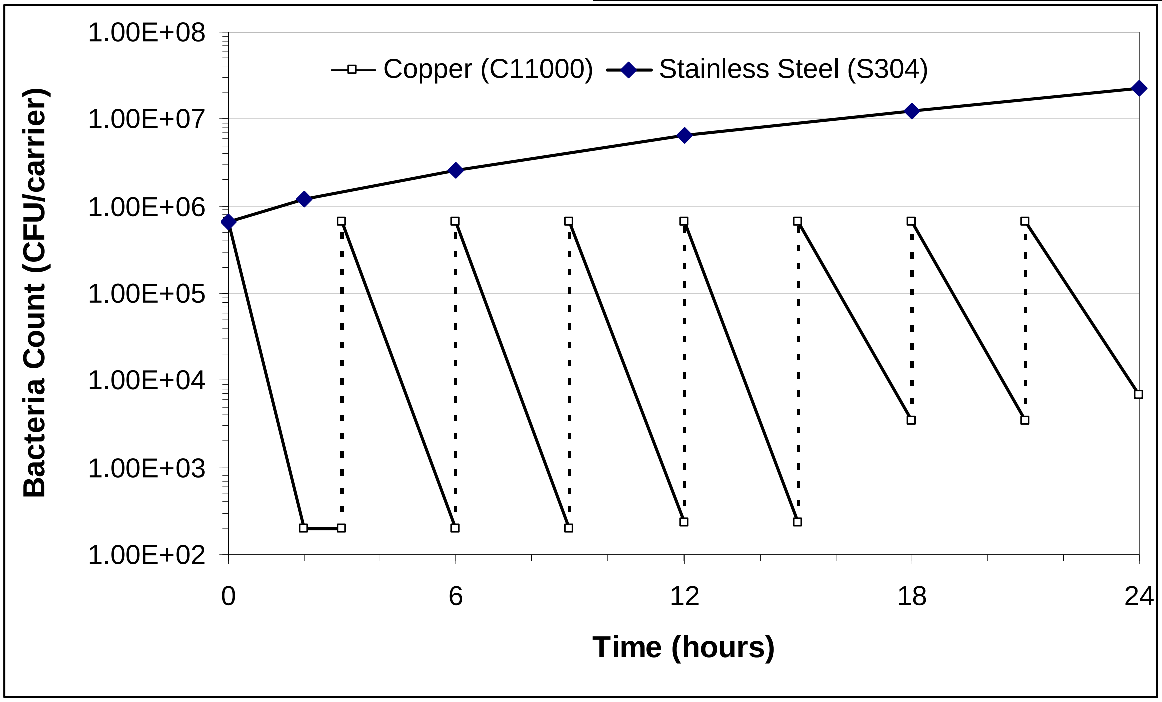

Figure 2: Continuous Reduction Test Results of MRSA on alloy C110

During the first five inoculations, the bacterial count drops from approximately 700,000 CFU/coupon to the minimum detection level of <200 CFU/carrier, which was plotted as 200. Survivors, measurably above the minimum detection limit, are seen after the sixth, seventh and eighth inoculations. However, this represents a greater than 99.9% reduction relative to S304.

The efficacy test results summary in Table II show reductions of >99.9% in 174 out of 180 tests, and reductions from 99.3% to 99.9% in the remaining six tests. These results indicate that the antimicrobial response of copper alloys is consistent, strong, enduring and reproducible.

Initially, copper alloys will be introduced into hospitals as surfaces and objects that humans frequently touch. Specific applications within hospitals include door hardware, sink faucet handles, IV drip stands, bed rails and footboards, over-the-patient tables, nurse's call buttons and work stations, furniture pulls, instrument knobs, arms of chairs and other routinely touched surfaces within the healthcare setting. Copper alloys should also be used in nursing homes, assisted living facilities, schools, public buildings, exercise facilities, shopping malls, mass transit systems, airports, the interiors of passenger aircrafts and cruise ships and even in the home.

The attainment of EPA approval is the first of several barriers to introducing copper alloys into hospitals. The applications with the highest likelihood of success are being identified, and the entire supply chain is being engaged. Clinical trials are underway. Copper alloy components are being fabricated and made readily available. Insight into the decision-making process in hospitals must be gained and understood. It is necessary to gain acceptance among hospital administrators, infection control practitioners, government healthcare officials and the general public, as well as architectural firms engaged in hospital construction. Widespread application of copper alloys should significantly decrease the number of viable bacteria found on human touch surfaces. The net result should be an increase in copper alloy utilization, a reduction in nosocomial infections and ultimately, the saving of lives. Success will be attained when it is generally understood and appreciated that copper alloys should be utilized in those applications where their unique and intrinsic antimicrobial properties will benefit human health.

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